Title: COMBINATION FOR TREATING METABOLIC DISORDERS

Abstract: Methods of treating, preventing, reducing or mitigating inflammation and/or oxidative stress, enhancing zinc uptake, regulating the lipid profile to produce a favourable lipoprotein / lipid profile and/or improving glycaemic control in an individual, comprising administering a therapeutically effective amount of a combination of zinc and an omega-3 fatty acid to the individual.
COMBINATION FOR TREATING METABOLIC DISORDERS

The present invention relates to a combination of zinc and omega-3 fatty acids which can be used to reduce or mitigate oxidative stress and/or improve the lipid profile in individuals. The invention is useful in the treatment of various conditions including metabolic disorders such as cardiovascular disease, diabetes and renal disease, as well as obesity, diseases associated with dyslipidaemia and/or oxidative stress; individuals with zinc deficiency and/or inadequate zinc intake (such as children, older or elderly people, vegetarians/vegans and others with reduced intakes of meat, seafood, or dairy); and/or individuals with inadequate intake of omega 3 fatty acids.

Metabolic disorders are considered the main cause of death worldwide and include cardiovascular disease (CVD) and diabetes mellitus (DM).

DM is one of the most common chronic diseases worldwide and is the fourth or fifth leading cause of death in the developed world. There are approximately 230 million people worldwide who currently have diabetes and approximately 3.2 million people die each year from complications associated with the diseases. There are various clinical forms of DM with the most common being type 1 or type 2 DM.

Type 1 DM, sometimes called juvenile diabetes, begins most commonly in childhood or adolescence. The onset of type 1 DM occurs rapidly and patients require daily injections of insulin. Type 1 diabetes is also known as insulin-dependent DM.

Type 2 DM is more common and accounts for 90% of all diabetics. It is sometimes called adult-onset diabetes although it is now known to occur in younger individuals and most often occurs in people who are overweight and do not engage in physical activity. Type 2 DM generally has a slow onset, often over the course of several years, but has become one of the major causes of premature illness and death, mainly through the increased risk of cardiovascular disease (CVD) which is responsible for up to 80% of these deaths.

The characterising features of DM include: the presence of chronic hyperglycaemia, consequent on decreased secretion or action of insulin, dyslipidaemia and enhanced levels of oxidative stress.
CVD itself is the leading cause of death in the western world and is responsible for over 1 million deaths a year in the US alone. The two most important components of CVD are coronary heart disease and cerebrovascular disease. Key risk factors for cardiovascular disease are high blood pressure, blood cholesterol levels, physical inactivity, obesity, smoking and diabetes. With regard to blood cholesterol levels, there is a positive correlation between the amount of cholesterol in low-density lipoproteins (LDLs) and the risk of CVD, and a negative correlation between the amount of cholesterol in high-density lipoproteins (HDLs) and the risk of CVD. According, one method to reduce the risk of CVD is to try to lower an individuals LDL levels and increase HDL levels.

Metabolic syndrome is a cluster of the most dangerous CVD risk factors: DM and raised fasting plasma glucose, abdominal obesity, high cholesterol and high blood pressure. It is estimated that around 20-25% of the worlds adult population have metabolic syndrome and they are twice as likely to die from a heart attack and three times as likely to die from a stroke compared to people without the syndrome.

Over the previous decades research has been undertaken to consider whether oxidation, or more specifically oxidative stress, is a primary cause or a secondary phenomenon of these, and other, chronic diseases. Oxidative stress is traditionally defined as an imbalance between oxidants and antioxidants (with an excess of oxidants). Oxidative stress has been linked to a broad range of disease states including obesity, autoimmune diseases, cancer (including breast, colon, liver, bowel), inflammatory bowel disease, chronic obstructive pulmonary disease, diabetes mellitus, cardiovascular disease, chronic infection, allergy, metabolic alterations, acute respiratory distress syndrome, asthma, rheumatoid arthritis, stroke, coeliac disease, renal disease, alcohol induced liver injury and other liver diseases, gastrointestinal disease, Alzheimer's disease, obstructive sleep apnoea, Parkinson's disease, cerebrovascular disease, neurodegenerative disease, cystic fibrosis and thyroid diseases.

Dyslipidemia is also a characterising feature of type 2 DM and insulin resistance. The perturbed lipid metabolism of dyslipidemia, including perturbed postprandial lipid metabolism, is a risk factor for CVD. The major changes in lipid profile in Type 2 DM are an increase in triglycerides, a reduction in HDL cholesterol (HDL-c), and the increased appearance of small, dense LDL particles, which are particularly susceptible to oxidative modification.
However, there is still a need for effective treatments to mitigate or treat the effects of oxidative stress, improve the lipid profile and mitigate or treat metabolic disorders such as DM, CVD, renal disease and/or metabolic syndrome.

In work leading to the present invention the inventors have surprisingly found for the first time that a combination product containing zinc and an omega-3 fatty acid can be used to combat oxidative stress. The combination of the two active agents both target and mitigate oxidative stress and the combination provides advantageous properties over prior art treatments. Furthermore, for the first time there is provided a combination which can be used to regulate or improve the lipid profile in an individual.

It has been found that the combination of zinc and omega-3 has as enhanced effect as compared against the effect seen with each component individually. This surprising finding means that use of the two active agents together has a beneficial effect on patients beyond the effect of each component individually. The combination has been found to lower total cholesterol levels in patients and lower LDL levels in patients. It has also been shown to have a beneficial effect in glycaemic control and to reduce the effects of oxidative stress and inflammation. Finally, the administration of omega-3 has been surprisingly found to increase the uptake of zinc.

Accordingly, in an embodiment of the present invention there is provided a method of treating, preventing, reducing or mitigating oxidative stress in an individual comprising administering a combination of zinc and an omega-3 fatty acid to the individual.

In a further embodiment, there is provided a method of treating, preventing, reducing or mitigating inflammation in an individual comprising administering a combination of zinc and an omega-3 fatty acid to the individual.

In a further embodiment, there is provided a method of enhancing zinc uptake in an individual comprising administering an omega-3 fatty acid to the individual. Preferably, the composition further comprises zinc.

In a further embodiment, there is provided a method of improving an individual's lipoprotein/lipid profile comprising administering a combination of zinc and an omega-3 fatty acid to the individual.
In one embodiment, the combination can increase the levels of high density lipoproteins (HDLs) and/or also decrease the levels of triglycerides. Thus, the combination of the present invention can be used to regulate lipid metabolism. In a particular embodiment, the combination increases HDL-c levels and decreases triglyceride levels in the individual.

The combination of zinc and omega-3 as defined in the present invention have application in reducing oxidative stress and/or improving the lipid profile. This has a number of therapeutic applications.

Accordingly, in a yet further embodiment there is provided a method of treating, preventing, reducing or mitigating type 2 diabetes comprising administering a combination of zinc and an omega-3 fatty acid to an individual.

Patients with type 2 diabetes are likely to have increased levels of oxidative stress and are also likely to have lipoprotein imbalance. The combination of zinc and omega-3 is believed to be useful in tackling both of these problems. The zinc and omega-3 may both work to reduce the oxidative stress associated with diabetes. At the same time, they may also work together to increase the HDL-c and reduce the triglyceride levels in the individual. Thus, the combination of zinc and omega-3 provides enhanced benefits over current treatments.

In a yet further embodiment there is provided a method of treating, preventing, reducing or mitigating cardiovascular disease in an individual, comprising administering a combination of zinc and an omega-3 fatty acid to the individual.

For the first time, the combination of zinc and an omega-3 fatty acid is provided. This specific combination has a number of advantages over prior art therapies and counters many of the risk factors associated with CVD, for example countering oxidative stress, increasing HDL-c and/or decreasing triglycerides. It may also be useful in improving insulin sensitivity and glycaemic control and positively impacting leptin signaling.

In a yet further embodiment there is provided a method of treating, preventing, reducing or mitigating renal disease in an individual, comprising administering a combination of zinc and an omega-3 fatty acid to the individual.
In a yet further embodiment there is provided a method of treating, preventing, reducing or mitigating dyslipidemia in an individual comprising administering a combination of zinc and an omega-3 fatty acid.

In a yet further embodiment there is provided a method of treating, preventing, reducing or mitigating metabolic syndrome in an individual, comprising administering a combination of zinc and an omega-3 fatty acid to the individual.

In one embodiment, the zinc and omega-3 fatty acids are administered concurrently. In a further embodiment, the zinc and omega-3 fatty acids are administered consecutively. In a yet further embodiment, the zinc and omega-3 fatty acids are administered in a single formulation.

In embodiments of the invention, the combination may be used on any individual in need thereof. In a particular embodiment, the compositions of the present invention are given to individuals who are zinc deficient. Zinc deficiency, as referred to herein, includes both severe and marginal deficiency. Marginal zinc deficiency is common even in otherwise healthy populations and can be caused by a number of factors, including the consumption of foods high in zinc inhibitors such as phytate or inadequacy of zinc intake. In a yet further embodiment, the compositions are given to individuals whose zinc intake levels are lower than needed. In a yet further embodiment, the compositions are given to individuals whose zinc homeostasis is perturbed. Examples of individuals who might present with zinc deficiency and/or inadequate zinc intake include: children, older or elderly people, vegetarians/vegans and others with reduced intakes of meat, seafood, or dairy. In further embodiments, the compositions of the present invention are given to individuals who are omega-3 deficient. In a yet further embodiment, the compositions are given to individuals whose omega-3 intake levels are lower than optimal.

Thus, in a further embodiment there is provided a method of determining if an individual is zinc deficient, and if so administering a combination of zinc and an omega-3 fatty acid to the individual. In one embodiment, measuring the plasma levels of zinc in the individual is taken to determine zinc deficiency.
In a yet further embodiment there is provided a method of measuring the plasma levels of HDL-c in an individual to determine their HDL-c level and if this level is low to administer a combination of zinc and an omega-3 fatty acid to the individual.

In a yet further embodiment of the present invention, the combination is given to individuals who are experiencing oxidative stress. In a yet further embodiment of the present invention, the combination is given to individuals who have type 2 DM.

The combination may be administered in a composition comprising zinc, an omega-3 fatty acid and a therapeutically acceptable carrier. In certain embodiments, the combination may be administered in a composition consisting zinc, an omega-3 fatty acid and a therapeutically acceptable carrier. Thus, in some embodiments the zinc and omega-3 may be administered alongside other agents whereas in other embodiments the zinc and omega-3 are provided in isolation of other active agents.

In one embodiment, the zinc is administered as elemental zinc or one or more zinc salts such as zinc citrate, zinc acetate, zinc picolinate, zinc sulphate, or other zinc salts like amino acids, dipeptides, gluconates, halides, nitrates, oxides or acetates.

The omega-3 fatty acid may be obtained from any available source. In one embodiment, the omega-3 fatty acid is derived from a marine oil. In a further embodiment, the omega-3 fatty acid is derived from a fish oil. In a further embodiment, the omega-3 fatty acid is derived from a marine algal oil. In a further embodiment, the omega-3 fatty acid is derived from a plant source. In a further embodiment, the omega-3 fatty acid is derived from an animal source. In a further embodiment, the omega-3 fatty acid is derived from a fish source. In a further embodiment, the omega-3 fatty acid is derived from an algal source, for example a microalgal source. In a yet further embodiment, the omega-3 fatty acid is ALA. In a yet further embodiment, the omega-3 fatty acid is EPA. In a yet further embodiment, the omega-3 fatty acid is DPA. The omega-3 of the present invention includes mixtures of omega-3 fatty acids which may be obtained from different sources.

For the avoidance of doubt, the present invention encompasses the use of a single omega-3 fatty acid as well as the use of two or more omega-3 fatty acids. As such, the term "an omega-3 fatty acid" can mean a single omega-3 fatty acid as well as two or
more omega-3 fatty acids. In one embodiment of the present invention the term means a single fatty acid. In an alternative embodiment, the term means two or more fatty acids.

In a further embodiment there is provided a composition comprising a combination of zinc and an omega-3 fatty acid, for use in treating, preventing, reducing or mitigating oxidative stress in an individual.

In a further embodiment there is provided a composition comprising an omega-3 fatty acid for use in enhancing zinc uptake in an individual. Preferably, the composition further comprises zinc.

In a further embodiment there is provided a composition comprising a combination of zinc and an omega-3 fatty acid, for use in regulating the lipid profile to produce a favourable lipoprotein/lipid profile in an individual.

In a further embodiment there is provided a composition comprising a combination of zinc and an omega-3 fatty acid, for use in treating, preventing, reducing or mitigating type 2 diabetes.

In a further embodiment there is provided a composition comprising a combination of zinc and an omega-3 fatty acid, for use in treating, preventing, reducing or mitigating cardiovascular disease in an individual.

In a further embodiment there is provided a composition comprising a combination of zinc and an omega-3 fatty acid, for use in treating, preventing, reducing or mitigating renal disease in an individual.

In a further embodiment there is provided a composition comprising a combination of zinc and an omega-3 fatty acid, for use in treating, preventing, reducing or mitigating dyslipidemia in an individual.

In a further embodiment there is provided a composition comprising a combination of zinc and an omega-3 fatty acid, for use in treating, preventing, reducing or mitigating metabolic syndrome in an individual.
In a further embodiment there is provided the use of a combination of zinc and an omega-3 fatty acid, for the manufacture of a medicament for treating, preventing, reducing or mitigating oxidative stress in an individual.

In a further embodiment there is provided the use of a composition comprising an omega-3 fatty acid for the manufacture of a medicament for use in enhancing zinc uptake in an individual. Preferably, the composition further comprises zinc.

In a further embodiment there is provided the use of a combination of zinc and an omega-3 fatty acid, for the manufacture of a medicament for improving an individual’s lipoprotein/lipid profile.

In a further embodiment there is provided the use of a combination of zinc and an omega-3 fatty acid, for the manufacture of a medicament for treating, preventing, reducing or mitigating type 2 diabetes.

In a further embodiment there is provided the use of a combination of zinc and an omega-3 fatty acid, for the manufacture of a medicament for treating, preventing, reducing or mitigating cardiovascular disease in an individual.

In a further embodiment there is provided the use of a combination of zinc and an omega-3 fatty acid, for the manufacture of a medicament for treating, preventing, reducing or mitigating renal disease in an individual.

In a further embodiment there is provided the use of a combination of zinc and an omega-3 fatty acid, for the manufacture of a medicament for treating, preventing, reducing or mitigating dyslipidemia in an individual.

In a further embodiment there is provided the use of a combination of zinc and an omega-3 fatty acid, for the manufacture of a medicament for treating, preventing, reducing or mitigating metabolic syndrome in an individual.

In particular embodiments of the present invention the composition contain additional components.
In an embodiment, the composition further comprises cofactors to modulate the conversion of short-chain to long-chain omega-3 fatty acids. Cofactors include but are not limited to B3, B6, Vitamin C, and magnesium.

The essential fatty acids of the omega-3 series (e.g. ALA, EPA, DHA) are precursors for different signalling molecules that mediate, for example, anti-inflammatory, anti-steatotic, and vascular protective effects through several mechanisms, including modifications in cell membrane composition and function, gene expression modulation, and production of specific eicosanoids. Conversion of essential fatty acids in the body from one form to another (e.g. conversion of the short-chain fatty acid ALA to the long chain fatty acids EPA and DHA) is limited. Initial steps in fatty acid metabolism are desaturations catalysed by rate-limiting 6 and 5 desaturases. In humans, 5 and 6 desaturase activities, and thereby the conversion rate of ALA to EPA/DHA, are low and can further be modulated by dietary co-factors which, in addition to zinc, include magnesium, vitamin B3, vitamin B6, and vitamin C.

In a further embodiment the composition further comprises antioxidants. Examples of antioxidants include but are not limited to retinyl esters, glutathione, vitamin C, vitamin E, and carotenoids.

Addition of antioxidants and/or phytochemicals can be used to enhance the effects of the multi-nutrient supplement in reducing oxidative stress. Adults with metabolic disorders often have suboptimal blood or tissue concentrations of several antioxidants, which may explain at least partially their increased risk for diabetes, cardiovascular disease, and related disorders. Antioxidants, such as flavonoids, carotenoids, ascorbic acid and tocopherols may inhibit the production of reactive species (RS) by a range of mechanisms, including the inhibition of several RS producing enzymes (i.e. xanthine oxidase, cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione-S-transferase, mitochondrial succinoxidase, NADH oxidase).

In a further embodiment the composition further comprises phytochemicals. Examples of phytochemicals include polyphenols (including catechins such as epigallocatechin gallate), isoflavones, isoprenoids, and anthocyanidins.

There is increasing evidence of potential benefits of phytochemicals in the regulation of cellular processes such as redox control and inflammatory responses. Phytochemicals,
including soy protein, proanthocyanidins, and polyphenols found in fruits, vegetables, berries, beverages and herbal medicines, may modify perturbed lipid and glucose homeostasis thereby having beneficial effects in weight management, glucose control and cardiovascular risk factors.

However, care should be taken with the addition of some antioxidants/phytochemicals to make sure that they do not reduce the effectiveness of the composition. For example, some phytochemicals may act as a chelator of zinc and this would need to be addressed in the composition formulation (i.e. particular antioxidants/phytochemicals may need to be included in a protected form, for example employing microencapsulation or timed-release techniques).

While some embodiments consider additional agents in the composition, in some embodiments the combinations comprise zinc and omega-3 in exclusion of other active ingredients / supplements. Such embodiments may include therapeutically acceptable carriers. Thus, for the avoidance of doubt where mention is made of combinations "comprising" zinc and an omega-3 fatty acid the present invention specifically includes the possibility that the word comprising can be replaced with "consisting" or "consisting essentially of."

Thus, for example, where the present invention refers to an embodiment providing: "a composition comprising a combination of zinc and an omega-3 fatty acid to the individual, for use in treating, preventing, reducing or mitigating oxidative stress in an individual", this also encompass "a composition consisting of a combination of zinc and an omega-3 fatty acid to the individual, for use in treating, preventing, reducing or mitigating oxidative stress in an individual" and "a composition consisting essentially of a combination of zinc and an omega-3 fatty acid to the individual, for use in treating, preventing, reducing or mitigating oxidative stress in an individual".

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step; or group of elements, integers or steps.
Throughout this specification, the term "consisting essentially of" is intended to exclude elements which would materially affect the properties of the claimed composition.

In embodiments of the invention the composition may exclude one or more agents. These optional embodiments apply to all aspects of the present invention and one or more of them may be combined. Furthermore, the agent exclusions may optionally be combined with the one or more of the use exclusions disclosed below.

In embodiments, the composition excludes ascorbic acid.

In further embodiments, the composition excludes carotenoids in general or lutein in particular.

In yet further embodiments, the composition excludes omega-6 fatty acids in general or gamma-linolenic acid in particular.

In yet further embodiments, the composition excludes omega-6 fatty acids in general or gamma-linolenic acid in particular.

In yet further embodiments, the composition excludes vitamin C and/or vitamin E and/or vitamin B6 and/or vitamin B12.

In yet further embodiments, the composition excludes proteins in general and insulin in particular.

In yet further embodiments, the composition excludes statins, co-enzyme Q10 and/or resveratrol.

In yet further embodiments, the composition excludes the zinc salt of the omega-3 fatty acid.

In yet further embodiments, the composition excludes an encapsulated pro-oxidant mineral.

In yet further embodiments, the composition excludes phospholipids.
In yet further embodiments, the composition is not a food product.

Furthermore, in embodiments of the invention, specific uses may optionally be excluded. These optional embodiments apply to all aspects of the present invention and one or more of them may be combined. Furthermore, the use exclusions may optionally be combined with the one or more of the agent exclusions disclosed above.

In embodiments, the methods and uses of the present invention exclude diabetic retinopathy.

In further embodiments, the methods and uses of the present invention exclude increasing horny layer cell maturation.

In yet further embodiments, the methods and uses of the present invention exclude dry eye syndrome.

It is believed that the combination may be beneficial when an individual exhibits increased zinc excretion (such as in Type 2 diabetes mellitus or renal disease), inadequate intake of zinc (such as may occur in the elderly, children, vegan/vegetarians, individuals who avoid consuming meat/seafood/dairy etc), inadequate intake of omega-3, malnutrition, and/or excessive alcohol intake.

In one embodiment, the combinations of the present invention may be used in the treatment of metabolic disorders such as cardiovascular disease, diabetes and renal disease; obesity, diseases associated with dyslipidaemia and/or oxidative stress; individuals with zinc deficiency and/or inadequate zinc intake (such as children, older or elderly people, vegetarians/vegans and others with reduced intakes of meat, seafood, or dairy); and/or individuals with inadequate intake of omega 3 fatty acids.

**Brief description of the Figures**

Figure 1a. Change in total cholesterol concentrations (mmol/L) after supplementation with zinc alone, omega 3 alone, zinc and omega 3 in combination, or placebo.
Figure 1b. Change in low density lipoprotein (LDL) cholesterol concentrations (mmol/L) after supplementation with zinc alone, omega 3 alone, zinc and omega 3 in combination, or placebo.

Figure 2. Change in plasma zinc concentrations (µmol/L) after supplementation with zinc alone, omega 3 alone, zinc and omega 3 in combination, or placebo.

Figure 3. Change in high-sensitivity c-reactive protein (hsCRP) concentrations (mg/L) after supplementation with zinc alone, omega 3 alone, zinc and omega 3 in combination, or placebo.

Figure 4. Change in Haemoglobin Ale (HbAlc) concentrations (%) after supplementation with zinc alone, omega 3 alone, zinc and omega 3 in combination, or placebo.

Zinc

Zinc plays a ubiquitous role in the body and is involved in many metabolic pathways. Perturbations of zinc homeostasis are now recognised as important contributors to the pathophysiology of an increasing number of chronic disorders, including CVD and DM. An extensive variety of genes are regulated by zinc, impacting such diverse processes as protein-protein interactions, fatty acid metabolism, apoptosis, and signal transduction. Many zinc-dependent biochemical pathways are also redox-sensitive and one of the consequences of the redistribution of zinc in disease states is an exacerbation of oxidative stress.

A number of pathways involved in redox metabolism require zinc for their function. Zinc ions are integral to the activities of superoxide dismutase (SOD) and metallothionein (MT), both of which protect against an accumulation of reactive species (RS) in cellular systems. Zinc affects a range of redox-sensitive cell signalling processes; it plays a role in the regulation of NF-κB and apoptosis and is involved in nitric oxide (NO) signalling pathways. Moreover, it has been suggested that zinc itself acts as a signalling molecule and may function to extend the signalling capacity of calcium and magnesium, the other redox-inert metal ions involved in redox metabolism.
However, the potential effects of inorganic nutrients, especially zinc, have received limited attention. Studies in rats have shown that a high intake of zinc, with a resultant increase in the dietary zinc/copper ratio, raises plasma cholesterol concentrations. In humans, the first reported randomised controlled trial investigating the effects of zinc supplementation on plasma lipids found that increasing zinc levels tended to reduce plasma HDL-c levels. These undesirable outcomes suggest away from the use of zinc since they would indicate an increased risk of CHD.

The present invention encompasses administration of all forms of zinc. The zinc can be included from any zinc source material which includes all appropriate sources of zinc for administration to humans and/or other animals. The zinc can be present as, for example, elemental zinc or one or more zinc salts such as zinc citrate, zinc acetate, zinc picolinate, zinc sulphate, or other zinc salts like amino acids, dipeptides, gluconates, halides, nitrates, oxides or acetates.

Particular forms of zinc encompassed by the present invention include zinc gluconate, zinc gluconate glycine, zinc acetate, zinc sulphate and/or zinc oxide.

The zinc may be present as a salt of the omega-3 fatty acid. In this instance, the zinc may be present as the counter ion to the acyloxy group of the fatty acid. The zinc and omega-3 fatty acid can be present in various stoichiometric amounts which can vary depending on various factors as like the degree of hydration and the presence of other compounds. Equally, the zinc may be present as separate forms. Therefore, in embodiments of the invention the omega-3 and zinc may not be present in the composition as a single salt, that is a zinc salt of an omega-3 fatty acid.

The present invention encompasses various doses of zinc. In one embodiment the zinc is administered daily.

The zinc may be administered in a daily dose of about 1 mg to about 100 mg, about 5 mg to about 100 mg, about 10 mg to about 100 mg, about 15 mg to about 100 mg, about 20 mg to about 100 mg, about 25 mg to about 100 mg, about 30 mg to about 100 mg, about 35 mg to about 100 mg, about 40 mg to about 100 mg, about 45 mg to about 100 mg, about 50 mg to about 100 mg, about 55 mg to about 100 mg, about 60 mg to about 100 mg, about 65 mg to about 100 mg, about 70 mg to about 100 mg, about 75 mg to about 100 mg, about 80 mg to about 100 mg, about 85 mg to about 100 mg, about 90 mg to about 100 mg, about
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20mg to about 15mg, about 5mg to about 10mg, or about 10mg to about 10mg.

In one embodiment of the present invention, daily doses of elemental zinc are about 1 to about 160 mg. In a further embodiment, daily doses are about 15 to about 160 mg. Particular daily doses of zinc include about 2 to about 50mg elemental zinc per day.

In a further embodiment, zinc doses were about 10mg, about 20mg, about 30mg, about 40mg, about 50mg, about 60mg, about 65mg, or about 70mg per day.

In a further embodiment, daily doses of elemental zinc are about 10 to about 100 mg per day. In a yet further embodiment, daily doses of elemental zinc are about 20 to about 90 mg, about 30 to about 80 mg, about 40 to about 60 mg, or about 50 mg per day.
In a further embodiment, daily doses of elemental zinc are about 10 to about 50 mg per day. In a yet further embodiment, daily doses of elemental zinc are about 20 to about 50 mg, about 30 to about 50 mg, about 35 to about 45 mg, or about 40 mg per day.

In one embodiment, the zinc anions can be sulphate, gluconate, acetate, oxide, and glycine chelate anions. The zinc anion may be organic or inorganic.

Omega-3 fatty acids

Omega-3 fatty acids are polyunsaturated fatty acids that are an essential part of a healthy diet. Some studies have suggested that an increased intake of omega-3 fatty acids may reduce the risk of cardiovascular disease. It has been suggested that omega-3 may also enhance glycaemic control and dietary supplementation has been shown to improve insulin sensitivity in subjects with DM. Furthermore, omega-3 directly activates transcription factors that regulate lipid metabolism and is known to decrease serum triglyceride levels.

Omega-3 fatty acids are long chain polyunsaturated fatty acids derived from various sources including certain fish oils and linseed oil. Particular omega-3 fatty acids encompassed by the present invention include a-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA).

The omega-3 fatty acid may be obtained from any available source. The source may be a marine oil, including fish oils and algal oils. The oil may be from a plant or animal. The omega-3 may be one or more of all-cis-7,10,13-hexadecatrienoic acid, a-linolenic acid (ALA), stearidonic acid (SDA), eicosatrienoic acid (ETA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), tetracosahexaenoic acid (THA, nisinic acid). The omega-3 may be in the form of form of: triglycerides (triacylglycerols), phospholipids, or ethyl esters.

The omega-3 fatty acid may be in a protected form to prevent oxidisation. One such form of protection includes encapsulation. Another way to prevent oxidation is to include one or more antioxidants, for example vitamin E.

The omega-3 may be present in liquid, powder or soft gel form.
The omega-3 may be administered in a daily dose of from about 0.1 g to about 20 g, from about 0.2 g to about 20 g, from about 0.3 g to about 20 g, from about 0.4 g to about 20 g, from about 0.5 g to about 20 g, from about 0.6 g to about 20 g, from about 0.7 g to about 20 g, from about 0.8 g to about 20 g, from about 0.9 g to about 20 g, from about 1 g to about 20 g, from about 2 g to about 20 g, from about 3 g to about 20 g, from about 4 g to about 20 g, from about 5 g to about 20 g, from about 6 g to about 20 g, from about 7 g to about 20 g, from about 8 g to about 20 g, from about 9 g to about 20 g, from about 10 g to about 20 g, from about 11 g to about 20 g, from about 12 g to about 20 g, from about 13 g to about 20 g, from about 14 g to about 20 g, from about 15 g to about 20 g, from about 16 g to about 20 g, from about 17 g to about 20 g, from about 18 g to about 20 g, from about 19 g to about 20 g, from about 0.1 g to about 19 g, from about 0.2 g to about 19 g, from about 0.3 g to about 19 g, from about 0.4 g to about 19 g, from about 0.5 g to about 19 g, from about 0.6 g to about 19 g, from about 0.7 g to about 19 g, from about 0.8 g to about 19 g, from about 0.9 g to about 19 g, from about 1 g to about 19 g, from about 2 g to about 19 g, from about 3 g to about 19 g, from about 4 g to about 19 g, from about 5 g to about 19 g, from about 6 g to about 19 g, from about 7 g to about 19 g, from about 8 g to about 19 g, from about 9 g to about 19 g, from about 10 g to about 19 g, from about 11 g to about 19 g, from about 12 g to about 19 g, from about 13 g to about 19 g, from about 14 g to about 19 g, from about 15 g to about 19 g, from about 16 g to about 19 g, from about 17 g to about 19 g, from about 0.1 g to about 18 g, from about 0.2 g to about 18 g, from about 0.3 g to about 18 g, from about 0.4 g to about 18 g, from about 0.5 g to about 18 g, from about 0.6 g to about 18 g, from about 0.7 g to about 18 g, from about 0.8 g to about 18 g, from about 0.9 g to about 18 g, from about 1 g to about 18 g, from about 2 g to about 18 g, from about 3 g to about 18 g, from about 4 g to about 18 g, from about 5 g to about 18 g, from about 6 g to about 18 g, from about 7 g to about 18 g, from about 8 g to about 18 g, from about 9 g to about 18 g, from about 10 g to about 18 g, from about 11 g to about 18 g, from about 12 g to about 18 g, from about 13 g to about 18 g, from about 14 g to about 18 g, from about 15 g to about 18 g, from about 16 g to about 18 g, from about 0.1 g to about 17 g, from about 0.2 g to about 17 g, from about 0.3 g to about 17 g, from about 0.4 g to about 17 g, from about 0.5 g to about 17 g, from about 0.6 g to about 17 g, from about 0.7 g to about 17 g, from about 0.8 g to about 17 g, from about 0.9 g to about 17 g, from about 1 g to about 17 g, from about 2 g to about 17 g, from about 3 g to about 17 g, from about 4 g to about 17 g, from about 5 g to about 17 g, from about 6 g to about 17 g, from about 7 g to about 17 g, from about 8 g to about 17 g, from about 9 g to about 17 g, from about 10 g to about 17 g, from about 11 g to about 17 g, from about 12 g to about 17 g, from about 13 g to about 17 g.
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A particular daily dose is about 100 mg to about 5 g. The lower doses often refer to a particular fatty acid such as DHA or EPA while higher doses often refer to total omega-3 fatty acids.
A further daily dose is about 1 to about 5g, about 1 to about 3g or about 2g of omega-3.

In an embodiment, the amount of omega-3 is based on short-chain ALA fatty acids.

In an embodiment of the present invention the amount of omega-3 fatty acid is selected to administer an amount of fatty acid that provides about 100-1000mg/day long chain fatty acid to the individual, or about 200-800mg/day, about 400-600mg/day or about 400 mg/day for a woman and 600mg/day for a man.

For both the zinc and the omega-3 the daily dose may be taken in a single administration or over multiple administrations in a single day to achieve the total daily dose.

Pharmaceutical formulations.

The combination of the present invention may be provided in any suitable pharmaceutical formulation which can comprise a combination of zinc and omega-3 as described herein and a pharmaceutically acceptable carrier. The formulations may be used therapeutically or prophylactically.

By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical formulation in which it is contained.

The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art. Pharmaceutical carriers and their formulations are known to those skilled in the art.

The pharmaceutical compositions according to the invention normally further comprise one or more physiologically acceptable excipients, i.e. a therapeutically inert substance or carrier.

The carrier may take a wide variety of forms depending on the desired dosage form and administration route.
The pharmaceutical composition comprising the combination of the present invention may be in the form of a solid, semi-solid or fluid composition. The solid composition may be in the form of tablets such as, e.g. conventional tablets, effervescent tablets, coated tablets, melt tablets or sublingual tablets, pellets, powders, granules, granulates, particulate material, gels, gel capsules, solid dispersions or solid solutions.

In one embodiment of the invention, the pharmaceutical composition may be in the form of a tablet. The tablet may have a shape that makes it easy and convenient for an individual to swallow. The tablet may thus e.g. have a rounded or a rod-like shape without any sharp edges. Furthermore, the tablet may be designed to be divided in two or more parts.

A semi-solid form of the composition may be a paste, a gel or a hydrogel.

The fluid form of the composition may be a solution, an emulsion including nano-emulsions, a suspension, a dispersion, a liposomal composition, a spray, a mixture, a syrup or an elixir.

Other suitable dosages forms of the pharmaceutical compositions according to the invention may be capsules, sachets, troches, devices etc.

The pharmaceutical compositions may be prepared by any of the methods well known to a person skilled in pharmaceutical formulation.

The pharmaceutically acceptable excipients may be e.g. fillers, binders, disintegrants, diluents, glidants, solvents, emulsifying agents, suspending agents, stabilizers, enhancers, flavours, colours, pH adjusting agents, retarding agents, wetting agents, surface active agents, preservatives, antioxidants etc. Details can be found in pharmaceutical handbooks such as, e.g., Remington's Pharmaceutical Science or Pharmaceutical Excipient Handbook. In those cases, where the combination is intended for controlled release, it may also comprise release controlling agents such as, e.g., material normally used in the formulation of matrix tablets (e.g. cellulose derivatives like hydroxypropyl methylcellulose and the like). Alternatively, the composition may be coated with a controlled release coating such as an enteric coating or a film coating.

A suitable coating may be a substantially water-insoluble but water-permeable coating.
The composition may be in the form of a tablet, a capsule, a multiparticulate form, or a unit dose packet such as a sachet.

The term "tablet" is intended to embrace compressed tablets, coated tablets, matrix tablets, osmotic tablets, and other forms known in the art.

The term "capsule" is intended to embrace hard and soft capsules, in which the shell of the capsule disintegrates after ingestion to release its content.

The term "multiparticulate" is intended to embrace a dosage form comprising a multiplicity of particles and/or granulates whose totality represents the intended therapeutically useful dose. The particles generally are of a diameter from about 50 microns to about 0.3 cm, with a preferred range of 100 μm to 1 mm. Multiparticulates represent a suitable embodiment for use in scaling dosage forms release because they are amenable according to the weight of an individual subject (e.g. a mammal such as a human).

Above are mentioned specific examples of the amounts of compounds administered. However, it will be understood that the amount of the compounds actually administered will be determined by a physician in light of the relevant circumstances including the condition to be treated, the choice of compounds to be administered, the age, weight, and response of the individual patient, the severity of the individual's symptoms and/or signs, and the chosen route of administration. While the present compounds are preferably administered orally, the compounds may also be administered by any other suitable route.

Foodstuffs
The combination may be incorporated into foodstuffs. Foodstuffs include items that can be consumed (e.g., eaten, drank, or ingested) by a subject. In one example, the compositions can be used as nutritional supplements that are added to a foodstuff. For example, the disclosed compositions can be added to food or beverages. In this sense, the disclosed compositions can be prepared in, for example, a powdered form and contained in articles such as sachets or shakers, which can be used to pour or sprinkle the disclosed compositions onto and into food and beverages.
In some examples, the foodstuff is a baked good, a pasta, a meat product, a frozen dairy product, a milk product, a cheese product, an egg product, a condiment, a soup mix, a snack food, a nut product, a plant protein product, a hard candy, a soft candy, a poultry product, a processed fruit juice, a granulated sugar (e.g., white or brown), a sauce, a gravy, a syrup, a nutritional bar, a beverage, a dry beverage powder, a jam or jelly, a fish product, or companion pet food. In other examples, the foodstuff is bread, tortillas, cereal, sausage, chicken, ice cream, yogurt, milk, salad dressing, rice bran, fruit juice, a dry beverage powder, liquid beverage, rolls, cookies, crackers, fruit pies, or cakes.

Foodstuffs can also include animal feed products, such as semi-dry pet food and moist pet food (e.g., dog and cat food). Foodstuff can also include livestock feed, e.g., ruminant feed.

**Supplements**

The combinations disclosed herein may be administered to the individual in the form of a nutritional supplement. A nutritional supplement is any compound or composition that can be administered to or taken by a subject to provide, supply, or increase a nutrient(s). In one aspect, disclosed herein are nutritional supplements comprising any of the compositions disclosed herein.

The nutritional supplement can comprise any amount of the compositions disclosed herein and can comprise other components such as preservatives, antimicrobials, antioxidants, chelating agents, thickeners, flavourings, diluents, emulsifiers, dispersing aids, or binders. The nutritional supplements are generally taken orally and can be in any form suitable for oral administration. For example, a nutritional supplement can typically be in a tablet, gel-cap, capsule, liquid, sachets, or syrup form.

The omega-3 oils of the present invention may be formulated to ensure that they remain stable. Omega-3 oils are prone to oxidisation and steps can be taken to protect the oxygen sensitive oils. Examples of such protecting steps are microencapsulation or other techniques to protect the oils. These techniques are well known to a formulator and are useful with the present invention.

Although the composition of the invention is preferably intended for administration to humans it should be understood that the formulation may also be utilized in veterinary applications for animals.
Experimental data
A randomised controlled trial was carried out to determine the effect of a combination
of zinc and omega-3 on three related conditions: hyperglycaemia, dyslipidemia and
increased oxidative stress, as well as the measurement of plasma zinc as an indicator of
zinc status.

Recruited participants follow a 12 week treatment schedule of:
   a) zinc,
   b) omega-3,
   c) zinc + omega 3, or
   d) placebo;

The effects of each treatment were monitored with respect to glycaemic control, lipid
profile and recognised biochemical markers of oxidative stress. In addition, plasma
zinc concentrations were monitored as well as dietary zinc intake to indicate changes
in zinc status in the group.

The study population consists of postmenopausal women diagnosed with type 2 DM
(fasting glucose > 8 mmol/L and HbAlC> 7.5%) who are not on insulin.

Inclusion criteria for the trial was as follows:
Female, postmenopausal
Have type 2 diabetes (controlled by diet and lifestyle or oral hypoglycaemic
medication)
Have normal Glomerular Filtration Rate (GFR) and normal microalbumin/creatinine ratio
Are not taking any nutritional supplements in the 6 weeks prior to the trial &
continuing through the trial period
Do not donate blood in the 6 weeks prior to the trial & continuing through the trial period
Non-smoking
Have not been diagnosed with any current major illness (other than diabetes)

Participants were randomly allocated to one of the four groups for a period of 12
weeks. The study protocol and nature of the intervention was explained to each
subject. Supplements comprise commercially available zinc and omega-3 nutrients.
The placebo matching the zinc supplement was formulated to contain mainly cellulose.
The placebo matching the omega-3 supplement contained oleic acid (a common dietary polyunsaturated fatty acid). The doses administered were 40 mg elemental zinc/day and 2g total omega-3 fatty acids/day.

During the trial period, usual dietary intake was assessed by 24 hour food records and a validated food frequency questionnaire administered by a research dietitian. Compliance with the intervention was determined by measuring plasma zinc and by counting unused capsules.

Blood samples were collected from all participants at the start of the intervention at week 0 and then at 4 weekly intervals (weeks 4, 8 and 12). Blood samples were analysed for plasma zinc, lipids (total cholesterol and LDL cholesterol) and a marker of oxidative stress (CRP) and glycaemic control (glucose, insulin, HbAlc).

Data is provided for the following participants:
Women with Type 2 Diabetes Mellitus (n=19), randomised to 1 of 4 groups:
Zn alone (n=5)
Omega 3 alone (n=2, after withdrawals)
Zn + omega 3 combination (n=6)
Placebo (n=6)

Outcome 1: Lipid Profile

The changes in lipid profile are shown in figure 1(a) and (b). Total cholesterol changes are shown in Figure 1(a) and it can be seen that the zinc and omega 3 combination decreased total cholesterol concentrations by approximately 0.3 mmol/L from baseline after 8 weeks of supplementation. In contrast, an increase of 0.3 mmol/L was observed with both the zinc alone and omega 3 alone interventions over the same time period. Total cholesterol concentrations were relatively constant over time in the placebo group.

Therefore, a beneficial decrease in total cholesterol concentrations was observed after supplementation with the zinc & omega 3 combination that was not apparent after supplementation with either zinc or omega 3 alone. The amount of the decrease (0.3 mmol/L) represents a significant improvement in heart disease risk; if maintained over time, a decrease in total cholesterol of as little as 0.1 mmol/L can improve heart disease.
risk and it has been estimated that a reduction in total cholesterol of 0.6 mmol/L lowers the risk of heart disease by as much as 50% (BMJ 1994;308(6925):367-72).

Change in low density lipoprotein (LDL-c) cholesterol concentrations (mmol/L) are shown in Figure 1(b). The zinc and omega 3 combination decreased LDL cholesterol concentrations by approximately 0.2 mmol/L from baseline and 0.3 mmol/L from placebo levels after 8 weeks of supplementation. In contrast, an increase in LDL cholesterol from baseline and placebo levels was observed with both the zinc alone and omega 3 alone interventions over the same time period. Total cholesterol concentrations demonstrated a slight increase at week 4 in the placebo group that was maintained at week 8.

In a similar trend to total cholesterol, a beneficial decrease in LDL cholesterol concentrations was observed after supplementation with the zinc & omega 3 combination. This beneficial effect was not apparent after supplementation with either zinc or omega 3 alone. As with total cholesterol, the decrease in LDL cholesterol represents a significant improvement in heart disease risk.

As such, it is believed that one of the advantages of the present invention, is the favourable effect the combination has on the lipid levels of the individual. For the first time, the present invention provides a combination which may effectively decrease total cholesterol and LDL-c concentrations. Furthermore, the combination may increase HDL-c levels and decrease triglyceride levels in individuals. Thus, the present invention may be used to lower total cholesterol in an individual. In a further embodiment, the present invention may be used to lower LDL-c in an individual. In a yet further embodiment, the present invention may be used to raise HDL-c levels in an individual. In a yet further embodiment, the combination may be used to decrease triglyceride levels. In a yet further embodiment, the present invention lowers total cholesterol and LDL-c in an individual. In a yet further embodiment, the present invention raises HDL-c levels and decreases triglyceride levels.

It has previously been believed that supplementation with zinc may have the undesirable effect of raising plasma LDL-c concentrations. It has also previously been believed that zinc supplementation may decrease plasma HDL-c concentrations. Neither of these outcomes is desirable.
However, the combination of the present invention has been shown to lower total plasma cholesterol concentrations. In particular, significantly lower plasma cholesterol levels may be found when the dose of zinc is ≥ 100 mg/d. Even more particularly, lower plasma cholesterol levels may be found when the dose of zinc is ≥ 50 mg/d.

However, in an alternative embodiment, the present invention does not significantly affect the total cholesterol level.

In addition, the combination of the present invention has been shown to lower LDL cholesterol (LDL-c) concentrations. In addition, in some embodiments, LDL-c concentrations may be reduced in either haemodialysis patients or individuals in the 40-55 year age category.

The combination of the present invention may increase plasma HDL-c levels. In one embodiment, the combination increases plasma HDL-c concentrations in individuals who are zinc deficient. In a further embodiment, the combination increases plasma HDL-c concentrations in individuals who are experiencing oxidative stress. In a yet further embodiment, the combination increases plasma HDL-c concentrations in individuals who have type 2 DM. In a yet further embodiment, the combination increases plasma HDL-c concentrations in individuals who are undergoing haemodialysis. Furthermore, it may be found that the combination of the present invention increases HDL-c concentrations in individuals aged 40 years and up, in particular, age 40 to 55.

The combinations of the present invention are believed to lower plasma triglyceride levels in individuals.

Without wishing to be bound by theory, it may be that the main effect of zinc is an increase in HDL cholesterol; the main effect of omega 3 is a decrease in triglycerides; with omega-3 also having an effect of increasing HDL cholesterol, decreasing total cholesterol and/or lowering LDL cholesterol.

**Outcome 2: Plasma Zn**

Changes in plasma zinc levels were measured and the results are shown in Figure 2. A decrease in plasma zinc concentrations was observed in the placebo group. A slight increase in plasma zinc was apparent at week 8 in the omega 3 alone group, which may
represent normal plasma zinc variation, whereas sustained increases in plasma zinc levels were observed in both the zinc alone and zinc/omega 3 combination groups. The increase in zinc levels with the combined supplement exceeded the increase in the zinc alone group.

5 Zinc plays numerous essential physiological roles. Plasma zinc levels have been shown to be reduced in populations with chronic disease. The beneficial increase in plasma zinc induced by the zinc and omega 3 combination was substantially higher than the increase in the zinc alone group. Therefore, the combinations of the present invention have been shown to increase plasma zinc concentrations in the individuals beyond the effects seen from zinc supplementation alone.

It is also believed that higher doses may give rise to higher zinc plasma concentrations and that the plasma zinc levels may show a greater increase as a result of zinc supplementation in individuals with Type 2 DM than in healthy individuals. Plasma levels may also differ with age, with higher increases seen with supplementation in younger individuals. Finally, the zinc plasma levels may depend on the anion chosen, for example the sulphate giving a higher rise than with acetate, gluconate, oxide and sulphate.

20 It is unexpected that supplementation with omega-3 would increase the uptake of zinc in an individual.

Because zinc plays an important role in HDL-c metabolism, the combinations of the present invention are believed to modulate cardiovascular disease risk in healthy individuals by influencing HDL-c metabolism. A divergent effect of plasma HDL-c concentrations may be found when comparing apparently healthy participants where administration may lower HDL-c levels against individuals with conditions known to influence zinc homeostasis where an increase in HDL-c is seen. The combination of the present-invention is believed to produce a dose-dependent increase in plasma zinc, and at doses > 100 mg zinc/d, supplementation maybe associated with a significant decrease in plasma total cholesterol concentrations. Alternatively, doses > 50 mg zinc/d, supplementation may be associated with a significant decrease in plasma total cholesterol concentrations. The combination is also believed to lower triglyceride levels in individuals.
In one embodiment of the present invention, administration of the combination of zinc and omega-3 increases HDL-c concentrations in subjects with type 2 DM. Whilst not wishing to be bound by theory, the mechanism may involve insulin, which has been proposed as an independent predictor of plasma HDL. Zinc is transported into the pancreas by ZnT8, and plays a role in the synthesis, storage, secretion, and action of insulin. Single nucleotide polymorphisms in the ZnT8 gene (SLC30A8), which may impair zinc flux into the pancreas, are associated with impaired proinsulin conversion and increased risk of developing type 2 DM. It is believed that the combination of the present invention when administered to individuals with type 2 DM results in a greater increase in plasma zinc levels than in healthy subjects, and this supports the notion that an underlying disturbance in zinc homeostasis exists in diabetes. It is further believed that a higher plasma zinc concentration protects those with type 2 DM from cardiovascular complications.

However, the combinations of the present invention have a greater effect than that seen with zinc alone. Thus, the combination of the present invention is believed to beneficially affect the lipid profile in individuals. In one embodiment, the combination can be used with individuals with an underlying zinc deficiency. The combination may also be specifically used with individuals with oxidative stress, diabetes, type 2 DM, CVD, and/or renal disease.

**Dyslipidaemia**

Dyslipidaemia may present as the combination of raised triglycerides (TG) and low concentrations of HDL-c together with elevated apolipoprotein B (ApoB), and small dense LDL particles, all of which are independently atherogenic, and which is commonly observed in people with both type 2 diabetes and the metabolic syndrome. Low HDL-c and high TG levels are frequently found with insulin resistance, with or without type 2 diabetes, and both are risk factors for coronary heart disease (CHD).

In some embodiments, the combination of the present invention may be used to mitigate, treat, prevent or ameliorate the effects of dyslipidaemia.

**Outcome 3: Inflammation/Oxidative Stress**

Inflammation and oxidative stress changes were measured by references to changes in high-sensitivity c-reactive protein (hsCRP) concentrations and the results are shown in
Figure 3. CRP concentrations are maintained at relatively constant levels in the zinc alone intervention group, with only a slight increase observed from baseline. The CRP levels fluctuate in both the placebo and omega 3 interventions; levels in the placebo group had reverted to baseline by week 8 but showed an increase at this timepoint in the omega 3 group. A clear and beneficial decreasing trend in CRP concentrations was observed in the zinc and omega 3 combination group over time.

CRP is a classic marker of inflammation that has been associated significantly with oxidative stress, independently of BMI and other CRP determinants (Atherosclerosis, 2005; 178:15-21). The decrease in CRP that is maintained over time with combined zinc and omega 3 supplementation, but which is not apparent with supplementation of zinc or omega 3 alone, is likely to afford protection against processes of oxidative stress and pro-atherosclerotic inflammation that are apparent in CHD development.

Perturbations of zinc homeostasis are recognised as important contributors to the pathophysiology of an increasing number of chronic disorders, including cardiovascular disease (CVD) and diabetes mellitus (DM). An extensive variety of genes are regulated by zinc, impacting such diverse processes as protein-protein interactions, fatty acid metabolism, apoptosis, and signal transduction. Many zinc-dependent biochemical pathways are also redox-sensitive and one of the consequences of the redistribution of zinc in disease states is an exacerbation of oxidative stress. It is believed that this interrelationship between zinc and redox effects, combined with the high prevalence of zinc deficiency worldwide, give rise to at least some of the advantages of the present combination of zinc and omega-3.

A number of pathways involved in redox metabolism require zinc for their function. Zinc ions are integral to the activities of superoxide dismutase (SOD) and metallothionein (MT), both of which protect against an accumulation of reactive species (RS) in cellular systems. Zinc affects a range of redox-sensitive cell signalling processes; it plays a role in the regulation of NF-κB and apoptosis and is involved in nitric oxide (NO) signalling pathways. Moreover, it has been suggested that zinc itself acts as a signalling molecule and may function to extend the signalling capacity of calcium and magnesium, the other redox-inert metal ions involved in redox metabolism.
In eukaryotes, three forms of SOD exist: copper, zinc SOD (CuZnSOD), which is the major intracellular SOD, present in the cytoplasm and nucleus; MnSOD, which is located primarily in the mitochondrial matrix; and extracellular SOD (EC-SOD), the predominant SOD in extracellular fluids. SOD plays a critical role in redox metabolism by accelerating the dismutation of the superoxide radical $O_2^-$ to H$_2$O$_2$. Both CuZnSOD and EC-SOD require zinc for their enzymatic activity. CuZnSOD comprises two 16-kDa protein subunits, each of which incorporates one zinc ion at its active site. The several isoforms of EC-SOD are tetrameric CuZnSODs, containing one zinc ion at each of the four 30-kDa subunits. It is believed that the combinations of the present invention may increase SOD activity and that influencing SOD activity is one of the mechanisms by which the present combination can provide protection against oxidative stress. Restoring or maintaining sufficient cellular zinc is believed to play an important role in the present combinations ability to reduce oxidative stress.

The levels of zinc supplementation may have an effect on the combinations ability to counter oxidative stress. It has been shown in animal studies that CuZnSOD activity has been decreased by both low and high zinc intakes. In rats, a zinc deficient diet has been shown to reduce erythrocyte CuZnSOD activity and serum zinc concentrations, while high dietary zinc resulted in a significant decrease in CuZnSOD activity in heart and liver tissue. The activity of EC-SOD, on the other hand, shows a positive association with zinc intake in animal models. Rats fed a diet supplemented with 60 mg Zn/kg demonstrated increased plasma EC-SOD activity, whereas dietary zinc deprivation was characterised by reduced plasma EC-SOD levels in rodent and rhesus macaque models. Omega-3 may also increase SOD. Furthermore, an interaction between zinc and omega-3 may contribute to an increase in omega-3 in phospholipids.

In humans, the relationship of CuZnSOD and EC-SOD activities to zinc intake is inconsistent. In randomised controlled supplementation trials, no effect of zinc on CuZnSOD was observed in healthy subjects supplemented with moderate amounts of zinc (30 mg/d or less). In contrast, zinc supplementation of 50 mg/d reduced CuZnSOD activity in men and women, most likely by limiting the bioavailability of copper ions. Supplementation with 150 mg elemental Zn/d, however, decreased CuZnSOD activity in healthy women but not in men. In relation to EC-SOD activity, augmented zinc intakes increased the enzyme activities in two studies in healthy individuals.
Conversely, supplementation with 25 mg/d elemental zinc did not influence the activity of plasma EC-SOD in healthy pregnant African-American women compared to controls and a cross-sectional study found no correlation between plasma EC-SOD activity and dietary zinc intake, again indicating that the relationship between zinc supplementation and SOD activity in humans is far from obvious. Diet-induced marginal zinc deficiency has been associated with a significant lowering of erythrocyte CuZnSOD activity in men, while plasma EC-SOD activity decreased during the first 6 weeks of zinc depletion in healthy males but then increased in the final week of depletion and in the first week of repletion before finally decreasing again. These findings may be due to the differing priorities of zinc redistribution in response to dietary zinc fluctuations.

It is therefore surprising that the combinations of the present invention have a beneficial effect on an individual in relation to reducing or mitigating oxidative stress.

Metallothioneins (MTs) comprise a class of ubiquitous, low molecular weight proteins, each of which has the ability to accommodate 7 zinc ions in its fully metallated conformation. MT participates in cellular zinc distribution and homeostasis, contributes to the antioxidant defence system by scavenging RS, and is critically involved in redox and zinc signalling. Although primarily a cytoplasmic protein, MT can translocate to the intermembrane space of mitochondria or to the nucleus and has been reported to occur in low levels in extracellular fluids.

Zinc co-ordination to the various sulphur ligands of MT links redox and zinc metabolism. Although the zinc ions are embedded within the conformational structure of the MT protein, the thiolate ligands are available to participate in redox reactions with concomitant release and binding of zinc. The released zinc may then facilitate a diverse range of other cellular processes, including the regulation of MT gene expression in a positive feedback mechanism. It is believed that MT levels are positively associated with zinc in cell cultures. For example, zinc supplementation increases and zinc depletion decreases MT mRNA and protein levels in monocytes and retinal pigment epithelial (RPE) cell-lines. In the majority of cell culture and in vivo experiments, MT induction is associated with protection against subsequent metal, chemical, and other stresses.
It is believed that the combination of the present invention may increase MT levels in the individuals.

Zinc is amphoteric and has the ability to form flexible coordination geometry, both characteristics which assist it in translating chemical structures into pervasive biological messages in various cell types. The transcription factor NF-κB is a key example of a protein complex that is involved in signal transduction and which is both zinc and redox-responsive. NF-κB is ubiquitously expressed and impacts an extensive assortment of cellular processes, including proliferation, immunity, inflammation, and apoptosis. Its expression varies according to zinc status. Both zinc and omega-3 can each individually contribute to an increase in NF-κB (depending on the cellular environment). This may result in a surprising and desirable increase in NF-κB. A decrease may be caused when zinc intake is too low or zinc supplementation too high.

Zinc has been shown to both inhibit and induce cell death, depending on the zinc and redox status of the cell. At physiological concentrations, zinc is able to prevent cell death by selectively inhibiting caspase-6, which otherwise activates the proenzyme form of caspase-3 and commits the cell to irreversible cell death. Zinc has also been shown to increase the ratio of bcl-2 to bax in U-937 cells pretreated with hydrogen peroxide, indicating enhanced cell survival. Further, physiological zinc concentrations facilitate cell survival through activation of the stress-responsive phosphatidylinositol 3-kinase (PI3K)/Akt signalling cascade. Activated Akt facilitates important cellular responses to growth factors and oxidative stress and promotes cell survival by reducing the activity of proapoptotic factors such as caspase-9.

In contrast, zinc deficiency has been linked to increased apoptosis in a multitude of cell and animal models. The mechanisms by which zinc deficiency promotes apoptosis may include a direct effect of zinc loss on critical proteins involved in the apoptotic cascade, such as caspases and p53, or indirect effects due to increased levels of oxidative stress.

In primary rat endothelial cells, zinc depletion induced by TPEN treatment significantly decreased the expression of GSH, while zinc supplementation protected against H2O2-induced cell death via Nrf2-dependent stimulation of GSH synthesis. A reduction in the translocation of NF-κB to the nucleus in zinc deficiency may also contribute to increased levels of apoptosis, given that NF-κB has been implicated in the transcriptional regulation of a range of anti-apoptotic proteins, including IAP, IEX-1 L, and the anti-apoptotic members of the Bcl-2 family.
Thus, it is believed that the combination of the present invention may prevent cell death.

The NO radical functions as a mediator or regulator of cell function in a variety of physiological systems and its redox-signalling roles are related to zinc in at least two ways. Firstly, the zinc-dependent enzymes CuZnSOD and EC-SOD function to protect NO bioavailability by controlling 02- levels, which if left unchecked react with NO to form ONOO-. The ONOO- radical in turn has been shown to oxidise the zinc-thiolate cluster of members of the nitric oxide synthase (NOS) family of enzymes, leading to the release of zinc and enzyme uncoupling. Secondly, NO is known to interact with the cysteine residues of MT, inducing a conformational change in the protein and a concomitant release of zinc ions. It is been suggested that this pathway provides a novel mechanism for NO-based signalling through the regulation of zinc homeostasis. Thus, the combinations of the present invention may mediate NO-based signalling. Omega-3 may also help mediate NO-based signalling because it can protect NO synthase and has been associated with an increase in NO.

Omega-3 also plays a role in reducing or mitigating oxidative stress. It is believed that omega-3 has anti-inflammatory properties which assist in reducing oxidative stress. Without wishing to be bound by theory it is believed that omega-3 can result in antagonistic production of inflammatory eicosanoids from arachidonic acid which results in the generation of less potent EPA-derived eicosanoids and, in some conditions, resolvins. Altering eicosanoid production may regulate production of inflammatory cytokines. Thus, omega-3 may affect the production of TNF-α, IL-1β, IL-6. Equally, the omega-3 may affect cytokines (and therefore oxidative stress) in a eicosanoid independent manner, for example by altering the activation of transcription factors involved in inducing transcription of inflammatory genes, for example NF-κB or PPAR-γ, which may occur as a result of altered plasma membrane signalling processes.

Oxidative stress has been identified as an important underlying factor in a number of chronic diseases, including CVD and DM. It is believed that since these disease states are associated with perturbed zinc homeostasis levels and given the interconnectedness and widespread effects of cellular zinc- and redox-dependent pathways, abnormal zinc levels and the subsequent redistribution of zinc in disease states exacerbates oxidative
stress. Providing a combination product containing zinc redresses this imbalance and counters oxidative stress. Omega-3 oils can also assist with oxidative stress as discussed above. It is therefore believed that the combinations of the present invention may be useful to treat or ameliorate a number of oxidative stress-related disorders.

Outcome 4: Glycaemic Control

Changes in glycaemic control as measured by changes in Haemoglobin Ale (HbAlc) are shown in Figure 4. HbAlc levels fluctuated over 8 weeks in the placebo group and experienced a disadvantageous increase in the zinc alone intervention group. Supplementation with omega 3 alone produced a reduction in the HbAlc concentration at week 4, but HbAlc levels had returned to baseline by week 8. The zinc and omega 3 combination appeared to maintain HbAlc levels over time, with only minimal changes from baseline observed in this group.

HbAlc is a measure of glycaemic control over time. A beneficial decrease in HbAlc was observed after 4 weeks in the omega 3 alone intervention group but this was no longer apparent after 8 weeks of supplementation. A detrimental increase in HbAlc was observed with the zinc alone intervention and in the placebo group. The combination of zinc and omega 3 maintained glycaemic control at baseline levels, which were lower than the HbAlc levels of the placebo group, and therefore represent a beneficial result. Although the zinc and omega 3 combination did not produce an effect of greater benefit than the omega 3 alone group, it did protect against a detrimental effect by zinc on HbAlc, such as that observed in the zinc alone intervention. The combination therefore allows benefits such as the decrease in total and LDL cholesterol to be realised without incurring the apparent detrimental effect of supplementation with zinc alone on glycaemic control.

Atherosclerosis

Atherosclerosis is a progressive inflammatory disorder characterised by the infiltration of monocytes and other inflammatory cells into the arterial intima, which become lipid-laden macrophages or foam cells, and culminating in the formation of a fibrinous plaque. Complications of atherosclerosis include myocardial infarction, peripheral vascular disease, and stroke, all of which remain major causes of death in Western countries. An altered distribution of zinc has been observed in atherosclerosis and the ease with which labile zinc is transported into endothelial cells suggests that the vascular
endothelium may be particularly affected by perturbations in zinc homeostasis and metabolism. Indeed, a number of the characteristic features of atherosclerosis are influenced by zinc, including enhanced apoptosis, disturbed NO and NF-κB-related signalling mechanisms, and the oxidative modification of LDL.

The induction of endothelial cell apoptosis in response to conditions of oxidative stress is a typical atherogenic trait. Endothelial cells rendered zinc deficient by exposure to the membrane-permeable chelator TPEN demonstrated considerably higher levels of apoptotic cell death and caspase-3 activity than control cells when stimulated with linoleic acid and TNF-α. This effect was completely blocked by concurrent administration of physiological amounts of zinc to the culture medium. Conversely, increases in intracellular free zinc levels induced by H2O2-stimulation resulted in a significant rise in oxidative stress-related apoptosis of endothelial cells, highlighting that a change in cellular zinc concentration in either direction can promote cell death in the endothelium.

Aberrant expression of NF-κB is another common feature of atherosclerosis that appears to be impacted by zinc. NF-κB is a key component of the adhesion molecule upregulation process and is also involved in the promotion of smooth muscle cell proliferation. Cellular zinc deficiency has been shown to upregulate NF-κB activity in endothelial and high levels of NF-κB have been found to be present in the smooth muscle cells of the atherosclerotic lesion.

The release of NO by the endothelium plays a key role in vascular homeostasis and a reduction in NO levels therefore has adverse implications in atherosclerosis. An altered zinc distribution in the disease process affects the activities of CuZnSOD and EC-SOD, impairing the ability of these enzymes to control 02- levels and thereby protect NO bioavailability. If not maintained at manageable levels by SOD, 02- is at liberty to react with NO to form ONOO-, which in turn promotes eNOS uncoupling and a further increase in 02- levels. NADPH oxidase has been proposed to play a central role in eNOS uncoupling and appears able to be activated by zinc. Numerous studies have reported that eNOS uncoupling is an important mechanism of pathologic 02-production in the vascular endothelium and increased expression of the p22phox subunit of NADPH oxidase has been demonstrated in the walls of human coronary atherosclerotic arteries.
Another means by which the relationship between NO and zinc may promote the progression of atherosclerosis relates to Nrf2 expression in vascular cells, which is a key factor in the cellular protection against oxidative stress and inflammation. A release of intracellular zinc induced by iNOS-derived NO has been shown to be a critical component of an Nrf2-dependent signalling pathway that activates the GSH redox cycle in endothelial cells, ultimately protecting against oxidative damage. Localised zinc deficiency or its redistribution during the atherosclerotic process believed to ameliorates this protective effect.

Atherosclerosis typically incorporates increased levels of oxidised lipids and lipoproteins in the vessel wall. Oxidised LDL (oxLDL) in particular has been demonstrated to play a critical role in abnormal endothelial vasorelaxation and excess uptake of oxLDL can disrupt the endothelium, injuring endothelial cells or committing them to apoptosis.

Zinc has been found to inhibit the formation of oxLDL and that zinc deficiency can enhance LDL oxidation in vitro. However, few studies have been carried out in vivo. In one study, healthy men supplemented with 50 mg zinc per day, saw no significant change in LDL oxidation levels and supplementation with 15 or 30 mg zinc per day had no effect on in vitro LDL oxidation parameters in healthy subjects aged 55-70 years. Despite this, the inventors surprisingly believe that the combinations of the present invention may inhibit LDL oxidation.

The omega-3 may lead to less progression and more regression of atherosclerosis. It may have a particular effect in patients with DM. Omega-3 intake may influence plaque growth because supplemental omega-3 is incorporated into the phospholipids and cholesteryl esters in atherosclerotic lesions. A study of patients awaiting carotid endarterectomy, showed that omega-3 had been incorporated into plaques. This was associated with a significant decrease in macrophage infiltration, which is consistent with greater plaque stability.

It is believed that the combinations of the present invention may prevent or mitigate the effects of atherosclerosis.
Studies have confirmed a role for changes in zinc distribution in the pathogenesis of arterial hypertension and zinc deficiency states have been associated with the disorder. Analysis of the NHANES-II data revealed significantly lower serum zinc levels in older hypertensive women and men with isolated systolic hypertension, which may result from lower dietary zinc intake, increased urinary zinc excretion, or redistribution of zinc into other compartments, such as red blood cells. Serum zinc levels have also been found to be lower in younger persons with hypertension, although the decrease did not reach statistical significance. A number of mechanisms have been proposed to explain the effects of altered zinc levels on the disease, many of which also impact redox metabolism, such as changes in SOD activity, an impaired vascular NO system, and leptin signalling effects.

In spontaneously hypertensive rats, a reduction in CuZnSOD activity in the thoracic aorta was observed in diet-induced zinc deficiency while zinc supplementation resulted in significantly increased CuZnSOD activity. In human hypertension, inverse correlations have been observed between diastolic blood pressure and CuZnSOD and reductions in EC-SOD also have been noted. Perturbations of zinc homeostasis in hypertension that compromise CuZnSOD and EC-SOD activities would help to explain the increased levels of RS, including the abnormal production of 02-, that have been associated with the disorder. Increased 02- production in conjunction with diminished NO bioavailability has been recognised in humans as has evidence for eNOS uncoupling, which involves an attack on the zinc-thiolate cluster of eNOS by ONOO-, where the binding site of the essential BH4 cofactor is located, and a concomitant release of zinc. In rat models, higher than normal 02- concentrations have been demonstrated in both angiotensin II-induced hypertension and hypertension known to be associated with low-renin states, with NADPH oxidase being shown to contribute to the increase.

It is believed that the combinations of the present invention may prevent or mitigate the effects of hypertension.

CVD

It is believed that the combinations of the present invention may be useful in treating, mitigating or reducing the risks and/or effects of CVD.
Zinc as part of the combination of the present invention is believed to be useful as a possible adjunct therapy for a range of CVD disorders where zinc intake is deficient or zinc homeostasis perturbed. Longitudinal studies describe the involvement of zinc deficiency in CVD mortality in the population generally as well as in cohorts at substantial risk for future cardiovascular events, including those with Type 2 DM. These studies are supported by a large prospective cohort study showing an inverse association between dietary zinc intake and CVD mortality among postmenopausal women who consume at least 10 g alcohol per day. In older patients with congestive heart failure, zinc intakes appear to fall short of recommended levels, while reduced zinc levels were recently reported in peripheral blood mononuclear cells of patients with atherosclerosis. In chronic cardiac failure, hyperzincuria accompanied by low plasma and erythrocyte zinc levels has been detected in patients with dilated cardiomyopathy, whereas plasma and erythrocyte zinc levels were depressed without increased zincuria in subjects with hypertrophic cardiomyopathy.

Despite these indications that zinc supplementation may be beneficial in CVD, studies exploring the effects of zinc nutrition on cardiovascular outcomes and oxidative stress-related risk factors in humans are few and inconsistent. Acute zinc depletion of healthy males has been found to impair platelet aggregation and prolong bleeding times, supporting a role for zinc in haemostasis. On the other hand, supplementation with 50 mg elemental Zn/d has been shown to increase platelet reactivity, and at lower doses of zinc (30 mg) fibrinolytic factors and platelet zinc concentrations are unaffected.

The supposition that the effect of zinc supplementation on CVD risk is dependent on zinc status is supported by studies exploring the effect of zinc on CVD as part of a multi-nutrient regime. Populations already at risk of CVD appear more likely than healthy individuals to benefit from micronutrient supplementation. On the one hand, a large primary cardiovascular and cancer prevention study of healthy participants which incorporated 20 mg of zinc as part of its antioxidant supplementation protocol, showed no effect of supplementation in the prevention of CVD or on carotid atherosclerosis and arterial stiffness. Conversely, treatment of elderly chronic heart failure patients with multiple micronutrient supplements that were inclusive of zinc demonstrated improved left ventricular function and quality of life. In addition, obese Chinese women with hypertension and/or hyperglycaemia and/or hyperlipidemia receiving multivitamin and mineral supplements, including 15 mg of zinc, demonstrated a reduction in blood pressure and serum C-reactive protein.
It is believed that individuals with an underlying perturbation in zinc homeostasis experience an increase in plasma HDL-c concentrations when administered with the combination of the present invention.

It is believed that the omega-3 in the combination of the present invention provides additional benefits in reducing the risks of cardiovascular disease. Omega-3 directly activates transcription factors that regulate lipid metabolism and is known to decrease serum triglyceride levels.

Overall, the combination of the present invention is believed to reduce or mitigate the effects of CVD and the inventors have found surprising benefits associated with the combination over and above the individual agents.

Diabetes Mellitus
Diabetes Mellitus (DM) is a major risk factor for CVD, with 50% of diabetes-associated deaths being attributed to cardiovascular complications. Disturbances in zinc homeostasis and increased levels of oxidative stress each appear to play a major role in the pathogenesis of DM. The involvement of oxidative processes in the disease is corroborated by the impact of antioxidants, such as MT, on the development of diabetic complications. Overexpression of MT in the pancreatic β-cells of STZ-treated transgenic mice has been shown to reduce DNA damage and protect against DM, while in humans a mutation in the MT-1A gene has been associated with the development of DM and its cardiovascular complications.

It is believed that a positive association between zinc and MT expression is one example of how a perturbed zinc distribution in DM might exacerbate oxidative stress. Other critical pathways that are affected in the disease and which are influenced by zinc include those of insulin signalling and lipid metabolism.

The characterising feature of DM is the presence of chronic hyperglycaemia, which is known to enhance oxidant production and impair antioxidant defence mechanisms. Hyperglycaemia is consequent upon the decreased secretion or action of insulin. Zinc has long been known to elicit insulin-like effects and it is this property that is perhaps most obviously affected by the alterations in zinc distribution and metabolism associated with DM. Under normal conditions zinc is abundant throughout the
pancreas, but is particularly concentrated in the secretory vesicles of the β-cells where it forms an integral component of the insulin crystalline structure, serving to stabilise the insulin granule by rendering it less soluble. Zinc has been shown to stimulate lipogenesis and inhibit lipolysis, attenuate hyperglycaemia, and stimulate GLUT4 translocation to the cell membrane. Recent evidence has also demonstrated a role for zinc in the induction of the PI3K/Akt cascade which, in addition to its roles in regulating apoptosis and proliferation and promoting resistance to oxidative stress, is a major mediator of insulin signalling. The serine/threonine kinase Akt directly targets and inactivates a number of the forkhead box (FoxO) transcription factors, which are potent transactivators of genes involved in glucose metabolism, such as glucose-6-phosphatase (G6Pase), glucokinase, and phosphoenolpyruvate carboxykinase (PEPCK), and as such are important targets of insulin action. The ability of zinc to activate the PI3K/Akt signalling pathway is achieved via attenuation of protein tyrosine phosphatase (PTPase) activity, which otherwise acts to inhibit the PI3K-dependent activation of Akt.

When zinc homeostasis is altered, the insulinomimetic effects of zinc have been shown to be impaired. Zinc depletion of the insulin molecule leaves insulin vulnerable to structural modification and increases its susceptibility to a free radical-induced reduction in biological activity. In addition, recent evidence suggests that single nucleotide polymorphisms in the ZnT8 gene (SLC30A8), which are involved in zinc flux into the pancreas, are associated with impaired proinsulin conversion and increased risk of developing type 2 DM. An overall state of zinc deficiency in DM is evidenced in part by the state of hyperzincuria consistently reported to be present in the disease. It has also been suggested that oxidative stress-induced zinc release may result in a cellular zinc deficiency in insulin-responsive tissue. One of the effects of low zinc availability is a reduction in CuZnSOD levels. It has been proposed that CuZnSOD functions to protect insulin and β-cells from oxidative damage. Further, CuZnSOD protects the bioavailability of NO, which has a role in promoting glucose uptake and supply in skeletal muscle and facilitates the binding of insulin to its receptor.

It is believed that the omega 3 in the composition is likely to play a role with insulin, for example it is believed to increase insulin sensitivity. It is theorised that it increases unsaturation of muscle membrane fatty acids. It may also interact with insulin signalling via an effect on insulin and/or leptin levels.
Type 2 DM and insulin resistance are associated with perturbed postprandial lipid metabolism, which itself is a risk factor for CVD. The major changes in lipid profile in Type 2 DM are an increase in triglycerides, a reduction in HDL-c, and the increased appearance of small, dense LDL particles, which are particularly susceptible to oxidative modification. The roles of zinc and omega-3 have been discussed above in relation to lipid metabolism and it is believed that the present combination may be used to restore and/or improve the lipid profile in patents.

It is believed that both omega-3 and zinc can affect triglyceride levels in individuals. Both are also believed to affect lipoprotein metabolism and hence impact DM and CVD risk. While the mechanisms for zinc have yet to be established, they may again involve zinc's role in insulin action, which has been proposed as an independent predictor of plasma HDL and triglyceride concentrations. Moreover, as with glucose metabolism, many of the effects of insulin on lipid metabolism are mediated by the zinc and redox-responsive PI3K/Akt pathway and its regulation of FoxO-dependent gene transactivation. The mechanism by which zinc influences the PI3K/Akt pathway in lipid metabolism appears to differ, however, from that of glucose metabolism. While in glucose-related signalling PTPase is the key inhibitor targeted by zinc, the inactivation of lipid products appears instead to be modulated by the lipid phosphatase PTEN. The ability of zinc to inhibit PTEN has been demonstrated in epithelial cells, where zinc treatment resulted in both the downregulation of PTEN mRNA levels and a reduction in PTEN protein levels as a result of proteasomal degradation. The many roles ascribed to FoxO proteins in lipid metabolism that may be affected by changes to zinc homeostasis in DM include the stimulation of lipoprotein lipase expression, which is responsible for the breakdown of plasma triglycerides, the suppression of lipogenesis in the liver, and the transcriptional regulation of microsomal triglyceride transfer protein (MTP), which is involved in hepatic lipoprotein assembly. FoxO has also been shown to induce the translocation of the fatty acid transport protein CD36 to the plasma membrane where it can promote fatty acid uptake.

An interaction between zinc and leptin has also been proposed in DM. Among its many roles in lipid metabolism, leptin is critically involved in fatty acid oxidation and the regulation of intracellular triglyceride levels, the excessive accumulation of which can induce lipotoxicity in non-adipocytes and ultimately result in insulin resistance. Like insulin, leptin is able to invoke PI3K/Akt-dependent signalling (Figure 2) and crosstalk has been observed between the two pathways. Most recently, leptin-deficient
Akt knockout mice were shown to display hyperglycaemia and reduced insulin levels, both of which are able to be normalised by the restoration of leptin despite the existence of β-cell dysfunction. As with insulin, PTPase has been shown to be a key inhibitor of the leptin PI3K signalling pathway, intimating a role for zinc in leptin signalling via its ability to inactivate this enzyme. It is believed that there may be a direct link between the combination of the present invention on patient leptin concentrations. Zinc deficiency in humans is accompanied by a reduction in leptin concentrations, although this relationship may be complicated by such factors as baseline zinc status, oxidative stress levels, body weight, and gender. In postmenopausal women with type 2 diabetes, for example, while both obese and non-obese subjects demonstrated lower plasma zinc levels than the non-obese controls, only obese subjects demonstrated a difference in leptin levels, which were negatively correlated with plasma zinc.

In embodiments of the present invention, the combination may be used to prevent type 2 DM. In a further embodiment of the present invention the combination may be used to treat or mitigate DM. In particular, in some embodiments, the combinations of the present invention may lower the risk of type 2 DM in women.

Furthermore, in embodiments of the present invention, the combination may ameliorate oxidative stress-related parameters in the established disease. In a further embodiment, the combination may generate beneficial effects in diabetic neuropathy and/or to reduce oxidative stress in Type 1 diabetic patients with retinopathy.

Metabolic syndrome
The metabolic syndrome is a cluster of the most dangerous heart attack risk factors: diabetes and raised fasting plasma glucose, abdominal obesity, high cholesterol and high blood pressure.

In most people with glucose intolerance or type II diabetes, there is a multiple set of risk factors that commonly appear together, forming what is called the 'Metabolic Syndrome'. This cluster of metabolic abnormalities confers a substantial additional cardiovascular risk to the individual over and above the sum of the risks associated with each abnormality. Said another way, an individual presenting with the metabolic syndrome their cardiovascular disease risks are higher than that which would be predicted from the sum of each risk factor individually.
Metabolic syndrome can occur even before the blood glucose levels of an individual are high enough to be diagnosed with diabetes. Hyperglycaemia and related changes in blood lipids (an increase in triglycerides and a decrease in HDL-c) also increase a person's risk of cardiovascular disease.

The more components of metabolic syndrome present in an individual, the higher the cardiovascular mortality rate.

Metabolic syndrome is defined as being present if the individual fulfils the following criteria:

<table>
<thead>
<tr>
<th>Table 1: The new International Diabetes Federation (IDF) definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>According to the new IDF definition, for a person to be defined as having the metabolic syndrome they must have:</td>
</tr>
<tr>
<td>Central obesity (defined as waist circumference *with ethnicity specific values) plus any two of the following four factors:</td>
</tr>
<tr>
<td><strong>Raised triglycerides</strong></td>
</tr>
<tr>
<td>≥ 150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality</td>
</tr>
<tr>
<td><strong>Reduced HDL cholesterol</strong></td>
</tr>
<tr>
<td>&lt; 40 mg/dL (1.04 mmol/L) in males &lt; 50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality</td>
</tr>
<tr>
<td><strong>Raised blood pressure</strong></td>
</tr>
<tr>
<td>Systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg or treatment of previously diagnosed hypertension</td>
</tr>
<tr>
<td><strong>Raised fasting plasma glucose</strong></td>
</tr>
<tr>
<td>(FPG) ≥ 100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes. If above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of the syndrome.</td>
</tr>
</tbody>
</table>

* If BMI is ≥ 30kg/m², central obesity can be assumed and waist circumference does not need to be measured.
<table>
<thead>
<tr>
<th>Population</th>
<th>Male Cut-off</th>
<th>Female Cut-off</th>
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<td>Europids*</td>
<td>Male &gt; 94 cm</td>
<td>Female ≥ 80 cm</td>
</tr>
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<td>In the USA, the ATP III values (102 cm male; 88 cm female) are likely to continue to be used for clinical purposes</td>
<td></td>
<td></td>
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<tr>
<td>South Asians</td>
<td>Male &gt; 90 cm</td>
<td>Female ≥ 80 cm</td>
</tr>
<tr>
<td>Based on a Chinese, Malay and Asian-Indian population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>Male ≥ 90 cm</td>
<td>Female ≥ 80 cm</td>
</tr>
<tr>
<td>Japanese **</td>
<td>Male ≥ 90 cm</td>
<td>Female ≥ 80 cm</td>
</tr>
<tr>
<td>Ethic South and Central Americans</td>
<td>Use South Asian recommendations until more specific data are available.</td>
<td></td>
</tr>
<tr>
<td>Sub-Saharan Africans</td>
<td>Use European data until more specific data are available.</td>
<td></td>
</tr>
<tr>
<td>Eastern Mediterranean and Middle East (Arab) Populations</td>
<td>Use European data until more specific data are available.</td>
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* In future epidemiological studies of populations of Europid origin, prevalence should be given using both European and North American cut-points to allow better comparisons.

** Originally different values were proposed for Japanese people but new data support the use of the values shown above.

In some embodiments, the combination of the present invention may be used to mitigate, treat, prevent or ameliorate the effect of metabolic syndrome.

Overall, the combinations of the present invention may ameliorate, treat or prevent one or more disorders which exhibit increased levels of oxidative stress and/or lipid imbalance. This includes CVD, DM, particularly type 2 DM or renal disease. It also includes one or more of the CVD and DM-related disorders, including atherosclerosis, hypertension, insulin resistance, and dyslipidemia.
The combinations of the present invention may be used in the treatment of metabolic disorders such as cardiovascular disease, diabetes and renal disease; obesity, diseases associated with dyslipidaemia and/or oxidative stress; individuals with zinc deficiency and/or inadequate zinc intake (such as children, older or elderly people, vegetarians/vegans and others with reduced intakes of meat, seafood, or dairy); and/or individuals with inadequate intake of omega 3 fatty acids.

The combinations of the present invention may be used to treat one or more of the broad range of disease states linked to oxidative stress including obesity, autoimmune diseases, cancer (including breast, colon, liver, bowel), inflammatory bowel disease, chronic obstructive pulmonary disease, diabetes mellitus, cardiovascular disease, chronic infection, allergy, metabolic alterations, acute respiratory distress syndrome, asthma, rheumatoid arthritis, stroke, coeliac disease, renal disease, alcohol induced liver injury and other liver diseases, gastrointestinal disease, Alzheimer's disease, obstructive sleep apnoea, Parkinson's disease, cerebrovascular disease, neurodegenerative disease, cystic fibrosis and thyroid diseases.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.
CLAIMS:

1. A composition comprising a combination of zinc and an omega-3 fatty acid for use in treating, preventing, reducing or mitigating oxidative stress in an individual.

2. A composition comprising a combination of zinc and an omega-3 fatty acid for use in treating, preventing, reducing or mitigating inflammation in an individual.

3. A composition comprising an omega-3 fatty acid for use in enhancing zinc uptake in an individual.

4. The composition of claim 3 which further comprises zinc.

5. A composition comprising a combination of zinc and an omega-3 fatty acid, for use in regulating the lipid profile to produce a favourable lipoprotein / lipid profile in an individual.

6. A composition comprising a combination of zinc and an omega-3 fatty acid for use in improving glycaemic control in an individual.

7. A composition comprising a combination of zinc and an omega-3 fatty acid, for use in:
   - treating, preventing, reducing or mitigating type 2 diabetes;
   - treating, preventing, reducing or mitigating cardiovascular disease;
   - treating, preventing, reducing or mitigating renal disease;
   - treating, preventing, reducing or mitigating dyslipidemia; and/or
   - treating, preventing, reducing or mitigating metabolic syndrome in an individual.

8. The composition of claim 5 for use in:
   - increasing the levels of high density lipoproteins (HDLs) including HDL-c;
   and/or
   - decreasing the levels of triglycerides in an individual.
9. The composition of claims 1 or 2 or any one of claims 4 to 8, wherein the composition consists essentially of zinc and an omega-3 fatty acid, together with a therapeutically acceptable carrier.

10. The composition of claims 1 or 2 or any one of claims 4 to 8, wherein the composition consists of zinc and an omega-3 fatty acid, together with a therapeutically acceptable carrier.

11. The composition of any one of claims 1 to 7, further comprising cofactors to modulate the conversion of short-chain to long-chain omega-3 fatty acids, such as B₃, B₆, Vitamin C and/or magnesium.

12. The composition of any one of claims 1 to 7, or 10 further comprising antioxidants and/or phytochemicals, for example retinyl esters, glutathione, vitamin C, vitamin E, or carotenoids and/or polyphenols (including catechins such as epigallocatechin gallate), isoflavones, isoprenoids or anthocyanidins.

13. The composition according to any preceding claim wherein the zinc and omega-3 fatty acids are administered:
   - concurrently;
   - consecutively; or
   - in a single formulation.

14. The composition according to any preceding claim wherein the compositions are given to individuals who:
   - are zinc deficient;
   - are omega-3 deficient;
   - are experiencing oxidative stress; and/or
   - have type 2 DM.

15. The composition according to any preceding claim wherein the zinc is elemental zinc or one or more zinc salts such as zinc citrate, zinc acetate, zinc picolinate, zinc sulphate, or other zinc salts like amino acids, dipeptides, gluconates, halides, nitrates, oxides or acetates.
16. The composition according to any preceding claim wherein the omega-3 fatty acid is:
   - derived from a marine oil;
   - derived from a fish oil;
   - derived from a marine algal oil;
   - derived from a plant source;
   - derived from an animal source;
   - derived from a fish source; and/or
   - derived from an algal source, such as a microalgal source.

17. The composition according to any preceding claim wherein the omega-3 fatty acid is:
   - ALA;
   - EPA;
   - DHA; and/or
   - DPA.

18. A method of:
   - treating, preventing, reducing or mitigating inflammation and/or oxidative stress;
   - enhancing zinc uptake;
   - regulating the lipid profile to produce a favourable lipoprotein / lipid profile
   - improving glycaemic control;
   - treating, preventing, reducing or mitigating type 2 diabetes;
   - treating, preventing, reducing or mitigating cardiovascular disease;
   - treating, preventing, reducing or mitigating renal disease;
   - treating, preventing, reducing or mitigating dyslipidemia; and/or
   - treating, preventing, reducing or mitigating metabolic syndrome
in an individual, comprising administering a therapeutically effective amount of a
combination of zinc and an omega-3 fatty acid to the individual.

19. A method of measuring the zinc levels in an individual and if this level is low
administering a therapeutically effective amount of a combination of zinc and an
omega-3 fatty acid to the individual.
20. A method of measuring the plasma levels of HDL-c in an individual to determine their HDL-c level and if this level is low administering a therapeutically effective amount of a combination of zinc and an omega-3 fatty acid to the individual.

21. The use of a combination of zinc and an omega-3 fatty acid, for the manufacture of a medicament for:
   - treating, preventing, reducing or mitigating inflammation and/or oxidative stress;
   - enhancing zinc uptake;
   - regulating the lipid profile to produce a favourable lipoprotein / lipid profile
   - improving glycaemic control;
   - treating, preventing, reducing or mitigating type 2 diabetes;
   - treating, preventing, reducing or mitigating cardiovascular disease;
   - treating, preventing, reducing or mitigating renal disease;
   - treating, preventing, reducing or mitigating dyslipidemia; and/or
   - treating, preventing, reducing or mitigating metabolic syndrome.
Lipid Profile

Figure 1a. Change in total cholesterol concentrations (mmol/L) after supplementation with zinc alone, omega 3 alone, zinc and omega 3 in combination, or placebo.

Figure 1b. Change in low density lipoprotein (LDL) cholesterol concentrations (mmol/L) after supplementation with zinc alone, omega 3 alone, zinc and omega 3 in combination, or placebo.
Figure 2. Change in plasma zinc concentrations (μmol/L) after supplementation with zinc alone, omega 3 alone, zinc and omega 3 in combination, or placebo.
Figure 3. Change in high-sensitivity c-reactive protein (hsCRP) concentrations (mg/L) after supplementation with zinc alone, omega 3 alone, zinc and omega 3 in combination, or placebo.
Figure 4. Change in Haemoglobin A1c (HbA1c) concentrations (%) after supplementation with zinc alone, omega 3 alone, zinc and omega 3 in combination, or placebo.
A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 33/30 (2006.0) 1 A61K 31/202 (2006.0) 1 A61P 3/00 (2006.0) 1 A61P 1/00 (2006.0) 1 A61P 9/70 (2006.0) 1.

According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols).

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, Medline; Keywords: zinc, omega-3 fatty acid, cardiovascular disease, diabetes mellitus, oxidative stress, lipid profile (and related terms).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>DE 20 020 05 012 984 U1 (W &amp; B PHARMAMARKEN GMBH) 29 December 2005 X Paras. 0003, 0004, 0013, 0018-0020 and 0023.</td>
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* Further documents are listed in the continuation of Box C X See patent family annex

- Special categories of cited documents:
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) or other means
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed
  - "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  - "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  - "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  - "&" document member of the same patent family

Date of mailing of the international search report

09 December 2001

09 December 2001

Name and mailing address of the ISA/AU

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Authorized officer

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This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX