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(54) Title: METHODS OF TREATING VARIOUS CONDITIONS BY ADMINISTRATION OF SUSTAINED RELEASE L-ARGININE

(57) Abstract: The present invention provides methods for using L-arginine formulations, such as sustained release formulations, for various indications, including lowering triglyceride levels, inducing thermogenesis, weight loss and treatment and prevention of obesity and obesity related conditions, such as diabetes. Moreover, the present invention provides methods for treating or preventing other indications, such as asthma.

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METHODS OF TREATING VARIOUS CONDITIONS BY ADMINISTRATION OF SUSTAINED RELEASE L-ARGININE

Related Applications

This application claims priority to U.S. application No. 11/107395, entitled "Methods of Treating Various Conditions By Administration of Sustained Release L-Arginine" filed April 14, 2005, and U.S. application No. 11/042017, entitled "Methods of Treating Various Conditions By Administration of Sustained Release L-Arginine" filed January 24, 2005 which is a continuation-in-part of PCT/US2004/013255, entitled "Sustained Release L-Arginine Formulations and Methods of Manufacture and Use" filed April 28, 2004 which claims priority to PCT/US2003/033931, entitled "Sustained Release L-Arginine Formulations and Methods of Manufacture and Use" filed October 24, 2003, which further claims priority to U.S. Provisional Patent Application Serial No. 60/421,258, entitled "Methods and Compositions for the Treatment of Cerebrovascular and Cardiovascular Diseases and Disorders" filed October 24, 2002, U.S. Provisional Patent Application Serial No. 60/507,312, entitled "Methods and Compositions for the Treatment of Cerebrovascular and Cardiovascular Diseases and Disorders" filed September 29, 2003, and U.S. Provisional Patent Application Serial No. 60/512,035, entitled "Sustained Release L-Arginine Formulations and Methods of Manufacture and Use" filed October 17, 2003. The entire contents of each of the aforementioned applications are hereby expressly incorporated herein by reference in their entireties.

Background of the Invention

L-arginine is a semi-essential amino acid involved in multiple areas of human physiology and metabolism. Although arginine can be synthesized *de novo* from citrulline, glutamine, glutamate and proline, dietary intake of arginine is critical to maintain necessary plasma arginine levels.

In large part, L-arginine derives its importance from its role as the biologic precursor of nitric oxide (NO). Indeed, a family of enzymes called nitric oxide synthases (NOS) synthesize NO from L-arginine. NO is an endogenous messenger molecule involved in a variety of endothelium dependent physiological effects in the cardiovascular system. In addition, NO is responsible for the endothelium dependent relaxation and activation of soluble guanylate cyclase, neurotransmission in the central and peripheral nervous systems,

and activated macrophage cytotoxicity. Moreover, in response to a variety of vasoactive agents and even physical stimuli, the endothelial cells release a short-lived vasodilator called endothelium derived relaxing factor (EDRF) (also referred to as endothelium derived nitric oxide (EDNO)), which is known as nitric oxide (NO). Products of inflammation and platelet aggregation such as serotonin, histamine, bradykinin, purines, and thrombin exert all or part of their action by stimulating the release of NO. Endothelial cell-dependent mechanisms of relaxation are important in a variety of vascular beds, including the coronary circulation. In the vasculature, EDNO has several actions among which are the inhibition of platelet aggregation, adhesion of inflammatory cells, and the proliferation of smooth muscle cells. In particular, EDNO is an important regulator of vascular tone. Also, flow dependent dilation, a commonly used index of endothelial function, is largely mediated by NO.

Summary of the Invention

The present invention is based, in part, on the discovery that L-arginine, for example, a sustained release formulation of L-arginine, is useful for reducing triglyceride levels. In addition, the invention is based, in part, on the discovery that L-arginine, for example, a sustained release formulation of L-arginine, is useful in the prevention or treatment of various indications, including, obesity, obesity-related disorders, and asthma.

In one aspect, the present invention provides a method for lowering triglyceride levels in a subject by administering L-arginine to the subject, for example, a sustained release formulation of L-arginine. In various embodiments, the method can reduce triglyceride levels in a subject by less than about 100 mg/dL, 50 mg/dL or 25 mg/dL.

In one aspect, the present invention provides a method for inducing thermogenesis in a subject by administering to the subject L-arginine, for example, a sustained release formulation of L-arginine.

In another aspect, the present invention provides a method for maintaining a given weight or for inducing weight loss (for example, less than 20, 15, 10 or 5 pounds) in a subject by administering to the subject L-arginine, for example, a sustained release formulation of L-arginine.

In a further aspect, the present invention provides a method for preventing or treating obesity or an obesity related disorder, such as diabetes, in a subject by administering to the subject L-arginine, for example, a sustained release formulation of L-arginine.

In yet another aspect, the present invention provides a method for preventing or treating asthma in a subject by administering to the subject a sustained release formulation including L-arginine, for example, a sustained release formulation of L-arginine.

In yet another aspect, the present invention provides a method for preventing or treating erectile dysfunction, female infertility, male infertility, interstitial cystitis, Human Immunodeficiency Virus (HIV), Acquired Immunodeficiency Syndrome (AIDS), preeclampsia, burn or trauma injuries, cancer, gastrointestinal conditions including, for example, gastroesophageal Reflux Disease (GERD) and sphincter motility disorders, preterm delivery and senile dementia in a subject by administering to the subject a sustained release formulation of L-arginine. In yet another aspect, a sustained release formulation of L-arginine, may serve as a perioperative nutrition.

In various embodiments of the foregoing aspects of the invention, the sustained release formulations include about 25% to about 75% by weight of L-arginine or a pharmaceutically acceptable salt thereof; about 0.5% to about 5% by weight of polyvinylpyrrolidone; about 5% to about 40% by weight of hydroxypropyl methylcellulose; about 2% to about 20% by weight of microcrystalline cellulose; less than about 3% by weight of silicon dioxide; and less than about 3% by weight of magnesium stearate. For example, the formulation may include about 50% by weight of L-arginine, where the L-arginine is L-arginine monohydrochloride; between about 3% and about 4% by weight of polyvinylpyrrolidone; about 35% by weight of hydroxypropyl methylcellulose; about 10% by weight of microcrystalline cellulose; less than about 1% by weight of silicon dioxide, where the silicon dioxide is colloidal silicon dioxide; and less than about 1% by weight of magnesium stearate.

In another embodiment of the foregoing aspects of the invention, the sustained release formulation includes about 50% to about 90% by weight of L-arginine or a pharmaceutically acceptable salt thereof; about 0.5% to about 5% by weight of polyvinylpyrrolidone; and about 5% to about 40% by weight of hydroxypropyl methylcellulose. For example, the formulation may include about 70% by weight of L-arginine, where the L-arginine is L-arginine monohydrochloride; about 2% to about 3% by weight of polyvinylpyrrolidone; and about 27% to about 28% by weight of hydroxypropyl methylcellulose.

In yet another embodiment of the foregoing aspects, the sustained release formulation includes about 35% to about 90% by weight of L-arginine or a

pharmaceutically acceptable salt thereof; about 0.5% to about 5% by weight of polyvinylpyrrolidone; about 5% to about 40% by weight of hydroxypropyl methylcellulose; about 2% to about 20% by weight of microcrystalline cellulose; and less than about 1% by weight of silicon dioxide. For example, the formulation may include about 51% by weight of L-arginine, where the L-arginine is L-arginine monohydrochloride; about 3% to about 4% by weight of polyvinylpyrrolidone; about 35% by weight of hydroxypropyl methylcellulose; about 10% to about 11% by weight of microcrystalline cellulose; and less than about 1% by weight of silicon dioxide, where the silicon dioxide is colloidal silicon dioxide. Alternatively, the formulation may include about 56% by weight of L-arginine, where the L-arginine is L-arginine monohydrochloride; about 3% to about 4% by weight of polyvinylpyrrolidone; about 31% to about 32% by weight of hydroxypropyl methylcellulose; about 9% to about 10% by weight of microcrystalline cellulose; and less than about 1% by weight of silicon dioxide, where the silicon dioxide is colloidal silicon dioxide.

In yet another embodiment of the foregoing aspects, the sustained release formulation includes about 50% to about 90% by weight of L-arginine or a pharmaceutically acceptable salt thereof; about 0.5% to about 10% by weight of polyvinylpyrrolidone; about 5% to about 40% by weight of hydroxypropyl methylcellulose; and less than about 1% by weight of silicon dioxide. For example, the formulation may include about 69% by weight of L-arginine, where the L-arginine is L-arginine monohydrochloride; about 6% to about 7% by weight of polyvinylpyrrolidone; about 24% to about 25% by weight of hydroxypropyl methylcellulose; and less than about 1% by weight of silicon dioxide, where the silicon dioxide is colloidal silicon dioxide.

In yet another embodiment of the foregoing aspects, the sustained release formulation includes about 35% to about 70% by weight of L-arginine or a pharmaceutically acceptable salt thereof; about 0.5% to about 10% by weight of polyvinylpyrrolidone; about 40% to about 60% by weight of hydroxypropyl methylcellulose; and less than about 1% by weight of silicon dioxide. For example, the formulation may include about 50% by weight of L-arginine, where the L-arginine is L-arginine monohydrochloride; about 4% to about 5% by weight of polyvinylpyrrolidone; about 45% by weight of hydroxypropyl methylcellulose; and less than about 1% by weight of silicon dioxide, where the silicon dioxide is colloidal silicon dioxide.

In other aspects, the present invention provides capsules, tablets or food bars

including a sustained release formulation with L-arginine (for example, sustained release granulars of L-arginine) and red yeast rice extract. In various embodiments, the food bar is for use in lowering triglycerides, in maintaining a given weight or for inducing weight loss, for inducing thermogenesis, for treating or preventing obesity or an obesity related disorder, such as diabetes, for use in treating or preventing asthma, or for use in increasing Nitric Oxide in a subject. In a particular embodiment, the food bar further includes co-enzyme Q10.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1 is a graph depicting the release pattern of a formulation comprising L-arginine and simvastatin.

Figure 2 is photograph of NMR images of infarct size in a mouse brain treated with L-arginine and simvastatin versus in an untreated mouse brain.

Figure 3 is a bar graph depicting infarct volume in mice treated with L-arginine, simvastatin and both L-arginine and simvastatin.

Figure 4 is a bar graph depicting total infarct volume in mice treated with L-arginine and various levels of simvastatin.

Figure 5 is a flow chart depicting a method of manufacture of sustained release L-arginine tablets.

Figure 6 is a flow chart depicting a method of manufacture of sustained release L-arginine tablets.

Figure 7 is a bar graph comparing the performance of sustained release L-arginine formulations.

Figure 8 is a chart comparing the affect of administration of simvastatin with and without a sustained release L-arginine composition of the present invention on endothelium-dependent vasodilation in humans.

Figure 9 is a chart summarizing the synergistic effect of administration of simvastatin and a sustained release L-arginine composition of the invention on cholesterol levels in humans.

Figure 10 is a graph depicting the release profile of a sustained release L-arginine formulation in accordance with the present invention compared with that of an immediate release L-arginine formulation.

Figure 11 is a graph depicting the pharmocokinetics of a sustained release formulation in accordance with the present invention.

Figure 12 is a graph depicting the ratio of L-arginine to ADMA in subjects administered a sustained release formulation in accordance with the present invention.

Detailed Description of the Invention

The present invention provides methods for lowering triglyceride levels, for inducing thermogenesis, for maintaining a given weight or for inducing weight loss, and for treating or preventing asthma, obesity and obesity related conditions, such as diabetes, in a subject by administering L-arginine to the subject. In one embodiment, the L-arginine is a sustained release formulation of L-arginine. The present invention is based, in part, on the discovery that L-arginine, in particular, sustained release L-arginine, can reduce triglyceride levels in a subject. The present invention is based, in part, on the discovery that L-arginine and, in particular, sustained release L-arginine, has a thermogenic effect, and accordingly can serve to induce weight loss, maintain current weight, and/or prevent or treat obesity and obesity related conditions.

The present invention further provides methods for the treatment and prevention of at least the following diseases and disorders by administering to a subject a sustained release formulation of L-arginine: erectile dysfunction, female infertility, male infertility, interstitial cystitis, Human Immunodeficiency Virus (HIV), Acquired Immunodeficiency Syndrome (AIDS), preeclampsia, burn or trauma injuries, cancer, gastrointestinal conditions including, for example, gastroesophageal Reflux Disease (GERD) and sphincter motility disorders, preterm delivery and senile dementia. In yet another aspect, a sustained release formulation of L-arginine, may serve as a perioperative nutrition.

Moreover, the invention provides a sustained release formulation of L-arginine and methods of manufacture that render a composition with an optimal release profile. Furthermore, the formulation and methods of manufacture render a composition that is conveniently compressible, but not excessively friable.

In one embodiment, the formulations used in the methods of the invention comprise at least one sustained release agent (for purposes of the present invention, controlled release

and sustained release may be used interchangeably). In another embodiment, the L-arginine is slowly released into the system of a subject. The slow release of L-arginine creates a pharmacokinetic profile of L-arginine within the plasma that provides NOS with a substantially constant supply of L-arginine needed for the production of NO over an extended period. The formulations can, therefore, slowly dissolve *in vivo* and release a substantially uniform amount of L-arginine over a time period to be therapeutically effective for a subject.

The present invention further provides food supplemented with L-arginine. Preferably, the food is in the form of a bar such as a prescription health bar. Use of food enables the provision of larger amounts of L-arginine than could be incorporated into a single tablet. The present invention thus provides a bar that can provide more than 1 gram of L-arginine as well as other agents, as desired. In one embodiment, the L-arginine is added as an immediate release formulation, *e.g.*, immediate release granulars of L-arginine, to a food bar. In another embodiment, the bar includes a sustained release formulation that includes, *e.g.*, sustained release granulars of L-arginine. In another embodiment, the bar further contains additional agents, such as an HMG-CoA reductase inhibitor such as simvastatin or red yeast rice extract.

Definitions

Before further description of the invention, certain terms employed in the specification, examples and claims are, for convenience, collected here.

As used herein, unless otherwise specified, the term "subject" includes mammals. The term "mammals" includes, but is not limited to, dogs, cats, cattle, horses, pigs, and humans.

As used herein, the terms "treat", "treating", "treatment" and the like refer to the application or administration of a therapeutic agent or formulation to a subject, or application or administration of a therapeutic agent or formulation to an isolated tissue from a subject, who has a disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose of curing, healing, alleviating, relieving, altering, remedying, preventing, ameliorating, delaying onset of the disease or disorder and/or event, slowing the progression of the disease or disorder, improving or affecting the disease or disorder, the symptoms of disease or disorder or the predisposition toward a disease or disorder and/or event.

As used herein, the term "vascular disease" or "vascular disorder" generally refer to diseases or disorders of blood vessels and include, but are not limited to, cardiovascular, cerebrovascular, and peripheral vascular diseases or disorders. Cardiovascular disease refers to diseases of blood vessels of the heart. See, *e.g.,* Kaplan, R. M., et al., "Cardiovascular diseases" in Health and Human Behavior, pp. 206-242 (McGraw-Hill, New York 1993). Cardiovascular disease is generally one of several forms, including, for example, hypertension (also referred to as high blood pressure), coronary heart disease, stroke, and rheumatic heart disease. Diseases and disorders associated with cardiovascular disease, such as angina and congestive heart failure, are also intended to be encompassed by the term. Peripheral vascular disease or disorders refer to diseases of any of the blood vessels outside of the heart. For example, peripheral vascular disease may refer to a narrowing of the blood vessels that carry blood to leg and arm muscles. Cerebrovascular disease refers to diseases that affect the ability of blood vessels to supply blood to the brain.

The term "atherosclerosis" encompasses vascular diseases and disorders and conditions that are recognized and understood by physicians practicing in the relevant fields of medicine. Atherosclerotic cardiovascular disease, coronary heart disease (also known as coronary artery disease or ischemic heart disease), cerebrovascular disease and peripheral vessel disease are all clinical manifestations of atherosclerosis and are therefore encompassed by the terms "atherosclerosis" and "atherosclerotic disease".

As used herein the term "obesity" refers to a condition in which the body weight of a subject exceeds medically-recommended limits (*e.g.*, wherein the body mass index (BMI) is greater than that used to describe a healthy individual as defined by the NIH/WHO BMI Guidelines, which is incorporated by reference herein).

As used herein, the term "obesity related disorder" is any disease or condition that is caused by or associated with (*e.g.*, by biochemical or molecular association) obesity or that is caused by or associated with weight gain and/or related biological processes that precede clinical obesity. The phrase "to treat" or "treating" a disorder associated with obesity in a subject refers to reducing or ameliorating the disorder in a subject that suffers from the disorder or is at risk of acquiring the disorder. Preferably, the disorder, or the potential for developing the disorder, is reduced, optimally, to an extent that the subject no longer suffers from or does not develop the disorder or the discomfort and/or altered functions and detrimental conditions associated with such disorder.

As used herein, the term "asthma" is art recognized and generally includes the state in which excessive smooth muscle contraction of the airways in the lungs of a subject occurs.

As used herein, the term "erectile dysfunction" is art recognized and generally refers to certain disorders of the cavernous tissue of the penis and the associated facia which produce impotence, the inability to attain a sexually functional erection.

As used herein, the term "thermogenesis" is art recognized and generally refers to the oxidation of fatty acids with minimal or no ATP production. Thermogenesis is generally associated with weight loss or the prevention of weight gain.

As used herein the terms "coadministration" or "coadministered" when used to describe the administration of two or more compounds to a subject means that the compounds, which may be administered by the same or different routes, are administered concurrently (*e.g.,* as a mixture) or sequentially, such that the pharmacological effects of each overlap in time. As used herein, unless otherwise specified, when applied to the administration of at least two compounds, the term "sequentially" means that the compounds are administered such that the pharmacological effects of each overlap in time. In certain embodiments, agents are coadministered substantially simultaneously. By "substantially simultaneously," it is meant that the formulation of the invention is administered to the subject close enough in time with the administration of at least one additional agent, whereby the agents may exert an additive or even synergistic effect, *e.g.,* without limitation, increasing NOS activity, NO production, or vasodilation.

As used herein the term "precursor of NO" includes any substrate precursor of native NO, *e.g.,* L-arginine.

The term "native NO" as used herein refers to nitric oxide that is produced through the bio-transformation of L-arginine or the L-arginine dependent pathway. The terms "endothelium derived relaxing factor (EDRF)" or "endothelium derived nitric oxide (EDNO)" may be used interchangeably with "native NO".

As used herein the term "L-arginine" refers to L-arginine and all of its biochemical equivalents, *e.g.,* L-arginine hydrochloride, precursors, and its basic form, that act as substrates of NOS with resulting increase in production of NO. The term includes pharmaceutically acceptable salts of L-arginine. Also included are L-arginine containing peptides such as poly(L-arginine) and protamine.

The term "pharmaceutically acceptable salts" refers to salts prepared from

pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. Suitable non-toxic acids include inorganic and organic acids such as acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric acid, p-toluenesulfonic, and the like. Particularly preferred are hydrochloric, hydrobromic, phosphoric, and sulfuric acids, and most particularly preferred is the hydrochloride salt.

Since the L-arginine used in the methods of the present invention may be free base and/or hydrochloric acid, salts may be prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic and organic acids or inorganic and organic bases. Such salts may contain any of the following anions: acetate, benzensulfonate, benzoate, camphorsulfonate, citrate, fumarate, gluconate, hydrobromide, hydrochloride, lactate, maleate, mandelate, mucate, nitrate, pamoate, phosphate, succinate, sulfate, tartrate, bromide, fluoride, iodide, borate, hypobromite, hypochlorite, nitrite, hyponitrite, disulfate, sulfite, sulfonate, diphosphate, phosphite, phosphonate, diphosphonate, perchlorate, perchlorite, oxalate, malonate, carbonate, bicarbonate, tosylate, permanganate, manganate, propanolate, propanoate, ethandioate, butanoate, propoxide, chromate, dichromate, selenate, orthosilicate, metasilicate, pertechnetate, technetate, dimethanolate, dimethoxide, thiocyanate, cyanate, isocyanate, 1,4-cyclohexanedithiolate, oxidobutanoate, 3-sulfidocyclobutane-1-sulfonate, 2-(2-carboxylatoethyl)-cyclohexanecarboxylate, 2-amino-4-(methythio)-butanoate and the like. Particularly preferred are benzensulfonate, hydrobromate, hydrochloride, and sulfate. Such salts may also contain the following cations: aluminum, calcium, lithium, magnesium, potassium, sodium, zinc, benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, and procaine. Preferably, the cation is hydrogen.

As used herein the term "agonist" or "agonist of eNOS or cNOS" refers to an agent which stimulates the bio-transformation of a substrate such as, for example, L-arginine to NO. An agonist of eNOS or cNOS includes, for example, an HMG-CoA reductase inhibitor. "HMG-CoA reductase (3-hydroxy-3-methylglutaryl-coenzyme A)" is the microsomal enzyme that catalyzes the rate limiting reaction in cholesterol biosynthesis. An "HMG-CoA reductase inhibitor" inhibits HMG-CoA reductase. HMG-CoA reductase inhibitors are also referred to as "statins."

There are a large number of compounds described in the art that have been obtained

naturally or synthetically, which inhibit HMG-CoA reductase and are referred to as "statins," and which form the category of agents useful for practicing the present invention. Examples include, without limitation, those which are commercially available, such as simvastatin (U.S. Pat. No. 4,444,784), lovastatin (U.S. Pat. No. 4,231,938), pravastatin sodium (U.S. Pat. No. 4,346,227), fluvastatin (U.S. Pat. No. 4,739,073), atorvastatin (U.S. Pat. No. 5,273,995), cerivastatin, rosuvastatin, and numerous others such as compactin, dalvastatin, mevastatin, fluindostatin, pitavastatin, HR-780, GR-95030, CI 980, BMY 22089, BMY 22566, and those described in, for example, U.S. Pat. No. 5,622,985, U.S. Pat. No. 5,135,935, U.S. Pat. No. 5,356,896, U.S. Pat. No. 4,920,109, U.S. Pat. No. 5,286,895, U.S. Pat. No. 5,262,435, U.S. Pat. No. 5,260,332, U.S. Pat. No. 5,317,031, U.S. Pat. No. 5,283,256, U.S. Pat. No. 5,256,689, U.S. Pat. No. 5,182,298, U.S. Pat. No. 5,369,125, U.S. Pat. No. 5,302,604, U.S. Pat. No. 5,166,171, U.S. Pat. No. 5,202,327, U.S. Pat. No. 5,276,021, U.S. Pat. No. 5,196,440, U.S. Pat. No. 5,091,386, U.S. Pat. No. 5,091,378, U.S. Pat. No. 4,904,646, U.S. Pat. No. 5,385,932, U.S. Pat. No. 5,250,435, U.S. Pat. No. 5,132,312, U.S. Pat. No. 5,130,306, U.S. Pat. No. 5,116,870, U.S. Pat. No. 5,112,857, U.S. Pat. No. 5,102,911, U.S. Pat. No. 5,098,93 1, U.S. Pat. No. 5,081,136, U.S. Pat. No. 5,025,000, U.S. Pat. No. 5,021,453, U.S. Pat. No. 5,017,716, U.S. Pat. No. 5,001,144, U.S. Pat. No. 5,001,128, U.S. Pat. No. 4,997,837, U.S. Pat. No. 4,996,234, U.S. Pat. No. 4,994,494, U.S. Pat. No. 4,992,429, U.S. Pat. No. 4,970,231, U.S. Pat. No. 4,968,693, U.S. Pat. No. 4,963,538, U.S. Pat. No. 4,957,940, U.S. Pat. No. 4,950,675, U.S. Pat. No. 4,946,864, U.S. Pat. No. 4,946,860, U.S. Pat. No. 4,940,800, U.S. Pat. No. 4,940,727, U.S. Pat. No. 4,939,143, U.S. Pat. No. 4,929,620, U.S. Pat. No. 4,923,861, U.S. Pat. No. 4,906,657, U.S. Pat. No. 4,906,624 and U.S. Pat. No. 4,897,402, the disclosures of each of which are incorporated herein by reference. Additionally, red yeast rice extract may be utilized. Without wishing to be bound to any particular theory, red yeast extract may inhibit HMG-CoA Reductase through the action of mevinolin, which is chemically identical to lovastatin and similar to simvastatin. Any other member of the class of compounds that inhibits HMG-CoA reductase may be used in the methods of the invention. A combination of two or more HMG-CoA reductase inhibitors may also be used in the methods of the invention.

The term "eNOS activity", as used herein, means the ability of a cell to generate NO from the substrate L-arginine. Increased eNOS activity can be accomplished in a number of different ways. For example, an increase in the amount of eNOS protein or an increase in

the activity of the protein (while maintaining a constant level of the protein) can result in increased "activity." An increase in the amount of protein available can result from, for example and without limitation, increased transcription of the eNOS gene, increased translation of eNOS mRNA, increased stability of the eNOS mRNA, activation of eNOS, or a decrease in eNOS protein degradation.

The eNOS activity in a cell or in a tissue can be measured in a variety of different ways. A direct measure is to measure the amount of eNOS present. Another direct measure is to measure the amount of conversion of L-arginine to L-citrulline by eNOS or the amount of nitric oxide generation by eNOS under particular conditions, such as the physiologic conditions of the tissue. The eNOS activity also can be measured indirectly, for example by measuring mRNA half-life (an upstream indicator) or by a phenotypic response to the presence of NO (a downstream indicator). One phenotypic measurement employed in the art is measuring endothelial dependent relaxation in response to acetylcholine, which response is affected by eNOS activity. The level of NO present in a sample can be measured using a NO meter. All of the foregoing techniques are well known to those of ordinary skill in the art.

The methods of the present invention, by causing an increase in NO production, permit not only the re-establishment of normal base-line levels of eNOS activity, but also allow increasing such activity above normal base-line levels. Normal base-line levels are the amounts of activity in a normal control group, controlled for age and having no symptoms that would indicate alteration of endothelial cell NOS activity (such as hypoxic conditions, hyperlipidemia and the like). The actual level then will depend upon the particular age group selected and the particular measure employed to assess activity. In abnormal circumstances, endothelial cell NOS activity (and NO production) is depressed below normal levels. Accordingly, the formulations of the invention can not only restore normal base-line levels of NO production in such abnormal conditions, but can increase endothelial cell NOS activity (and NO production) far above normal base-line levels.

The term "carrier" refers to diluents, excipients and the like for use in preparing admixtures of a pharmaceutical composition.

As used herein, the term "dosage form" means a pharmaceutical composition that contains an appropriate amount of active ingredient for administration to a subject, *e.g.*, a subject either in single or multiple doses.

The unit "mg/Kg" as used herein means the mg of agent per Kg of subject body weight.

As used herein, unless otherwise indicated, the term "half-life" means the time taken to decrease the concentration of drug in the blood plasma of the organism by about one half from the drug concentration at the time of administration.

As used herein, unless otherwise specified, the term "immediate release" means that no extrinsic factors delay the *in vitro* release of one or more drugs.

As used herein, the terms "pharmaceutical composition" or "pharmaceutical formulation," used interchangeably herein, mean a composition that comprises pharmaceutically acceptable constituents.

As used herein, the term "pharmaceutically acceptable" means the type of formulation that would be reviewed and possibly approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

As used herein unless otherwise specified, the term "pharmaceutically acceptable carrier" means a carrier medium which does not interfere with the effectiveness of the biological activity of the active ingredient and which is not toxic to the subject to which it is administered. The use of such media and agents for pharmaceutically active formulations is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the formulations used in the methods of the invention is contemplated.

As used herein, the term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic acids, including inorganic acids and organic acids.

As used herein, unless otherwise specified, the term "sustained release" is defined as a prolonged release pattern of one or more drugs, such that the drugs are released over a period of time. A sustained release formation is a formulation with a release kinetics which results in measurable serum levels of the drug over a period longer than that obtained following IV injection or by administering an immediate release oral dosage form. A sustained release formulation is giving a continued effect to drugs of which biological half lives after administration are short; decreasing side effects of drugs which likely exhibit side effect C_{max} -dependently; and improving compliance by decreasing the number of times of administration. For purposes of the present invention, sustained release, slow release,

controlled release, extended release, prolonged release, controlled release and delayed release are used interchangeably.

As used herein, the term "salt or complex" is used to describe a compound or composition comprising two or more chemical moieties that are associated by at least one type of interaction including, but not limited to, Van der Waals, ionic and/or hydrogen bonding. A salt or complex may exist as a solid or in a liquid.

As used herein, the term "weight percent" when used to describe the amount of a component within a formulation means the weight of the specified component based upon the weight of all components within the formulation.

Various aspects of the invention are described in further detail in the following subsections:

I. Formulations

The methods of the invention include methods for maintaining a given weight or for inducing weight loss in a subject, methods for the treatment and prevention of asthma, obesity and obesity related conditions, *e.g.,* diabetes, by administering to a subject L-arginine. The methods of the invention further include methods of inducing thermogenesis by administering to a subject L-arginine. In one embodiment, the L-arginine is a sustained release formulation of L-arginine. Furthermore, the methods of the invention include methods of treating and preventing other indications described herein, by administering to a subject a sustained release formulation of L-arginine.

In one embodiment, the formulations used in the methods of the invention comprise L-arginine in a therapeutically effective amount and at least one sustained release agent. The formulations also can include additional ingredients necessary to modify the formulations for administration, preservation, esthetics and the like. In one embodiment, the formulation of the present invention also include binders, fillers and lubricants. In a preferred embodiment, the formulation comprises a sustained release L-arginine formula comprising L-arginine, a binder, one or more sustained release agents, a glidant, and a release agent or lubricant. The formulation may further comprise fillers and/or compression agents. The sustained release formulations of the present invention are particularly advantageous because their release profile allows the administration of lower dosages to maintain the same level of drug in the body than required with immediate release or commercially available sustained release agents.

L-arginine is commercially available from a number of sources known to the skilled practitioner. USP grade L-arginine, for example, is commercially available from various sources including Sigma-Aldrich (Milwaukee, WI). Suitable arginine and arginine derivative compounds include, but are not limited to, arginine salts such as arginine HCl, arginine aspartate, or arginine nicotinate. Other arginine compounds or derivatives may be chosen from di-peptides that include arginine such as alany-Larginine (ALA-ARG), valinyL-arginine (VAL-ARG), isoleucinyL-arginine (ISO-ARG), and leucinyL-arginine (LEU-ARG), and tri-peptides that include arginine such as argininyl-lysinyl-glutamic acid (ARG-LYS-GLU) and arginyl-glysyL-arginine (ARG-GLY-ARG). The L-arginine preferably is L-arginine monohydrochloride.

In one embodiment, the L-arginine is present at about 10% to about 90% by weight of the formulation. In another embodiment, the L-arginine is present at about 25% to about 75% by weight of the formulation. In various embodiments, the L-arginine is present at about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90%. In particular embodiments, the L-arginine is present at about 50, 51, 56, 69 or 70%. All ranges within each of the above ranges are within the scope of the present invention.

In certain embodiments, the formulation may contain less than about 7 g L-arginine, for example, less than about 6 g, about 5 g, about 4 g, about 3 g, about 2 g, or about 1 g L-arginine. For example, the formulation may contain from about 1 g to about 7 g, about 2 g to about 6 g or about 3 g to about 5 g L-arginine. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. Preferably, the formulation contains less than about 4 g L-arginine. While not risking to be bound by theory, the sustained release formulations of L-arginine allow for a small dosage to be employed, i.e. the total amount of L-arginine may be lower and yet still achieve a therapeutic effect.

In particular embodiments, the formulations of the present invention may also contain citrulline or a biological equivalent thereof. Citrulline is a biological precursor of L-arginine, *i.e.*, most endogenous arginine is derived from citrulline by processing within the kidney. Optionally, citrulline may be present in a sustained release form.

Use of one or more sustained release agents allows for the slow release of the L-arginine over an extended period of time. For example, the sustained release agent may release L-arginine at a rate that will not cause concentration peaks or lows that would

exacerbate side effects associated with high or low concentrations of L-arginine within the bloodstream. Sustained release agents suitable for the formulations used in the methods of the present invention include hydration agents, *e.g.*, such as cellulose, that partially hydrate when in contact with an aqueous environment to form a gelatinous barrier that retards dissolution of the agent that the hydration agent is coating. In other words, the sustained release agents form a temporary barrier to water such that water is slowly absorbed into the formulation thereby hydrating the formulation and subsequently releasing the active ingredient, *e.g.*, L-arginine, at a rate substantially slower than a formulation without sustained release agents. Additionally, the sustained release agents are present in a particle size where upon incorporation into a capsule or compaction or compression into a tablet, pill, or gelcap water slowly permeates into the structure.

In one embodiment, the sustained release agent or agents include, but are not limited to, cellulose ether products, polymethylmethacrylate, or polyvinylalcohol. In another embodiment, sustained release agents include celluloses including, but not limited to methylcellulose, hydroxypropyl methylcellulose, hydroxyethylcellulose, or combinations thereof. In a preferred embodiment, the sustained release agents include one or more hydroxypropyl methylcelluloses. Suitable sustained release agents are commercially available from The Dow Chemical Company under the trade designations METHOCEL® and ETHOCEL®. In a preferred embodiment, the sustained release agent is METHOCEL® K100 M CR Premium and/or METHOCEL® E 4M CR Premium.

The sustained release agent is typically present in an amount sufficient to release the active ingredient, *e.g.*, L-arginine, over a desired period of time. In one embodiment, the sustained release agent is present in an amount of about 5% to about 40% by weight of the formulation. In another embodiment, the sustained release agent is present in an amount of about 5% to about 75% by weight. In yet another embodiment, the sustained release agent is present in an amount of about 15% to about 50% by weight of the formulation. In various embodiments, the sustained release agent is present at about 5% to about 40%, for example, about 24% to about 25%, about 27% to about 28%, about 31% to about 32%, and about 35%. In alternative embodiments, the sustained release agent is present at about 40% to about 60%, for example, about 45%. All ranges within each of the above ranges are within the scope of the present invention.

In one embodiment, the sustained release agent releases L-arginine over a period of 10 hours, as depicted in Figure 1. In one embodiment, the formulation releases L-arginine substantially uniformly over a period from about 4 hours to about 24 hours. In another embodiment, the formulation of the present invention releases L-arginine substantially uniformly over a period of about 8 hours to about 24 hours. In yet another embodiment, the sustained release L-arginine formulation releases L-arginine substantially uniformly over a period of about 12 hours to about 48 hours.

In another embodiment, a formulation used in the methods of the present invention will release L-arginine in a manner to provide a pharmacokinetic profile wherein the half-life ($T_{1/2}$) and the T_{max} are sufficient to maintain L-arginine at a substantially constant level. In other words, in one embodiment, a sustained release formulation of the invention releases L-arginine such that a steady state of circulating L-arginine is achieved and remains constant. In one embodiment, the pharmacokinetic profile is such that $T_{1/2}$ is from about 4 hours to about 12 hours and the T_{max} is about 4 hours. In yet another embodiment, $T_{1/2}$ is from about 4 hours to about 8 hours and the T_{max} is about 4 hours. In yet another embodiment, $T_{1/2}$ is from about 6 hours to about 9 hours and the T_{max} is about 2 hours.

Binders useful in the formulation include those commonly known to the skilled practitioner. Binders include, but are not limited to, sugars, such as lactose, sucrose, glucose, dextrose, and molasses; natural and synthetic gums, such as acacia, guar gum, sodium alginate, extract of Irish moss, panwar gum, ghatti gum; other binders include a mixture of polyethylene oxide and polyethylene glycol, methylcellulose, sodium carboxymethylcellulose, hydroxypropyl cellulose (HPC), hydroxyethyl cellulose, hydroxypropyl methylcellulose, alginic acid, ethyl cellulose, microcrystalline cellulose, carbomer, zein, starch, dextrin, maltodextrin, gelatin, pregelatinized starch, polyvinlypyrrolidone (PVP) or povidone, and mixtures thereof. In a preferred embodiment, the binder is polyvinylpyrrolidone homopolymer.

In one embodiment, the binder is present at less than about 20% by weight of the formulation. In various embodiments, the binder is present at about .5% to about 10%, for example, about .5% to about 5%, about 2% to about 3%, about 3% to about 4%, about 4% to about 5%, about 5% to about 6%, about 6% to about 7%, about 7% to about 8%, about 8% to about 9%, or about 9% to about 10%. All ranges within each of the above ranges are within the scope of the present invention.

In a preferred embodiment, the formulation of sustained release L-arginine also includes a glidant. The glidant can be any known USP grade glidant including, *e.g.*, silicon dioxide. In a preferred embodiment, the glidant is colloidal silicone dioxide.

In one embodiment, the glidant is present at less than about 3% by weight of the formulation. In another embodiment, the glidant is present at less than about 2% of the formulation. In a preferred embodiment, the glidant is present at less than about 1% by weight of the formulation.

Fillers useful in the formulation include those commonly known to the skilled artisan. Typical fillers include, but are not limited to, sugars such as lactose, sucrose, dextrose, mannitol, and sorbitol, whey, dibasic calcium phosphate, tribasic calcium phosphate, calcium sulfate, and mixtures thereof. Other fillers include, but are not limited to, cellulose preparations such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropyl methylcellulose, sodium carboxymethylcellulose, polyvinylpyrrolidone, and mixtures thereof. Microcrystalline cellulose can also function as a compression agent as well as a filler. In a preferred embodiment the filler/compression agent is microcrystalline cellulose. More preferably, the microcrystalline cellulose is that sold under the designation AVICEL® PH 102 by The Dow Chemical Company.

In one embodiment, the filler is present at less than about 50% by weight of the formulation. In another embodiment, the filler is present at about 2% to about 20% by weight of the formulation including, for example, at about 8% to about 9%, at about 9% to about 10%, at about 10% to about 11%, at about 11% to about 12%, and at about 12% to about 13% by weight of the formulation. In a preferred embodiment, the filler is present at about 10% by weight of the formulation. All ranges within each of the above ranges are within the scope of the present invention.

Excipients can be added to increase the amount of solids present in the formulation. Among the excipients found useful for this purpose, often in combination, are sodium or potassium phosphates, calcium carbonate, calcium phosphate, sodium chloride, citric acid, tartaric acid, gelatin, and carbohydrates such as dextrose, sucrose, lactose, sorbitol, inositol, mannitol and dextran, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. In addition to those mentioned herein, others are known to those skilled in the art.

Release agents or lubricants useful in the formulation include those commonly known to the skilled artisan. Lubricants may be chosen so as to insure optimal absorption and utilization of nutrients. Typical lubricants include, but are not limited to, stearate, magnesium stearate, zinc stearate, calcium stearate, stearic acid, hydrogenated vegetable oils (*e.g.*, hydrogenated cottonseed oil), sodium stearyl fumarate, glyceryl palmitostearate, glyceryl behenate, sodium benzoate, sodium lauryl sulfate, magnesium lauryl sulfate, mineral oil, talc, and mixtures thereof. In a preferred embodiment, the lubricant is magnesium stearate. In a preferred embodiment, the lubricant is magnesium stearate.

In one embodiment, the lubricant is present at less than about 20% by weight of the formulation. In another embodiment, the lubricant is present at about 2% to about 20% by weight of the formulation. In a preferred embodiment, the lubricant is present at about 10% by weight of the formulation.

Disintegrants include, but are not limited to, citric acid alone or in combination with bicarbonate, sodium starch glycolate, croscarmellose sodium, crospovidone, cross-linked polyvinylpyrrolidone, corn starch, pregelatinized starch, microcrystalline cellulose, alginic acid, amberlite ion exchange resins, polyvinylpyrrolidone, polysaccharides, sodium carboxymethylcellulose, agar, salts thereof such as sodium alginate, Primogel, and mixtures thereof.

The compression agent allows for the formulation to be shaped into a tablet, troche, gelcap, or other presentation for administration in solid form. In one embodiment, the compression agent allows the formulation to be shaped into a tablet, troche, or gelcap. Compression agents include, but are not limited to, Avicel, magnesium stearate, wax, gums, celleusics, stearate, or combinations thereof. In a preferred embodiment, the compression agent is microcrystalline cellulose.

In one embodiment, the compression agent is present in an amount of about 0.01% to about 5% by weight percent of the formulation. In another embodiment, the compression agent is present in an amount of about 0.5% to about 3%. In yet another embodiment, the compression agent is present in an amount of about 1% to about 2% by weight of the formulation.

In one embodiment, the L-arginine formula includes L-arginine in a unit dosage that would be sufficient for about 5 mg/Kg to about 40 mg/Kg subject body weight. In another embodiment, the L-arginine formula includes L-arginine in a unit dosage that would be sufficient for about 20 mg/Kg to about 25 mg/Kg.

In one embodiment, an HMG-CoA reductase inhibitor (such as red yeast rice extract, a natural source of lovastatin) may be administered with the L-arginine formulation. For example, a subject may be administered formulations including L-arginine in a sustained release formulation, an HMG-CoA reductase inhibitor in a sustained release formulation (commercially available from, *e.g.*, Merck & Company, Inc. (Rahway, NJ)), or both L-arginine and an HMG-CoA reductase inhibitor in a sustained release formulation. In one embodiment, the invention encompasses formulations including L-arginine that may be administered either concurrently or sequentially with at least one HMG-CoA reductase inhibitor wherein the formulation releases L-arginine in a substantially constant concentration over a prolonged period of time and the HMG-CoA reductase inhibitor is present in an immediate release formulation. In another embodiment, the invention encompasses formulations including L-arginine in a high concentration and in a sustained release formulation wherein the pharmacokinetic profile is zero order release kinetics (*i.e.*, linear release rate over time). The release characteristics of both classes of drugs may be modified to provide release patterns that allow for the adaptation of the combination into a once daily single unit dosage.

In a particular embodiment, both L-arginine and an HMG-CoA reductase inhibitor are in a sustained release formulation. The amount of HMG-CoA reductase inhibitor may vary based on the specific inhibitor present in the formulation, as some inhibitors are more efficacious than others. For example, rosuvastatin may be present in an amount of about 0.1 mg to about 0.8 mg per tablet, simvastatin may be present in an amount of about 10 mg to about 80 mg per tablet, and/or red yeast rice extract may be present in an amount of about 1 mg to about 80 mg. Those skilled in the art will be able to determine a therapeutic amount based on the specific inhibitor employed. In one embodiment, the HMG-CoA reductase inhibitor is simvastatin and is present in a unit dosage that would be sufficient for about 0.5 mg/Kg to about 3 mg/Kg subject body weight. In another embodiment, the HMG-CoA reductase inhibitor is simvastatin and is present in a unit dosage that would be sufficient for about 1.2 mg/Kg to about 1.4 mg/Kg subject body weight. Because administration of the sustained release L-arginine with a HMG-CoA reductase inhibitor can also increase the effectiveness of the HMG-CoA reductase inhibitor, *e.g.*, simvastatin, the use of the formulations of the invention may also allow a lower dosage of HMG-CoA reductase inhibitor with an equivalent beneficial affect.

In a particular embodiment, the formulations of the invention may further include

Coenzyme Q_{10}. Coenzyme Q_{10} (also known as CoQ_{10}, Q_{10}, vitamin Q_{10}, ubiquinone, or ubidecarenone) is a compound that is made naturally in the body. Coenzyme Q_{10} is used by cells to produce energy needed for cell growth and maintenance. It is also used by the body as an antioxidant. Statins inhibit the enzyme HMG-CoA Reductase before the synthesis of cholesterol in the mevalonate pathway. This same pathway is used to synthesize the essential biochemical Coenzyme Q_{10}. Thus a major side effect predicted for statins is reduced Coenzyme Q_{10} levels resulting in potential damage to heart and skeletal muscle. The effect would be most pronounced in cells that have high metabolic rates, for instance muscle cells and nerve cells. Accordingly, providing Coenzyme Q_{10} will serve to offset the depletion of the enzyme.

Formulations used in the methods of the invention may comprise a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of the preparation desired for oral administration. In preparing the formulations for oral dosage form any of the usual pharmaceutical media may be employed. The most preferred oral solid preparations are tablets and gelcaps. Alternatively, the formulations of the present invention may be incorporated into a capsule. In this embodiment, the sustained release L-arginine granulars may be incorporated within a capsule.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are employed. Tablets or capsules may contain an L-arginine formulation in the same tablet or capsule in different configurations. Configurations include, a two-part half and half tablet or capsule, one formulation surrounding a second, dispersion of one formulation in another, granules of both formulations intermixed, and the like. If desired, tablets or capsules may be coated by standard aqueous or non-aqueous techniques.

The formulations used in the methods of the present invention may also comprise other pharmaceutically acceptable ingredients, such as those commonly used in the art. *See*, Remington: the Science & Practice of Pharmacy, by Alfonso R. Gennaro, 20th ed., Williams & Wilkins, 2000. Additional ingredients used in the formulations used in the methods of the present invention include, but are not limited to, water, glycols, oils, alcohols, starches, sugars, diluents, disintegrating agents, preservatives, excipients, lubricants, disintegrants, diluents, carriers, stabilizing agents, coloring agents, flavoring agents, and combinations thereof. Examples of suitable diluents include water, ethanol,

polyols, vegetable oils, injectable organic esters such as ethyl oleate, and combinations thereof. Formulations can also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the action of microorganisms can be insured by various antibacterial and antifungal agents including, but not limited to, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents including, but not limited to, sugars, sodium chloride, and the like.

In another embodiment of the invention, the formulations may be further co-administered with at least one other pharmaceutical agent. Examples of categories of pharmaceutical agents include: adrenergic agent; adrenocortical steroid; adrenocortical suppressant; aldosterone antagonist; amino acid; ammonia detoxicant; anabolic; analeptic; analgesic; androgen; anesthetic; anorectic; antagonist; anterior pituitary suppressant; anthelmintic; anti-acne agent; anti-adrenergic; anti-allergic; anti-amebic; anti-androgen; anti-anemic; anti-anginal; anti-anxiety; anti-arthritic; anti-asthmatic; anti-atherosclerotic; antibacterial; anticholelithic; anticholelithogenic; anticholinergic; anticoagulant; anticoccidal; anticonvulsant; antidepressant; antidiabetic; antidiarrheal; antidiuretic; anti-emetic; anti-epileptic; anti-estrogen; antifibrinolytic; antifungal; antiglaucoma agent; antihemophilic; antihemorrhagic; antihistamine; antihyperlipidemia; antihyperlipoproteinemic; antihypertensive; anti-infective; anti-inflammatory; antikeratinizing agent; antimalarial; antimicrobial; antimigraine; antimitotic; antimycotic, antinauseant, antineoplastic, antineutropenic, antiobessional agent; antiparasitic; antiparkinsonian; antiperistaltic, antipneumocystic; antiproliferative; antiprostatic hypertrophy; antiprotozoal; antipruritic; antipsychotic; antirheumatic; antischistosomal; antiseborrheic; antisecretory; antispasmodic; antithrombotic; antitussive; anti-ulcerative; anti-urolithic; antiviral; appetite suppressant; benign prostatic hyperplasia therapy agent; blood glucose regulator; bone resorption inhibitor; bronchodilator; carbonic anhydrase inhibitor; cardiac depressant; cardioprotectant; cardiotonic; cardiovascular agent; choleretic; cholinergic; cholinesterase deactivator; coccidiostat; cognition adjuvant; depressant; diuretic; dopaminergic agent; ectoparasiticide; emetic; enzyme inhibitor; estrogen; fibrinolytic; fluorescent agent; free oxygen radical scavenger; gastrointestinal motility effector; glucocorticoid; gonad-stimulating principle; hair growth stimulant; hemostatic; histamine H2 receptor antagonists; hormone; hypocholesterolemic; hypoglycemic; hypolipidemic; hypotensive; imaging agent; immunizing agent; immunomodulator; immunoregulator; immunostimulant; immunosuppressant; impotence therapy adjunct;

keratolytic; LNRII agonist; liver disorder treatment; luteolysin; mental performance enhancer; mood regulator; mucolytic; mucosal protective agent; mydriatic; nasal decongestant; neuromuscular blocking agent; neuroprotective; NMDA antagonist; non-hormonal sterol derivative; oxytocic; plasminogen activator; platelet activating factor antagonist; platelet aggregation inhibitor; potentiator; progestin; prostaglandin; prostate growth inhibitor; prothyrotropin; psychotropic; radioactive agent; regulator; relaxant; repartitioning agent; scabicide; sclerosing agent; sedative; selective adenosine A1 antagonist; serotonin antagonist; serotonin inhibitor; serotonin receptor antagonist; steroid; stimulant; suppressant; symptomatic multiple sclerosis; synergist; thyroid hormone; thyroid inhibitor; thyromimetic; tranquilizer; treatment of cerebral ischemia; treatment of Paget's disease; treatment of unstable angina; uricosuric; vasoconstrictor; vasodilator; vulnerary; wound healing agent; or xanthine oxidase inhibitor.

Another example of a pharmaceutical agent includes angiotensin converting enzyme inhibitors (ACE inhibitors). ACE is an enzyme that catalyzes the conversion of angiotensin I to angiotensin II. ACE inhibitors include amino acids and derivatives thereof, peptides, including di and tri peptides and antibodies to ACE which intervene in the renin-angiotensin system by inhibiting the activity of ACE thereby reducing or eliminating the formation of pressor substance angiotensin II. ACE inhibitors have been used medically to treat hypertension, congestive heart failure, myocardial infarction and renal disease. Classes of compounds known to be useful as ACE inhibitors include acylmercapto and mercaptoalkanoyl prolines such as captopril (U.S. Pat. No. 4,105,776) and zofenopril (U.S. Pat. No. 4,316,906), carboxyalkyl dipeptides such as enalapril (U.S. Pat. No. 4,374,829), lisinopril (U.S. Pat. No. 4,374,829), quinapril (U.S. Pat. No. 4,344,949), ramipril (US Pat. No. 4,587,258), and perindopril (U.S. Pat. No. 4,508,729), carboxyalkyl dipeptide mimics such as cilazapril (U.S. Pat. No. 4,512,924) and benazapril (U.S. Pat. No. 4,410,520), phosphinylalkanoyl prolines such as fosinopril (U.S. Pat. No. 4,337,201) and trandolopril. Estrogens upregulate NOS expression whereas ACE inhibitors do not affect expression, but instead influence the efficiency of the action of NOS on L-arginine. Thus, activity can be increased in a variety of ways. In general, activity is increased by the reductase inhibitors of the invention by increasing the amount of the active enzyme present in a cell versus the amount present in a cell absent treatment with the reductase inhibitors according to the invention.

II. Prophylactic and Therapeutic Methods

In one aspect, the invention provides methods for lowering triglyceride levels, by administering L-arginine to a subject, preferably a sustained release formulation of L-arginine. In one embodiment, the methods of the invention lower triglyceride levels in a subject by less than about 100, 90, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10 or 5 mg/dL (ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included by the teachings of the present invention).

In one aspect, the invention provides methods for maintaining a given weight or inducing weight loss. In another embodiment, the invention provides methods for treating or preventing obesity or obesity related conditions, such as diabetes. Without wishing to be bound to any particular theory, it is believed that the administration of arginine induces a thermogenic effect within a subject. Increased data in scientific literature indicates that inhibiting the formation of NO directly affects thermogenesis (for example, Kamerman *et al.*, Can J Physiol Pharmacol. 2003 Aug; 81(8):834-8) and that increased NO promotes thermogenesis (for example, Saha *et al.* Jpn J Physiol. 1996 Oct; 46(5): 375-382; Saha *et al.* Jpn J Physiol. 2000 Jun; 50(3):337-342). Generally, uncoupled thermogenesis involves the oxidation (*i.e.*, burning) of free fatty acids with minimal or no ATP production, so that the energy generated during this process is dissipated as heat into surrounding tissues. Because thermogenesis involves the breakdown of fatty acids with minimal corresponding energy production, thermogenesis is a wasteful or metabolically inefficient process and therefore results in weight loss or the prevention of weight gain. Indeed, reduction of body fat by breaking down fatty acids is considered an important means of weight control. The ability of L-arginine to stimulate thermogenesis to achieve the breakdown of fatty acids renders the administration of L-arginine as an effective weight loss method.

Additionally, administration of L-arginine may result in weight loss by other mechanisms. Obesity is characterized by increased levels of insulin (resulting, in part, from high glycemic foods and drinks) and by subnormal growth hormone (GH) release. Insulin promotes fat and carbohydrate storage while GH stimulates lipolysis (fat-burning). The insulin/GH ratio is significantly higher in obese humans than in lean humans. The combination of high insulin and low GH exacerbates obesity. Without wishing to be bound by any particular theory, L-arginine serves to enhance GH levels, thereby inducing lipolysis and reducing fat storage.

In performing certain embodiments of the present invention, a formulation of sustained release L-arginine is administered to a subject. While not wishing to be bound by theory, it is believed that sustained release formulations of arginine allow for an above baseline level of circulating L-arginine which enhances the flow of nutrients and oxygen into the cells so as to enhance metabolism and the thermogenic effect.

In various embodiments, the administration of L-arginine lowers the weight of the subject by less than about 50, 45, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, or 4 pounds.

In other aspects, the invention provides methods for preventing or treating asthma by administering to the subject arginine, preferably a sustained release formulation of L-arginine. Without wishing to be bound to any particular theory, it is believed that elevated levels of nitric oxide resulting from ingestion of arginine serves to prevent or treat or asthma.

In other aspects, the invention provides methods for treating acute chest syndrome in, for example, sickle cell disease by administering to the subject L-arginine, preferably a sustained release formulation of L-arginine. Pneumonia in patients with sickle cell disease can be particularly severe and has come to be called acute chest syndrome. Acute chest syndrome is a common cause of morbidity in sickle cell disease patients and is the most common cause of death in sickle cell disease. Multiple factors are involved in the severity of acute pulmonary injury in sickle cell disease. Without wishing to be bound to any particular theory, it is believed that elevated nitric oxide levels could impact favorably on acute chest syndrome in sickle cell as a result of the relationship between the L-arginine-nitric oxide pathway and vaso-occlusion in sickle cell disease. Low arginine levels during vaso-occlusive crisis could reflect a state of acute substrate depletion that results in a decrease in nitric oxide production. Accordingly, arginine supplementation would serve to elevate NO levels.

In yet another aspect, the invention provides methods for preventing or treating erectile dysfunction by administering arginine to the subjects, preferably a sustained release formulation of L-arginine as disclosed herein. Without wishing to be bound to any particular theory, it is believed that elevated levels of nitric oxide resulting from ingestion of arginine serves to prevent or treat erectile dysfunction.

Additionally, arginine may be utilized to treat or prevent female infertility, for example, improving ovarian response, endometrial receptivity and pregnancy rate. Such

treatment may be used with *in vitro* fertilization candidates. Similarly, arginine may be utilized to treat or prevent male infertility, for example, by enhancing spermatogenesis and increasing sperm counts and sperm motility.

In yet another aspect, arginine may be used to treat, prevent or alleviate the symptoms of interstitial cystitis, for example, by decreasing urinary voiding discomfort, lower abdominal pain, urinary frequency, and vaginal/urethral pain.

In yet another aspect, arginine, for example, the sustained release formulations of the present invention, may be used to treat, prevent or alleviate the symptoms of Human Immonodeficiency Virus (HIV) and/or Acquired Immunodeficiency Syndrome (AIDS). In particular embodiments of the treatment or prevention regimen, arginine may be administered with glutamine, hydroxymethylbutyrate and/or essential fatty acids such as omega 3 fatty acids.

According to the invention, arginine, such as sustained release formulations as described herein, may be used to treat or prevent preeclampsia. Additionally, according to the invention, arginine may be used to enhance physical performance. Without wishing to be bound to any particular theory, it is believed that arginine enhances secretion of growth hormone, thereby enhancing physical performance.

In addition, arginine (for example, sustained release L-arginine formulations such as those described herein) may be utilized to treat burn or trauma injuries. Without wishing to be bound to any particular theory, it is believed that burn victims suffer from arginine oxidation and a resulting decrease in arginine reserves. Accordingly, administering arginine to subjects with such injuries may serve to replenish the arginine reserves. In certain embodiments, arginine may be administered with fish oil, canola oil and/or nucleotides.

In yet another aspect, arginine (for example, sustained release L-arginine formulations such as those described herein) may be used to treat or prevent cancer. Without wishing to be bound to any particular theory, arginine can serve to interfere with tumor induction; to maintain or improve immune function, for example, generally or during chemotherapy; to enhance the activity of tumor infiltrating lymphocytes; and/ or to reduce chemotherapy induced suppression of NK-cell and lymphokine-activated killer cell cytotoxicity, and lymphocyte mitogenic reactivity in cancer subjects.

According to the invention, arginine (for example, sustained release L-arginine formulations such as those described herein) may further be used to treat or prevent gastrointestinal conditions. For example, arginine may be administered to treat or prevent

gastritis or ulcers, for example, by exhibiting hyperemic, angiogenic and growth promoting activity. Furthermore, arginine may be used to treat, prevent, or alleviate symptoms associated with gastroesophageal Reflux Disease (GERD) or sphincter motility disorders.

According to another aspect of the invention, arginine (for example, sustained release L-arginine formulations such as those described herein) may further be used as perioperative nutrition. For example, arginine may be used in catabolic conditions such as sepsis and postoperative stress. Without wishing to be bound to any particular theory, it is believed that arginine serves as an immunomodulator, can up-regulate immune function, and reduce the incidence of postoperative infection.

Additionally, arginine, including sustained release formulations as described herein, may be used to treat or prevent senile dementia, for example, by reducing lipid peroxidation and by increasing cognitive function.

Arginine may also be used to prevent preterm delivery in women, for example, by inhibiting uterine contractility and maintaining uterine quiescence.

In another aspect, the invention provides methods for preventing vascular diseases or disorders, such as cerebrovascular and/or cardiovascular diseases or disorders including, for example, angina pectoris, congestive heart failure, atherosclerosis, coronary heart disease, hypertension and intermittent claudication, in a subject by administering to a subject at risk for cerebrovascular and/or cardiovascular diseases or disorders a formulation comprising L-arginine. Subjects at risk for cerebrovascular and/or cardiovascular diseases and disorders (including events) can be identified by, for example, a predisposition to atherosclerosis, symptoms of atherosclerosis, or by the presence of risk factors such as, for example, cigarette smoking, high blood pressure, diabetes, family history, genetic factors, high cholesterol levels, advancing age and alcohol use.

Administration of a formulation used in the methods of the invention as a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the onset of the particular indication, such that the disease or disorder is prevented, its progression slowed, or its onset delayed.

Furthermore, methods of the present invention may be used to increase nitric oxide production and/or increase vasodilation in a subject with elevated asymmetrical dimethylarginine (ADMA). Asymmetrical dimethylarginine (ADMA) is an endogenous, competitive inhibitor of eNOS. ADMA is formed by post-tranlational methylation of L-arginine residues in protein and is released from the proteins following their hydrolysis.

Elevated levels of ADMA are associated with hypercholesterolemia, hypertension, diabetes, preeclampsia, smoking and aging. Elevation of ADMA may be due to altered metabolism of this substance by dimethylarginine dimethylaminohydrolase or DDAH. DDAH is the major enzyme involved in ADMA catabolism. Decreased levels of DDAH have been found in diabetic and hypercholesterolemic animal models.

Without wishing to be bound by theory, it is believed that the inhibitory effect of ADMA is overcome by L-arginine. Increasing levale of L-arginine overcome the inhibition of NOS by ADMA. Moreover, administration of L-arginine, optionally with an HMG-CoA reductase inhibitor can stimulate the expression of endothelial NO synthase (eNOS) *in vitro* and enhance endothelium-dependent, NO-mediated vasodilation *in vivo*. Accordingly, such a therapeutic regimen can enhance endothelial function in subjects with elevated ADMA.

By administering L-arginine to a subject with elevated ADMA, the methods of the present invention can increase nitric oxide production and/or increase vasodilation. Such administration can increase endothelial function by about 5% to about 15% or alternatively, by about 7% to about 12%. In one embodiment according to the invention, the subject has endothelial dysfunction.

For any mode of administration, the actual amount of compound delivered, as well as the dosing schedule necessary to achieve the advantageous pharmacokinetic profiles described herein, will depend, in part, on such factors as the bioavailability of the compound (and/or an active metabolite thereof), the disorder being treated, the desired therapeutic dose, and other factors that will be apparent to those of skill in the art. The actual amount delivered and dosing schedule can be readily determined by those of skill without undue experimentation by monitoring the blood plasma levels of administered compound and/or an active metabolite thereof, and adjusting the dosage or dosing schedule as necessary to achieve the desired pharmacokinetic profile.

The formulations used in the methods of the invention, as described herein, or pharmaceutically acceptable addition salts or hydrates thereof, can be delivered to a subject so as to avoid or reduce undesirable side effects according to the invention using a wide variety of routes or modes of administration. In one embodiment, the subject is an animal. In another embodiment, the subject is a mammal. In yet another embodiment, the subject is a human. The most suitable route in any given case will depend on the nature and severity of the condition being treated. The preferred route of administration of the present invention is the oral route. The compositions may be conveniently presented in unit dosage

form, and prepared by any of the methods well known in the art of pharmacy. Techniques and formulations for administering the compositions may be found in Remington: the Science & Practice of Pharmacy, by Alfonso R. Gennaro, 20th ed., Williams & Wilkins, 2000.

The formulations of the invention will generally be used in an amount effective to achieve the intended purpose, *e.g.,* to induce thermogenesis, to maintain a given weight or to induce weight loss, to treat or prevent obesity or an obesity related disorder, or to treat or prevent asthma. By therapeutically effective amount is meant an amount effective to treat a disease, disorder, symptom related to a disease or disorder, or predisposition toward a disease or disorder. As described earlier, the term "treat" refers to the application or administration of a therapeutic agent or formulation to a subject, or application or administration of a therapeutic agent or formulation to an isolated tissue from a subject, who has a disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, delaying onset of the disease or disorder and/or event, slowing the progression of the disease or disorder, improving or affecting the disease or disorder, the symptoms of disease or disorder or the predisposition toward a disease or disorder and/or event. Determination of a therapeutically effective amount is well within the capabilities of those skilled in that art, especially in light of the detailed disclosure provided herein.

Pharmaceutical formulations suitable for use with the present invention include formulations wherein L-arginine is contained in a therapeutically effective amount, *i.e.*, an amount effective to achieve the intended purpose. In general, an effective amount is that amount of a pharmaceutical preparation that alone, or together with further doses, produces the desired response. This may involve only slowing the progression of the disease temporarily. In another embodiment, it involves halting the progression of the disease permanently or delaying the onset of or preventing the disease or condition from occurring. The effect of the dosage on any particular disease can be monitored by routine methods. Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual subject parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner.

Generally, doses of active compounds would be from about 0.01 mg/kg per day to about 1000 mg/kg per day. In one embodiment, it is expected that doses ranging from about 50 to about 500 mg/kg will be suitable. In another embodiment, administration is oral and in one or several administrations per day.

In another embodiment, the subject will receive less than about 10 g sustained release L-arginine per day for example, less than about 9 g, about 8 g, about 7 g, about 6 g, about 5 g, about 4 g, about 3 g, about 2 g, or about 1 g sustained release L-arginine per day. For example, the subject may receive a daily dosage of from about 1 g to about 7 g, about 2 g to about 6 g or about 3 g to about 5 g sustained release L-arginine. Ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. Preferably, the subject receives less than about 4 g sustained release L-arginine per day. While not risking to be bound by theory, the sustained release formulations of L-arginine allow for a small dosage to be employed, i.e. the total amount of L-arginine may be lower and yet still achieve a therapeutic effect.

Of course, the actual amount of L-arginine will depend on, among other things, the condition of the subject, and the weight and metabolism of the subject. Indeed, formulations will be tailored to contain an amount of L-arginine effective to, *inter alia*, ameliorate the harmful effects of the particular targeted disease or disorder, *i.e.*, prevent the development of or alleviate the existing symptoms of, or prolong the survival of, the subject being treated. Determination of an effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure herein.

Therapeutically effective amounts for use in humans can also be estimated from animal models. For example, a dose for humans can be formulated to achieve a plasma concentration found to be effective in animals.

A therapeutically effective dose can also be estimated from human pharmacokinetic data. While not intending to be bound by any particular theory, it is believed that efficacy is related to a subject's total exposure to an applied dose of administered drug, and/or an active metabolite thereof, as determined by measuring the area under the blood concentration-time curve (AUC). Thus, a dose administered according to the methods of the invention that has an AUC of administered compound (and/or an active metabolite thereof) within about 50% of the AUC of a dose known to be effective for the indication being treated is expected to be effective. A dose that has an AUC of administered compound (and/or an active metabolite thereof) within about 70%, about 80% or even about 90% or more of the AUC

of a known effective dose is preferred. Toxicity and therapeutic efficacy of such agents can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD50/ED50. Formulations that exhibit large therapeutic indices are preferred. While formulations that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such formulations to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. In one embodiment, the dosage of such formulations of the instant invention lies within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any formulation used in the therapeutic or prophylactic methods of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Adjusting the dose to achieve maximal efficacy in subjects based on the methods described above, particularly on the blood concentration and duration of administered compound and/or its active metabolites is well within the capabilities of the ordinarily skilled artisan.

III. Methods of Manufacture

It has been discovered that efficient and substantial incorporation or coverage of L-arginine granules within a matrix improves the sustained release characteristics of the compositions of the present invention. In the case of a cellulosic matrix, upon contact with water, the matrix is partially hydrated, forming a gel layer that controls the rate of release of the L-arginine. Efficient coating or incorporation of the L-arginine granules creates a

temporary barrier to dissolution that prolongs the delivery of the L-arginine. Substantial gaps in the matrix allow the L-arginine to dissolve too quickly. The methods of the present invention result in a product with improved properties versus products made by direct compaction. Further, the present method is advantageous over methods that include fluidization dispersions as these methods are time-consuming and expensive.

The key to effective and efficient coverage is in performing the granulating, milling, and blending steps of the present invention. Referring to Figure 5, in a preferred embodiment, tablets are manufactured according a method that includes the steps of granulating the L-arginine (step 110), milling the L-arginine (steps 125, 140), blending the L-arginine with the remainder of the ingredients (steps 145, 150, 155), and compressing the ingredients to form a tablet (step 160). Preferably, the method also includes either or both of the steps of screening the ingredients (step 105), and/or drying the L-arginine during the milling step (step 135).

If the ingredients are screened prior to use (step 105), a #20 and/or a #30 mesh screen can be used for some or all of the ingredients. In a preferred embodiment, the granules are screened before granulation (step 105), and again before milling (not shown). Screening provides granules with a narrower particle size distribution in a range that is advantageous for coating and/or compaction.

The step of granulating is advantageous in that it provides more uniform particles. An active agent can be pelletized or granulated using any suitable methods known in the art. Pelletization or granulation is commonly defined as a size-enlargement process in which small particles are gathered into larger, permanent aggregates in which the original particles can still be identified and renders them into a free flowing state. Prior to granulation, a binder can be added to the active agent to improve the granulation process. Other additives can be added during granulation. These include, *e.g.*, sweeteners, flavors, color agents, antioxidants, etc.

Optionally, water or other solvent can be added to aid the granulation process. The amount of water or solvent added depends on, for example, the selection of a granulation process, and is readily determinable by those of skill in the art. Water or other solvent may be added at any suitable time point during the granulation process. For example, a binder may be mixed with a solvent (*e.g.*, water) to form a granulating agent, and then the granulating agent can be sprayed onto active agents. Alternatively, if a granulating agent is too viscous to be uniformly sprayed onto active agents, it may be desirable to blend the

binder with the active agent first and then spray water or other solvent to produce a uniform pattern of active agent granules or pellets.

Any suitable granulation method can be used to produce particles comprising an active agent. Wet granulation and/or dry granulation methods can be used.

Dry granulation refers to the granulation of a formulation without the use of heat and solvent. Dry granulation technology generally includes slugging or roll compaction. Slugging consists of dry-blending a formulation and compressing the formulation into a large tablet or slugs on a compressing machine. The resulting tablets or slugs are milled to yield the granules. Roller compaction is similar to slugging, but in roller compaction, a roller compactor is used instead of the tableting machines. See, *e.g.*, Handbook of Pharmaceutical Granulation Technology, D. M. Parikh, eds., Marcel-Dekker, Inc. pages 102-103 (1997). The dry granulation technique is useful in certain instances, for example, when the active agent is sensitive to heat or solvent.

Alternatively, wet granulation can be used. In wet granulation, solvents and binders are typically added to a formulation to provide larger aggregates of granules. The temperature during granulation can be set at any suitable point, generally not exceeding the melting point of any components of the formulation. Typically, the mixture is granulated at a temperature of about 35° C to about 65° C for about 20 to about 90 minutes. In a preferred embodiment, the mixture is granulated for less than about 20 minutes, more preferably for about 1 to about 10 minutes at room temperature (see, Example 11). Then the granules are typically air dried for a suitable duration (*e.g.*, one or more hours).

Preferably, the active agents are granulated by high shear mixer granulation ("HSG") or fluid-bed granulation ("FBG"). Both of these granulation processes provide enlarged granules or pellets but differ in the apparatuses used and the mechanism of the process operation. These granulation techniques can be performed using commercially available apparatuses.

In HSG, blending and wet massing are accomplished by high mechanical agitation by an impeller and a chopper. Mixing, densification, and agglomeration of wetted materials are achieved through shearing and compaction forces exerted by the impeller. The primary function of the chopper is to cut lumps into smaller fragments and aid the distribution of the liquid binder. The liquid binder is either poured into the bowl or sprayed onto the powder to achieve a more homogeneous liquid distribution.

On the other hand, fluidization is the operation by which fine solids are transformed into a fluid-like state through contact with a gas. At certain gas velocities, the fluid will support the particles, giving them freedom of mobility without entrainment. Such a fluidized bed resembles a vigorously boiling fluid, with solid particles undergoing extremely turbulent motion, which increases with gas velocity. Fluidized bed granulation is thus a process by which granules are produced in a fluidized bed by spraying a binder solution onto a fluidized powder bed to form larger granules. The binder solution can be sprayed from, for example, a spray gun positioned in any suitable manner (*e.g.*, top or bottom). The spray position and the rate of spray may depend on the nature of the active agent and the binder used, and are readily determined by those skilled in the art.

In a preferred method according to the invention, granulating the L-arginine (step 110) includes the steps of premixing the L-arginine with a binder such as povidone to form a blend (step 115), and granulating the blend with a granulating agent (granulating vehicle) in a granulator (step 120). The granulating agent can be, *e.g.*, povidone dissolved in purified water. Preferably, a high-shear granulator such as a Niro PMA 65 High Shear Granulator is employed. The granulator can be used both to mix the L-arginine and binder, and also to granulate the blend while spraying the granulating vehicle on the blend.

After the granulation of one or more components of the formulation, optionally, the granulated formulation can be milled. Milling can be performed using any suitable commercially available apparatus (*e.g.*, CoMil equipped with a 0.039 inch screen). The mesh size for the screen can be selected depending on the size of the granules desired. After the granulated active agents are milled, they may be further dried (e.g., in the air) if desired.

In a preferred embodiment, milling the L-arginine includes the steps of milling the wet granules or wet milling (step 125), drying the granules (step 130), and milling the dry granules or dry milling (step 140), in accordance with techniques well known in the art (see generally, U.S. Pat. No. 5,145,684 and European Patent Application 498,482, the contents of both of which are hereby incorporated by reference). A mill such as a CoMil can be employed to wet mill and dry mill the granules. In one embodiment, the mill is equipped with a '375Q screen for wet milling and a '062R screen for dry milling. The drying step can be accomplished by drying the granules in a bed dryer, *e.g.*, an Aeromatic S-2 Fluid Bed Dryer, to a desired Loss on Drying (LOD) level, *e.g.*, a $\leq$ 3% LOD. The drying steps can be accomplished in stages (step 135) until the desired LOD is reached.

Blending the L-arginine with the remainder of the ingredients can include a pre-blending step (step 145), a blending step (step 150), and a final blending step (step 155). The pre-blending step can include blending the L-arginine/povidone granules with a filler and a glidant, *e.g.*, microcrystalline cellulose and colloidal silicon dioxide. The pre-blending step can be accomplished, *e.g.*, in an 8 quart V-Blender, by blending for about 5 minutes at 25 rpm. The blending step can include adding to this blend one or more sustained release agents, *e.g.*, one or more hydroxypropyl methylcelluloses, and a filler, *e.g.*, microcrystalline cellulose. The blending step can be accomplished, *e.g.*, in a 2 cubic foot V-Blender, by blending for about 20 minutes at 25 rpm. The final blending step can include adding a release agent/lubricant, *e.g.*, magnesium stearate, to the blend in the 2 cubic foot V-blender and blending for about 5 minutes at 25 rpm.

After preparing the formulation as described above, the formulation is compressed (step 160) into a tablet form. This tablet shaping can be done by any suitable means, with or without compressive force. For example, compression of the formulation after the granulation step can be accomplished using any tablet press (*e.g.*, a Manesty Beta Press equipped with a 0.748" x 0.380" oval shaped, convex, plain tooling), preferably if the formulation composition is adequately lubricated with lubricant (*e.g.*, magnesium stearate). Many alternative means to affect this step are available, and the invention is not limited by the use of any particular apparatus. The compression step can be carried out using a rotary type tablet press. The rotary type tableting machine has a rotary board with multiple through-holes, or dies, for forming tablets. The formulation is inserted into the die and is subsequently press-molded.

Alternatively, the tablets can be made by molding. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The diameter and shape of the tablet depends on the molds, dies and punches selected for the shaping or compression of the granulation composition. Tablets can be discoid, oval, oblong, round, cylindrical, triangular, and the like. The tablets may be scored to facilitate breaking. The top or lower surface can be embossed or debossed with a symbol or letters.

The compression force can be selected based on the type/model of press, what physical properties are desired for the tablet product (*e.g.*, desired hardness, friability, etc.), the desired tablet appearance and size, and the like. Typically, the compression force

applied is such that the compressed tablets have a hardness of at least about 2 kp. These tablets generally provide sufficient hardness and strength to be packaged, shipped or handled by the user. If desired, a higher compression force can be applied to the tablet to increase the tablet hardness. However, the compression force is preferably selected so that it does not deform (*e.g.*, crack or break) the active agent-containing particles within the tablet. Preferably, the compression force applied is such that the compressed tablet has a hardness of less than about 10 kp. In certain embodiments, it may be preferred to compress a tablet to a hardness of between about 3 kp to about 7 kp, optionally between about 3 kp to about 5 kp, or about 3 kp.

Typically, the final tablet will have a weight of about 50 mg to about 2000 mg, more typically about 200 mg to about 1000 mg, or about 400 mg to about 700 mg.

The particular formulation and methods of manufacturing the formulation of the present invention impart unique advantages on the sustained release L-arginine composition. In particular, the formulation and the methods of the present invention render a composition that achieves a desirable sustained release dissolution profile. Optimally, a sustained release L-arginine formulation would sustain *in vitro* drug release at least up to 14 hours, preferably about 10% to about 40% at about 1 hour, about 30% to about 70% at about 4 hours, about 55% to about 75% at about 6 hours, about 65% to about 85% at about 8 hours, about 75% to about 95% at about 12 hours and about 80% to about 100% at 14 hours. As demonstrated by Figure 7, the formulation of the present invention achieves such optimal dissolution. Furthermore, as shown in Example 11 and Example 17, dissolution and stability studies demonstrate that the formulation of the present invention displays an optimal dissolution profile one and two months following manufacturing.

Furthermore, the formulation and methods of the present invention render a sustained release L-arginine composition that is not excessively friable. Furthermore the formulation and methods of the present invention render a sustained release L-arginine composition that is sufficiently compressible to allow for convenient manufacturing of the composition.

If desired, other modifications can be incorporated into embodiments of the tablet. For example, modification of active agent release through the tablet matrix of the present invention can also be achieved by any known technique, such as, e.g., application of various coatings, e.g., ion exchange complexes with, *e.g.*, Amberlite IRP-69. The tablets of the invention can also include or be coadministered with GI motility-reducing drugs. The active

agent can also be modified to generate a prodrug by chemical modification of a biologically active compound that will liberate the active compound in vivo by enzymatic or hydrolytic cleavage, etc. Additional layers or coating can act as diffusional barriers to provide additional means to control rate and timing of drug release.

If an HMG CoA-reductase inhibitor (*e.g.*, simvastatin) and/or additional agents are included, preferably these agents are added in the blending steps (steps 145, 150, 155). When the tablet comprises a sustained release L-arginine formulation and an HMG-CoA reductase inhibitor formulation, the tablet may have a core of slow release L-arginine formulation and a second outer cover or coating of a formulation comprising at least one HMG-CoA reductase inhibitor. Alternatively, the tablet may comprise an L-arginine formulation, *e.g.*, a sustained release L-arginine formulation, and a HMG-CoA reductase inhibitor formulation sharing one surface.

When L-arginine is administered either sequentially or concurrently with HMG-CoA reductase inhibitors, each tablet, cachet, troche, or capsule contains from about 0.01 mg to about 200 mg of the HMG-CoA reductase inhibitors. The amount of an HMG-CoA reductase inhibitor will vary depending on the particular HMG-CoA reductase inhibitor utilized.

In another aspect of the present invention, a composition for the treatment of various indications such as inducing thermogenesis, inducing weight loss, maintaining a given weight, treating or preventing obesity or an obesity related disorder such as diabetes, or treating or preventing asthma as described herein is provided in the form of food. Preferably, the food is in the form of a bar such as a prescription health bar. Use of food enables the provision of larger amounts of L-arginine than could be incorporated into a single tablet, *e.g.*, it is difficult to incorporate more than 1 gram of L-arginine in a single tablet. Thus, multiple tablets are required for delivery of amounts of L-arginine in excess of 1 gram. The present invention provides a bar that can provide more than 1 gram of L-arginine as well as other agents, as desired. In one embodiment, the L-arginine is added as an immediate release formulation, *e.g.*, immediate release granulars of L-arginine, to a food bar. Preferably, the bar includes a sustained release formulation that includes, for example, sustained release granulars of L-arginine. In a preferred embodiment, the granulars include taste masking constituents, *e.g.*, taste making coatings. In another embodiment, the bar further contains additional agents, for example, an HMG-CoA reductase inhibitor such as simvastatin. Additionally, red yeast rice extract may be incorporated within the health bar.

Red yeast rice provides a natural source of lovastatin. Combining L-arginine with statins in a food vehicle form would provide continence and an easy to administer the formulation. Use of food also can reduce the need for taking multiple tablets of L-arginine when a higher dose is desired. In a particular embodiment, the food bar may further include Coenzyme Q10.

In one embodiment, the bars have between about 1 and about 10 grams of L-arginine. In a preferred embodiment, bars are provided having a total of about 4 g per bar of L-arginine or its salts in conjunction with sugars, fruit components, protein, and vitamins and minerals. The bar weighs in the range of about 25 to about 100 g. In a particular process, the bar is produced by combining sugars and fruit paste at an elevated temperature and then combining the syrup at a reduced temperature with the minor ingredients. After blending the minor ingredients in the syrup, the L-arginine is added, particularly in conjunction with a protein extender, followed by bulking and food agents, particularly fruit pieces or other particulate edible ingredients providing the desired texture and flavor, and soy proteins. The resulting product is storage stable, has desirable organoleptic properties in being tasty, and provides a healthy combination of ingredients in collaboration with the L-arginine. Methods and formulations for manufacturing health bars with L-arginine and L-lysine are described in, *e.g.*, U.S. Patent No. 6,063,432, incorporated in its entirety by this reference. Optionally, about 1 to about 80 g, preferably about 10 mg, of simvastatin or red rice yeast extract may be added concurrently with the addition of L-arginine. Optionally, about 1 to about 100 mg, preferably about 10 mg, of Co Q_{10} may be added concurrently with the addition of L-arginine and red rice yeast extract.

Another aspect of the present invention is a method of manufacturing the bar described above. The method would include granulating the L-arginine as described above in connection with Figure 5, step 110. Preferably the granulating step would include the pre-mixing step (step 115) and the granulating step (step 120). Preferably, the method also includes the wet milling step (step 125) described above. Such bar would be obtained by wet granulation of the L-arginine with appropriate excipients, such as detailed above. The resulting granulars would be either used as is or be coated with taste masking cellulosics.

This invention is further illustrated by the following examples that should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

EXAMPLE 1: Tablet Formulation 1

About 250 grams of L-arginine was placed in a mixer and as it was slowly mixed at 100 RPM, 100 g EUDRAGIT RS 30D low permeability methacrylic aqueous polymer dispersion (Röhm America, Piscataway, NJ) was added to form a wet mass. The wet mass was passed through 18-20 sieves and allowed to dry at 50°C for 24 hours. The resulting dry L-arginine granulars (250 g) were dry mixed with 84 g METHOCEL K100 M CR methylcellulose (The Dow Chemical Company, Danbury, CT) and 3 g magnesium stearate to form a blend. The resulting blend was compressed into tablets using 7/16 concave punches.

EXAMPLE 2: Tablet Formulation 2

250 g of L-arginine was placed in a mixer and as it was slowly mixed, 84 g METHOCEL K100 M CR methylcellulose and 3 g magnesium stearate were added. The resulting blend was compressed into tablets using 7/16 concave punches.

EXAMPLE 3: Capsule Formulation 1

250 g L-arginine was placed in a mixer and as it was slowly mixed, 100 g EUDRAGIT RS 30D low permeability methacrylic aqueous polymer dispersion was added to form a wet mass. The wet mass was passed through 18-20 sieves and allowed to dry at 50°C for 24 hours. The resulting dry L-arginine granulars (250 g) were dry mixed with 84 g METHOCEL K100 M CR methylcellulose and 3 g magnesium stearate to form a blend. The resulting blend was placed into 00 gel capsules.

EXAMPLE 4: Capsule Formulation 2

250 g L-arginine was placed in a mixer and as it was slowly mixed, 84 g METHOCEL K100 M CR methylcellulose and 3 g magnesium stearate were added. The resulting blend was placed into 00 gel capsules.

EXAMPLE 5: Tablet Formulation 3

250 g L-arginine and 50 g METHOCEL K100 M CR methylcellulose were mixed and homogenized using a Kitchen Aid® mixer on low speed for 10 minutes to form a dry blend. To the dry blend, 115 g EUDRAGIT RS 30D low permeability methacrylic aqueous polymer dispersion was added in 5 g increments until the mass was homogeneously wet. The wet mass was passed through a 12 mesh sieve followed by a 20 mesh sieve and subsequently, allowed to dry at 30°C for 24 hours until the moisture content was 1% by weight. The resulting dry L-arginine granulars were dry-mixed with 7 g magnesium stearate and then compressed, using a Beta Manesy press, into tablets using 7/16 concave punches.

EXAMPLE 6: Capsule Formulation 3

500 g free base arginine and 30 g Kollidon 30 were mixed for 4 minutes. A solution of 15 g Kollidon 30 and 63.3 g purified water was prepared. This solution was added to the mixer and mixed for a roll time of 6.5 minutes. The granulate was subsequently dried. About 5 g silica, qs, was added to the granulate in the blender. 375 g Methocel K100M PCR and 75 g Methocel E4M CR were added and blended as before. The material was encapsulated as described above.

EXAMPLE 7: Capsule Formulation 4

500 g free base arginine and 30 g Kollidon 30 were mixed for 4 minutes. A solution of 15 g Kollidon 30 and 63.3 g purified water was added to the mixer and mixed for a roll time of 6.5 minutes. The granulate was dried. About 5 g silica, qs, was added to the granulate in the blender. 137.5 g Methocel K100M PCR and 37.5 g E4M CR was added to the blender and blended as before. The material was encapsulated as described above.

EXAMPLE 8: L-Arginine Formulations

An L-arginine formulation including 50.75% L-arginine base, 3.5% Kollidon 30, 27.5% Methocel K100M PCR, 7.5% Methocel E4M CR, 10.25% MCC 102 and 0.5% silicone dioxide was made by techniques described above.

Additionally, an L-arginine formulation including 55.9% L-arginine base, 3.1% Kollidon 30, 24.6% Methocel K100M PCR, 6.7% Methocel E4M CR, 9.2% MCC 102, and 0.46% silicone dioxide was made by techniques described above.

Another L-arginine formulation including, in part, 70% L-arginine base, 2.8% Kollidon 30, 21.7% Methocel KM100 PCR, and 5.5% Methocel E4M CR was made by similar techniques.

EXAMPLE 9: Manufacturing of a Sustained Release Tablet

About 1000 g L-arginine and about 200 g METHOCEL K100 M CR methylcellulose were mixed in a GP-1 high shear mixer (granulator) for about 5 minutes at 100 RPM. About 138 g EUDRAGIT RS 30D low permeability methacrylic aqueous polymer dispersion was then added with the impeller running at 200 RPM and a pressure of 1.5 bar. The mixture was granulated for 1 minute at 200 RPM. The granulation was then dried in an MP-1 Fluid Bed Granulator at 45°C inlet temperature with an air flow of 100 CMH to approximately 2% moisture content. The dried granules were then milled using a Comil 197S with size 55R screen and round impeller at 90% speed. In an 8 Qt. V-Blender, about 27 g magnesium stearate was added to the milled granules and mixed for 2 minutes. The material was then compressed into tablets with a target weight of 682.5 mg to highest possible hardness using a Beta Manesty Press with 7/16" standard concave tooling. The tablets were hand-packaged at 60 tablets per bottle in 75 cc HDPE Bottles.

The release profile of the tablet versus commercially available sustained release L-arginine tablets purchased from BioEnergy (Warren, NJ), was generated using high performance liquid chromatography (HPLC). Figure 7 is a chart depicting the release profiles of both formulations.

EXAMPLE 10: Evaluation of Pharmacokinetics of L-arginine

A randomized, four-way crossover study to evaluate the pharmacokinetics of L-arginine sustained release tablets versus immediate release capsules was conducted on 14 healthy adult volunteers under fasting conditions. "Healthy" as used herein means nonhypercholestermic subjects with no cardiovascular risk factors. The study compared the sustained release L-arginine tablet (L-arginine SR) of Example 9 and commercially available immediate release L-arginine capsules (L-arginine IR) purchased from Montiff (Los Angeles, CA).

The study goal was to determine the pharmacokinetic parameters of sustained release L-arginine. As depicted in Table I below, based on the p-values from the two-tailed paired t-test performed on each pharmacokinetic parameters, there was a statistically significant difference between treatments for C_{max} and T_{max}. As expected, sustained release L-arginine tablets had a lower C_{max} (14.9 ug/mL versus 24.1 ug/mL) and a longer T_{max} (4.4 h versus 1.4 h) compared with the immediate release capsules.

Table I: PK Parameters of L-arginine SR v. L-arginine IR

L-arginine	C_{max}	AUC_{0-t}	AUC_{0-10}	$T_{max\ 0-t}$	$T_{max\ 0-10}$
L-Arg SR	14.9	143	68.56	4.4	3.27
L-Arg IR	24.1	147	92.23	1.4	1.35
% Ratio	0.62	0.97	0.74	3.2	2.43
P-value	0.0005	0.677	0.0382	0.0133	0.0073

EXAMPLE 11. Manufacturing of an Improved Sustained Release L-arginine Tablet

Table II lists the ingredients assembled to manufacture an improved sustained release tablet, as well as the amounts used of each ingredient.

Table II: Ingredients

Component	Mg/ tablet	Percentage (%)	Weight/ Batch (Kg)
L-arginine monohydrochloride	500	50	12.5
Povidone (K 29/32)	35	3.5	0.88
Purified Water	-	-	2*
Hydroxypropyl Methylcellulose (METHOCEL K100M P CR)	275	27.5	6.87
Hydroxypropyl Methylcellulose (METHOCEL E 4M CR)	75	7.5	1.88
Microcrystaline Cellulose (AVICEL PH 102)	102.5	10.2	2.56
Colloidal Silicon Dioxide	5	0.5	0.13
Magnesium stearate	7.5	0.75	0.18
TOTAL:	1000	100.0	25

*Water is used in granulation and then the mixture was dried

All ingredients, except the magnesium stearate, were screened in a #20 mesh screen. The magnesium stearate was screened in a #30 mesh screen. Approximately half of the

povidone (polyvinylpyrrolidone) was dissolved in purified water and set aside as a granulating agent. The L-arginine and the remainder of the povidone were dry mixed for 4 minutes in a Niro PMA 65 High Shear Granulator, and then granulated for about 6.5 minutes by spraying the granulating agent into it. The wet granules were then milled in a CoMil mill equipped with a '375Q screen. The milled granules were then dried in an Aeromatic S-2 Fluid Bed Dryer to a LOD of ≤ 3%. The dried granules were then milled in the CoMil equipped with a '062R screen. Approximately half of the microcrystalline cellulose and the collodial silicon dioxide were then blended in an 8 quart V-Blender for 5 minutes at 25 rpm and transferred to a 2 cubic foot V-Blender. The remaining portion of the microcrystalline cellulose and the hydroxylpropyl methylcellulose were then also added to the 2 cubic foot V-Blender and blended for 20 minutes at 25 rpm. The magnesium stearate was then added to the 2 cubic foot V-Blender and blended for 5 minutes at 25 rpm. Finally, the blend was compressed into tablets with a target weight of 1000 mg using a Manesty Bet Press equipped with 0.748" x 0.380" oval shaped, convex, plain tooling. Figure 6 is a schematic flow diagram of this method.

Standard in-process controls tests and specifications can be used during the manufacturing process, the ones used for this example are listed in Table III below.

Table III: L-arginine SR Tablets In-process Controls: Specifications and Methods

Specification	Method	Acceptance Criteria
Blend Uniformity	CTMLP-663	Mean: 90.0% - 110.0% of Label Claim RSD% NMT 5.0%
Bulk & Tap Density	SOP Lab 2010	Report results
Particle Size Distribution	SOP LAB 2018	Report results
Moisture	SOP Lab 2059	NMT 3.5%

Standard release methods and specifications can be used, the ones used for this example are provided in Table IV below.

Table IV: L-arginine SR Tablets Release Methods and Specifications

Specification	Method	Acceptance Criteria
Physical Appearance	Visual Inspection	White to off-white tablets Oval shaped, convex tablet
Identification	CTMPLP-663	The retention time and on-line UV spectrum (200-400 nm) of the sample, correspond to those of the reference standard
Potency	CTMLP-663	90.0 – 110.0% of label claims
Related Substances	CTMLP-663	Individual: NMT 0.5% Total: NMT 2.0%
Moisture	SOP LAB 2059	NMT 3.5%
Dissolution Profile	CTMLP-663	1 hr 10 -40% 4 hr 30-70% 12 hr ≥ 75% Record Profile
Content Uniformity	CTMLP-663	USP <905>
Microbial Limits	USP <61>	Total Aerobic Microbial count ≤100 cfu/mL Total Combined Molds and Yeast count ≤50 cfu/mL Absence of E. coli Absence of S. aureus Absence of P. aeruginosa Absence of Salmonella species

Furthermore, the studies have demonstrated desirable physical characteristics, including friability and content uniformity for the sustained release L-arginine formulations of the present invention.

Table V: Physical Testing, Potency, Content Uniformity and Dissolution for Two batches of the SR L-arginine formulation

Batch #	1	2
Average tablet weight n = 20, mg	1003.3	1014.5
Tablet hardness n =20, kp	11.0	12.4
Tablet thickness n =20, mm	7.89	7.70
Tablet friability, %	0.1	0.1
Potency, %	98.4	100.5
Content Uniformity n = 10; %	99.0	100.8
Content Uniformity, %RSD	1.5	1.8

Dissolution Time, hr	% Release	
0	0	0
1	27.3	26.8
2	42.1	42.1
4	59.9	60.2
6	73.0	73.6
8	82.8	83.4
10	90.3	90.3
12	95.1	94.9
14	98.4	92.5

EXAMPLE 12: Evaluation of pharmacokinetics of L-arginine SR with and without Simvastatin and Simvastatin with and without L-arginine SR

The pharmokinetics of L-arginine SR with and without simvastatin, and simvastatin with and without L-arginine SR were studied. The L-arginine SR tablets of Example 9 were used as well as commercially available simvastatin tablets purchased from BioEnergy (Warren, NJ).

As can be see in Table VI, based on the p-values from the two-tailed paired t-test performed on each pharmokinetic parameter, there was not a statistically significant difference between treatments for C_{max}, AUC_{0-10}, and T_{max}. As depicted in Table VII, L-arginine SR has no statistically significant effect on the single dose pharmokinetics of simvastatin.

Table VI. L-arginine PK Paramaters with and without Simvastatin

L-arginine	**C_{max}** (mg/ml)	**AUC_{0-10}** (mg-hr/ml)	**T_{max}** (hr)
L-Arg SR	14.77	68.56	3.27
L-Arg SR with Simvastatin	13.49	51.55	3.23
% Ratio	1.09	1.33	1.01
P-value	0.5001	0.0713	0.9716

Table VII. Simvastatin PK Paramaters with and without L-arginine

Simvastatin	C_{max} (ng/ml)	AUC_{0-10} (ng-hr/ml)	T_{max} (hr)	k_{elim} (1/hr)	$t_{1/2}$ (hr)
simvastatin w/o L-arginine SR	21.15	107.93	2.68	0.1248	6.56
simvastatin with L-arginine SR	18.95	114.36	2.29	0.0950	10.01
P-value	0.5360	0.6302	0.4758	0.1526	0.1059

EXAMPLE 13: Effect of Administration of Simvastatin with L-arginine Upon Infarct Size in Mice

The effect of administration of both simvastatin and L-arginine upon infarct size was studied in mice. Mice were given interperitoneal injections comprising simvastatin, and simvastatin and L-arginine, dissolved in saline solution in the amounts indicated in Figure 3. The results of infarct size on these mice versus a control group are depicted in Figure 2 and Figure 3.

EXAMPLE 14: Dose Optimization of Combination of Simvastatin and L-arginine

Dose optimization of combined administration of simvastatin and L-arginine was studied in mice. Mice were injected with varying levels of simvastatin and L-arginine as shown in Figure 4. The results of this study are also shown in Figure 4. Statistical analysis predicted that the optimal range of the combination to be 1.2-1.4 mg/Kg simvastatin with about 20-25 mg/Kg L-arginine.

EXAMPLE 15: Improvement of Endothelium-dependent Vasodilation by Simvastatin is Potentiated by Combination with L-arginine Sustained Release in subjects with Elevated ADMA Levels

Statins stimulate the expression of endothelial NO synthase (eNOS) in vitro and enhance endothelium-dependent, NO-mediated vasodilation in vivo. Asymmetrical dimethylarginine (ADMA) is an endogenous, competitive inhibitor of eNOS. The presence of elevated plasma ADMA levels is associated with endothelial dysfunction. It was discovered that simvastatin enhances endothelial function in subjects with elevated ADMA

only if the inhibitory effect of ADMA is overcome by supplemental L-arginine sustained release.

15 clinically asymptomatic, elderly subjects with elevated ADMA levels received, in a randomized order, simvastatin (40 mg/day), L-arginine sustained-release (3 g/day) prepared as described in Example 11, or a combination of both, each for 3 weeks, in a three period crossover design with at least three weeks of wash-out between treatments. Endothelium-dependent vasodilation was assessed by brachial artery ultrasound using computer-assisted image analysis; ADMA and L-arginine plasma concentrations were determined by a validated HPLC method.

Analysis of 15 subjects who completed the study revealed that both sustained release L-arginine alone or in combination with simvastatin increased percentage endothelial-dependent vasodilation, from pre-treatment measurements. The combination significantly increased the change from pre-treatment percentage endothelial-dependent vasodilation by 3.87% over that observed with simvastatin alone ($p<0.025$). The difference in the change in percentage endothelial-dependent vasodilation between the combination and sustained release L-arginine alone was small. Endothelium-independent vasodilation by glyceryl trinitrate was not affected by any of the treatments. L-arginine sustained release, either alone or in combination with simvastatin, significantly improved plasma L-arginine/ADMA ratio (baseline, 82.3±4.0 vs. 102.8±9.2 and 102.6±10.8, respectively, each $p<0.05$). These results are summarized in Figure 8.

Simvastatin does not enhance endothelial function in subjects in whom eNOS is blocked by elevated ADMA levels; combination of simvastatin with oral L-arginine sustained release has a synergistic effect on endothelial function. As NO-mediated effects may play a major role in therapeutic effects of statins, combination with L-arginine sustained release should be considered in subjects with elevated ADMA concentration.

EXAMPLE 16: Improvement in Cholesterol and Triglyceride Levels by Treatment with L-arginine Sustained Release

In the study described in Example 15, the change in total cholesterol (TC), LDL cholesterol, HDL cholesterol, and triglycerides was analyzed pre- and post-treatment. Tables VIII through X show the results of treatment with the indicated regimens on triglyceride levels.

Table VIII. Triglyceride Levels (mg/dL) Before Treatment with Indicated Regimen

	Sustained Release L-Arginine & Simvastatin	Simvastatin	Sustained Release L-Arginine
No. of subjects	15	15	15
No. of subjects outside normal range	5	5	5
Mean	178.2	165.1	161.1
Median	124.0	155.0	143.0
Standard Deviation	107.10	77.40	87.04
Min-Max	66 – 450	51 – 337	67 – 332

Table IX. Triglyceride Levels (mg/dL) After Treatment with Indicated Regimen

	Sustained Release L-Arginine & Simvastatin	Simvastatin	Sustained Release L-Arginine
No. of subjects	15	15	15
No. of subjects outside normal range	2	1	3
Mean	113.7	140.2	162.0
Median	105.0	134.0	135.0
Standard Deviation	52.11	94.12	89.42
Min-Max	43 – 212	58 – 440	60 - 385

Table X. Change in Triglyceride Levels (mg/dL) Resulting from Treatment with Indicated Regimen

	Sustained Release L-Arginine & Simvastatin	Simvastatin	Sustained Release L-Arginine
No. of subjects	15	15	15
Mean	-64.5	-24.9	0.9
Median	-36.0	-38.0	-10.0
Standard Deviation	78.87	87.40	44.51
Min-Max	-241 – 17	-143 – 205	-80 - 97

Tables XI through XIII show the change in total cholesterol levels, low density lipoprotein cholesterol levels and high density lipoprotein cholesterol levels resulting from treatment with the indicated regimen.

Table XI. Change in Total Cholesterol Levels (mg/dL) Resulting from Treatment with Indicated Regimen

	Sustained Release L-Arginine & Simvastatin	Simvastatin	Sustained Release L-Arginine
No. of subjects	15	15	15
Mean	-89.0	-76.3	-11.9
Median	-87.0	-78.0	-2.0
Standard Deviation	20.99	29.50	20.67
Min-Max	-138 - -53	-134 - -14	-42 - 15

Table XII. Change in Low Density Lipoprotein Cholesterol Levels (mg/dL) Resulting from Treatment with Indicated Regimen

	Sustained Release L-Arginine & Simvastatin	Simvastatin	Sustained Release L-Arginine
No. of subjects	14	14	15
Mean	-77.9	-70.7	-12.0
Median	-77.5	-78.0	-5.0
Standard Deviation	18.49	27.45	25.48
Min-Max	-115 - -50	-121 - -20	-64 - 30

Table XIII. Change in High Density Lipoprotein Cholesterol Levels (mg/dL) Resulting from Treatment with Indicated Regimen

	Sustained Release L-Arginine & Simvastatin	Simvastatin	Sustained Release L-Arginine
No. of subjects	15	15	15
Mean	2.8	1.2	-0.3
Median	3.0	0.0	-2.0
Standard Deviation	5.77	7.05	7.88
Min-Max	-9 – 11	-7 – 20	-13 –18

The results of this analysis are shown graphically in Figure 9. As the results demonstrate, the administration of sustained release L-arginine lowers triglyceride levels.

EXAMPLE 17: Determination of Dissolution Release of Arginine HCl in Sustained Release Arginine HCl 500 mg Tablets by HPLC

The mobile phase was prepared as follows. Initially, one liter of pH 3.3 buffer solution was prepared by weighing about 0.9 g of 1-pentanesulfonic acid sodium salt, monohydrate and 3.5 g of sodium phosphate monobasic, monohydrate into a suitable container. About 100 mL of deionized water was added to dissolve. The pH was adjusted to 3.3 by the addition of phosphoric acid. Subsequently, 850 mL of the pH 3.3 buffer was combined with 150 mL of methanol into a suitable container and mixed. The mixture was filtered through a 0.45 μm nylon membrane filter. Finally the mixture was degassed before use.

The dissolution medium (50 mM phosphate buffer at a pH of 6.8) was prepared as follows. Initially 20.0 mL of 10 M NaOH was pipetted into a 1000 mL volumetric flask and diluted with deionized water to prepare 0.2 M NaOH. Subsequently 54.44 g of Potassium Dihydrogen Phosphate, Anyhydrous was weighed into a suitable container, and dissolved and diluted with 2000 mL of deionized water. 896 mL of the 0.2 M NaOH was added to the container and diluted to 8000 mL with deionized water. Finally the mixture was degassed before use.

The dissolution sample was prepared as follows. Six Arginine HCl 500 mg tablets, prepared as described in Example 11, were weighed. Each tablet was placed in a stainless steel sinker with 900 mL of Phophate buffer (pH 6.8). The sinker was subsequently dropped into a vessel of a USP Apparatus 2 (paddle) for immediate rotation at 75 rpm at about 37° C ± 0.5° C. 10 mL of the solution from the vessel was removed at 1, 2, 4, 6, 8, 10, 12 and 14 hour time points for respective dissolution analysis at each time point. Each of these samples solutions were filtered through 0.45 μm PVDF syringe filters. The filtrate was collected into HPLC vials for analysis, wherein the first 1-2 mL were discarded. Using a 10 μm Full Flow Filter, 10 mL of the dissolution medium pre-warmed to 37° C ± 0.5° C was replaced back to the dissolution vessel after every sampling point. The practitioner should be aware that the sample solution is stable up to 1 day at room temperature and is stable up to 3 days at 4° C.

The Arginine HCl standard solution was prepared as follows. 28 ± 2 mg of Arginine HCl reference standard is accurately weighed into a 50 mL volumetric flask. The standard was dissolved in and diluted to volume with dissolution medium.

HPLC was conducted using a BDS Hypersil C18 column (5 μm, 250 mm x 4.6 mm) detecting using UV at 210 nm. The column temperature was set to ambient. Generally, the run time was 9 minutes, the injection volume was 10 μL, the flow rate was 0.8 mL/min and the mobile phase was pH 3.3. Buffer/Methanol (85/15, v/v), prepared as described above.

Each trial proceeded as follows. One injection of dissolution medium followed by five consecutive injections of Arginine HCl standard solution and finally one injection of each sample solution were performed. Arginine HCl standard solution was reinjected after every six sample injections and at the end of the sequence run. The system drift throughout the run (*i.e.*, the percent recovery of the standard solution compared to the mean of five consecutive injections of Arginine HCl standard solution) should be from about 97% to about 103%.

In determining the percent of arginine released, the practitioner must be careful to ensure that the USP trailing factor (T) for Arginine HCl peak in the injection of working standard solution is less than 2. T is calculated as follows:

$$T = W_{.05}/2f$$

where $W_{.05}$ is the peak width of Arginine HCl peak at 5% of the peak height from the baseline, and f is the distance from the peak maximum to the leading edge of the peak (the distance being measured at a point 5% of the peak height from the baseline.

The percent Arginine HCl released is calculated as follows:

$$\% \text{ Release} = [(C_s)(V)(R_u/R_s) + \sum_{i=1}^{n-1} C_i V_r]/(LC)$$

where n is the total number of measurements, V_r is the volume of dissolution medium for each measurement (10 mL), V is the initial volume of dissolution medium (900 mL), C_s is the concentration, in mg/mL, of Arginine HCl in the Working Standard Solution, C_i is the concentration, in mg/mL, of Arginine HCl in each sample solution (where, i=1 to i=n-1), R_u is the peak area response of Arginine HCl peak obtained from the sample solution, R_s is the average peak area response of Arginine HCl peak obtained from the consecutive injections of Working Standard Solution, and LC is the label claim of Arginine HCl (500 mg).

The percent released was calculated at 1, 2, 4, 6, 8, 10, 12 and 14 hours. Tables V and XIV summarize the results for various dissolution studies.

Table XIV: Dissolution Profiles of L-arginine SR Tablets at about 40° C/75%RH Stability

Time Point	Initial	1 month	2 months
Dissolution Time , hr	% Release		
0	0	0	0
1	20.4	21.8	28.1
2	36.4	36.6	41.1
4	53.5	54.3	58.5
6	66.8	67.5	71.5
8	76.6	77.9	81.3
10	83.1	85.5	88.3
12	87.2	89.7	92.9
14	89.1	92.4	96.0

EXAMPLE 18: Manufacturing an L-arginine Food Bar

L-arginine is granulated as described above in connection with Figure 5, step 110 including both the pre-mixing step (step 115) and the granulating step (step 120). Subsequently the granulation is wet milled (step 125) with appropriate excipients as described above. The resulting granulars are either used as is or are coated with taste masking cellulosics.

Sugars and fruit paste are blended at an elevated temperature and then combined with the syrup at a reduced temperature with the minor ingredients. The L-arginine granulars, bulking agents and food agents including fruit pieces or edible ingredients are added so as to achieve the desired texture and flavor. A protein extruder is utilized to form the food bar.

EXAMPLE 19: Weight Loss Resulting from Treatment with Sustained Release L-arginine

A 45 year old female volunteer took 3 capsules twice daily of a time release L-arginine formulation. The formulation consisted of 350 mg L-arginine, cellulose, kollidon, leucine and silica. The volunteer noted no other significant change in diet during this period. After two months of this L-arginine regimen, the volunteer lost 9 lbs.

A 53 year old male volunteer took 3 capsules twice daily of the same time release L-arginine formulation. The volunteer noted no other significant change in diet during this period. After two months, the volunteer lost 4 lbs. Moreover, the volunteer noted an

increased body temperature of .25° C. Body temperature increase is indicative of thermogenesis.

EXAMPLE 20: Thermogenic Effect of L-Arginine

The consumption of oxygen by animals to produce heat is a principle well known to one of ordinary skill in the art. See, for example, M. Kleiber, "The Fire of Life", Robert E. Kreiger Pub. Co., New York, New York, 1975. During increased energy expenditure, metabolic fuels, *e.g.* glucose or fatty acids, are oxidized to CO_2 and H_2O with concomitant evolution of heat, i.e. thermogenesis. Thus, the measurement of oxygen consumption in animals, including humans and companion animals, is an indirect measure of thermogenetic effect. In this regard, indirect calorimetry has been demonstrated to be a valid method for the measurement of energy expenditure and has been employed extensively in animals, including humans.

The ability of the L-arginine to generate a thermogenic response and, therefore, to have utility in the treatment of obesity is demonstrated in the following protocol.

The protocol is designed to measure oxygen consumption by dosing fatty Zucker rats for 6 days. Male fatty Zucker rats having a body weight range of about 400-500 g are housed at least 3-7 d in individual cages under standard laboratory conditions prior to the initiation of the study. An L-arginine formulation is administered by oral gavage as a single daily dose given between 3 and 6 p.m. for 6 days in a suitable form and dosage.

Oxygen consumption is measured the day after the last dose using an open circuit, indirect calorimeter (Oxymax, Columbus Instruments, 950 North Hague Ave., Columbus, Ohio 43204). The Oxymax gas sensors are calibrated with N_2 gas and gas mixture (0.5% CO_2, 20.5% O_2, 79% N_2) before each experiment. Rats are removed from their home cages, their body weights are recorded and they are placed in sealed chambers (43x43x10 cm) of the calorimeter and the chambers are placed in activity monitors. Air flow rate through the chambers is set at 1.6-1.7 l/min. The Oxymax calorimeter software calculates the oxygen consumption (ml/kg/h) based on the flow rate of air through the chambers and difference in oxygen content at inlet and output ports. The activity monitors have 15 infrared light beams spaced one inch apart on each axis; ambulatory activity is recorded when two consecutive beams are broken and the results are recorded as counts. Oxygen consumption and ambulatory activity are measured every 10 minutes for 5-6.5 hours. Resting oxygen consumption is calculated on individual rats by averaging the values excluding the first 5

values and values obtained during time periods where ambulatory activity exceeds 100 counts.

EXAMPLE 21: In vitro Release profile of Sustained Release Formulations of L-Arginine

In vitro analyses of the release profile of a commercially available generic formulation of L-arginine and a sustained release capsule formulation of L-arginine made in accordance with the present disclosure and including 350 mg L-arginine, cellulose, kollidon, leucine and silica were performed. Figure 10 graphically depicts the release profile of the two tests. The sustained release formulation of the present invention released L-arginine over 10 hours.

EXAMPLE 22: Pharmocokinetic profile of Sustained Release Formulations of L-Arginine

Subjects were administered a sustained release formulation of L-arginine. L-arginine levels in the subjects were determined at numerous time points. Figure 11 depicts the pharmacokinetic profile of the sustained release formulation. Administration of the sustained release formulation produced a significant increase in circulating L-arginine levels above base line levels for at least 8 hours.

In addition, Figure 12 depicts the improved ratio of L-arginine to ADMA in subjects administered sustained release formulations of the present invention.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Claims

What is claimed:

1. A method for lowering triglyceride levels in a subject, the method comprising administering sustained release L-arginine to the subject.

2. The method of claim 1, wherein the method lowers triglyceride levels by less than about 100 mg/dL.

3. The method of claim 1, wherein the method lowers triglyceride levels by less than about 50 mg/dL.

4. The method of claim 1, wherein the method lowers triglyceride levels by less than about 25 mg/dL.

5. The method of claim 1, wherein the subject is administered sustained releases L-arginine orally.

6. The method of claim 1, wherein the subject is administered less than 10 g sustained release L-arginine per day.

7. The method of claim 1, wherein the subject is administered from about 1 g to about 7 g sustained release L-arginine per day.

8. The method of claim 1, wherein the subject is administered from about 2 g to about 6 g sustained release L-arginine per day.

9. The method of claim 1, wherein the subject is administered from about 3 g to about 5 g sustained release L-arginine per day.

10. The method of claim 9, wherein the subject is administered about 3 g sustained release L-arginine per day.

11. The method of claim 9, wherein the subject is administered about 1 g to about 2 g sustained release L-arginine twice per day.

12. The method of claim 1, wherein the sustained release formulation comprises:

(a) about 25% to about 75% by weight of L-arginine or a pharmaceutically acceptable salt thereof;
(b) about 0.5% to about 5% by weight of polyvinylpyrrolidone;
(c) about 5% to about 40% by weight of hydroxypropyl methylcellulose;
(d) about 2% to about 20% by weight of microcrystalline cellulose;
(e) less than about 3% by weight of silicon dioxide; and
(f) less than about 3% by weight of magnesium stearate.

13. The method of claim 1, wherein the sustained release formulation comprises:
(a) about 50% by weight of L-arginine, wherein the L-arginine comprises L-arginine monohydrochloride;
(b) between about 3% and about 4% by weight of polyvinylpyrrolidone;
(c) about 35% by weight of hydroxypropyl methylcellulose;
(d) about 10% by weight of microcrystalline cellulose;
(e) less than about 1% by weight of silicon dioxide, wherein the silicon dioxide comprises colloidal silicon dioxide; and
(f) less than about 1% by weight of magnesium stearate.

14. A method for inducing thermogenesis in a subject, the method comprising administering L-arginine to the subject.

15. A method for maintaining a given weight or for inducing weight loss in a subject, the method comprising administering L-arginine to the subject.

16. The method of claim 15, wherein the method lowers the weight of the subject by less than about 20 pounds.

17. The method of claim 15, wherein the method lowers the weight of the subject by less than about 10 pounds.

18. The method of claim 15, wherein the method lowers the weight of the subject by

less than about 5 pounds.

19. A method for preventing or treating obesity or an obesity related disorder in a subject, the method comprising administering L-arginine to the subject.

20. The method of claim 19, wherein the obesity related disorder is diabetes.

21. A method for preventing or treating asthma in a subject, the method comprising administering L-arginine to the subject.

22. The method of any one of claims 14, 15, 19, or 21, wherein the L-arginine comprises a sustained release formulation of L-arginine.

23. The method of claim 22, wherein the sustained release formulation comprises:

(a) about 25% to about 75% by weight of L-arginine or a pharmaceutically acceptable salt thereof;
(b) about 0.5% to about 5% by weight of polyvinylpyrrolidone;
(c) about 5% to about 40% by weight of hydroxypropyl methylcellulose;
(d) about 2% to about 20% by weight of microcrystalline cellulose;
(e) less than about 3% by weight of silicon dioxide; and
(f) less than about 3% by weight of magnesium stearate.

24. The method of claim 22, wherein the sustained release formulation comprises:
(a) about 50% by weight of L-arginine, wherein the L-arginine comprises L-arginine monohydrochloride;
(b) between about 3% and about 4% by weight of polyvinylpyrrolidone;
(c) about 35% by weight of hydroxypropyl methylcellulose;
(d) about 10% by weight of microcrystalline cellulose;
(e) less than about 1% by weight of silicon dioxide, wherein the silicon dioxide comprises colloidal silicon dioxide; and
(f) less than about 1% by weight of magnesium stearate.

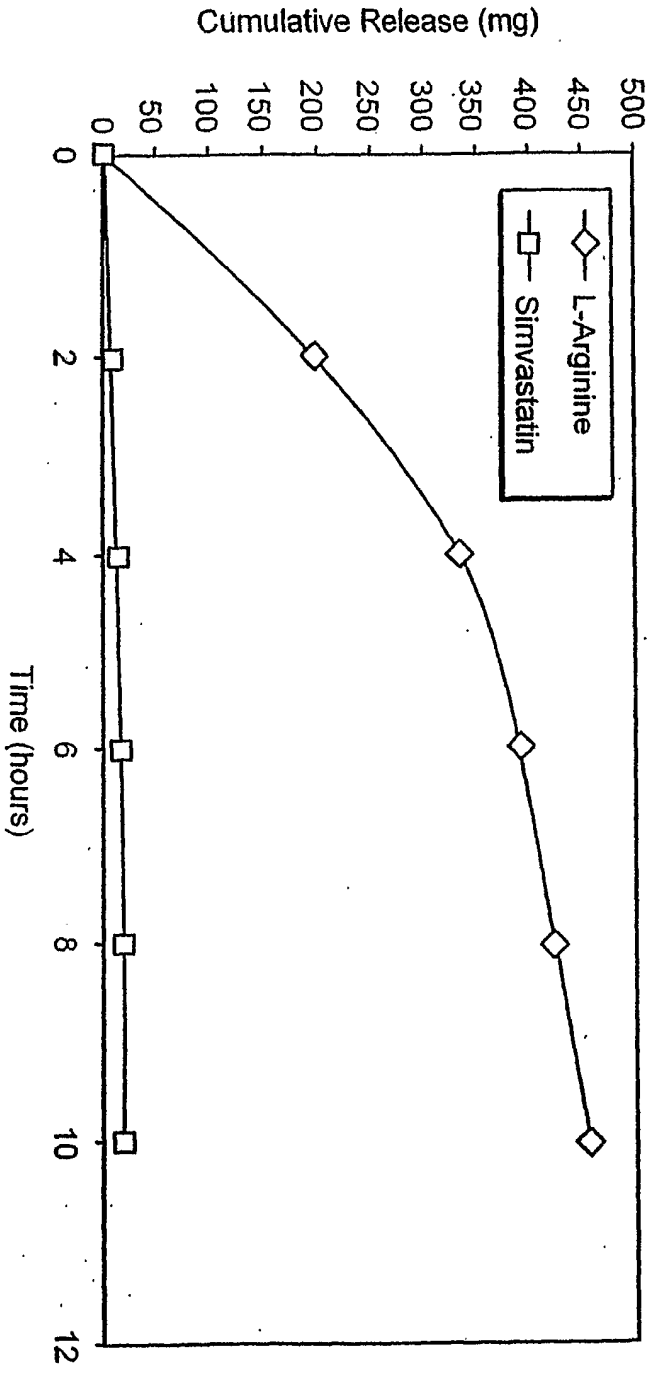

Cumulative Release (mg)
0
50
100
150
200
250
300
350
400
450
500
0
2
4
6
8
10
12
Time (hours)
L-Arginine
Simvastatin

FIG. 1

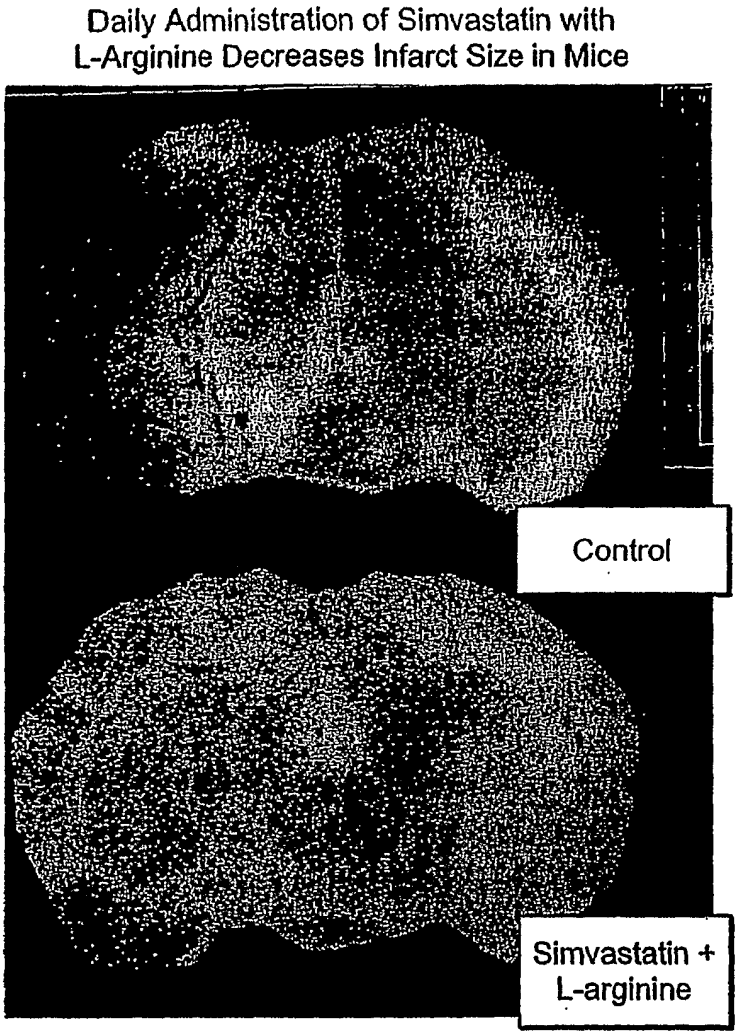

Daily Administration of Simvastatin with
L-Arginine Decreases Infarct Size in Mice
Control
Simvastatin +
L-arginine

FIG. 2

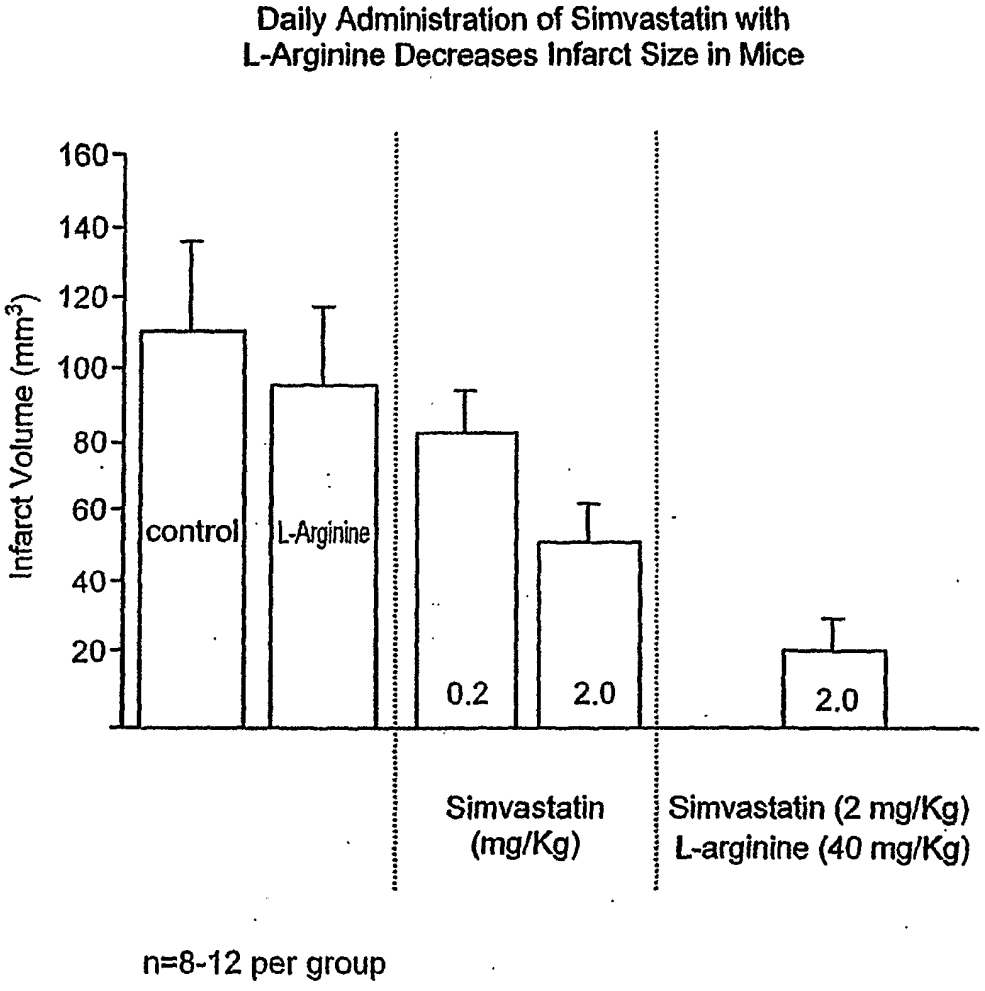

Daily Administration of Simvastatin with
L-Arginine Decreases Infarct Size in Mice
Infarct Volume (mm3)
160
140
120
100
80
60
40
20
control
L-Arginine
0.2
2.0
2.0
Simvastatin
(mg/Kg)
Simvastatin (2 mg/Kg)
L-arginine (40 mg/Kg)
n=8-12 per group

FIG. 3

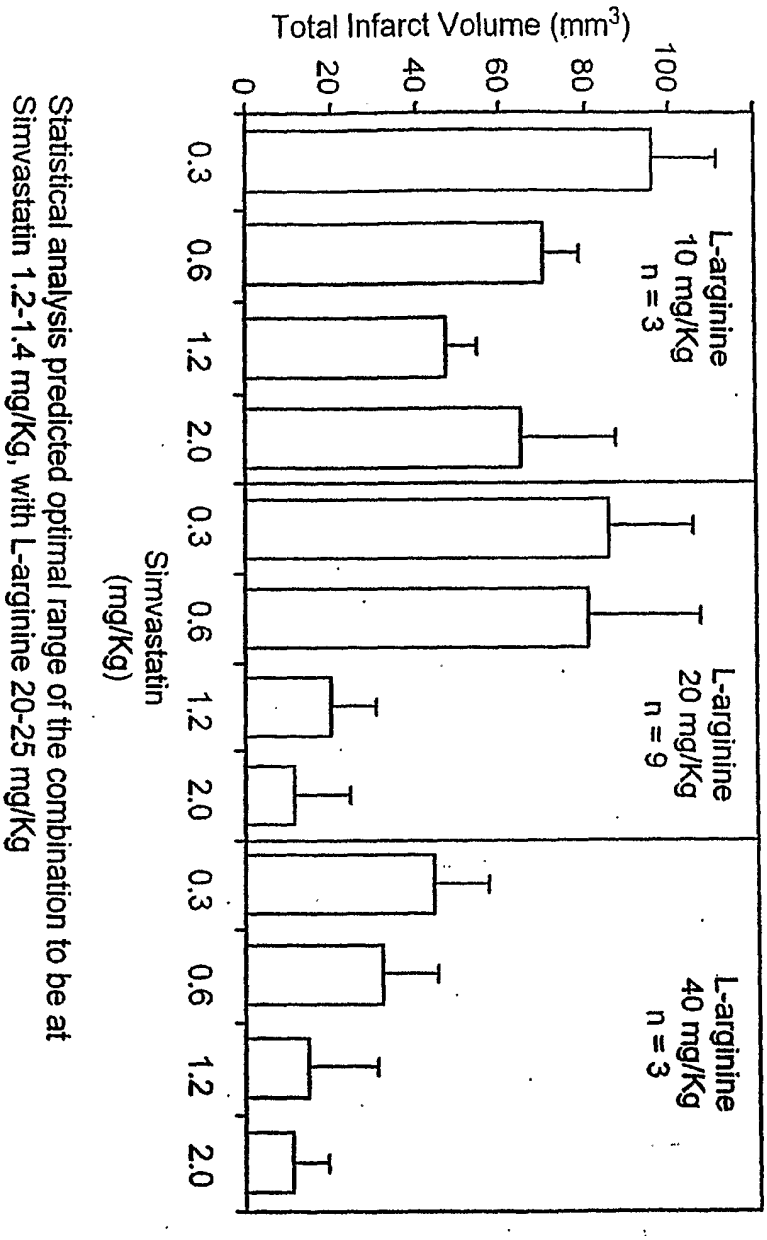


FIG. 4

Statistical analysis predicted optimal range of the combination to be at Simvastatin 1.2-1.4 mg/Kg, with L-arginine 20-25 mg/Kg

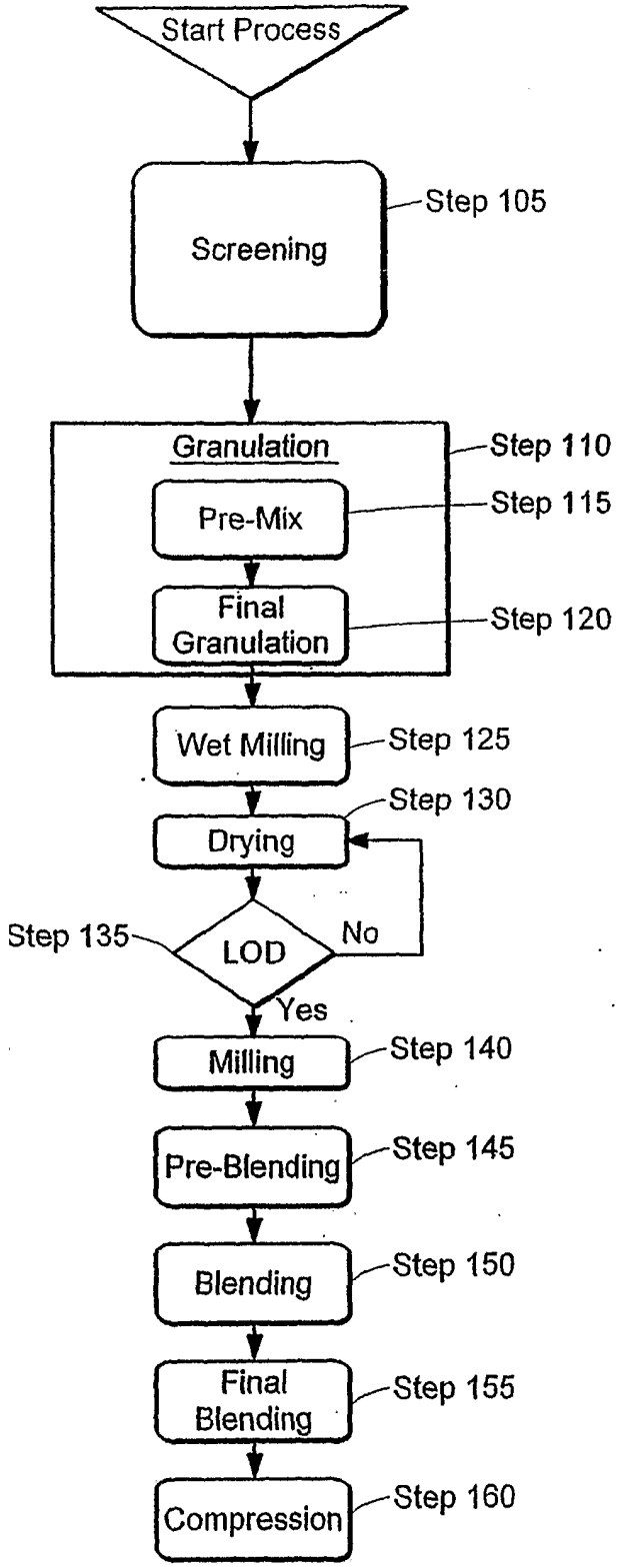
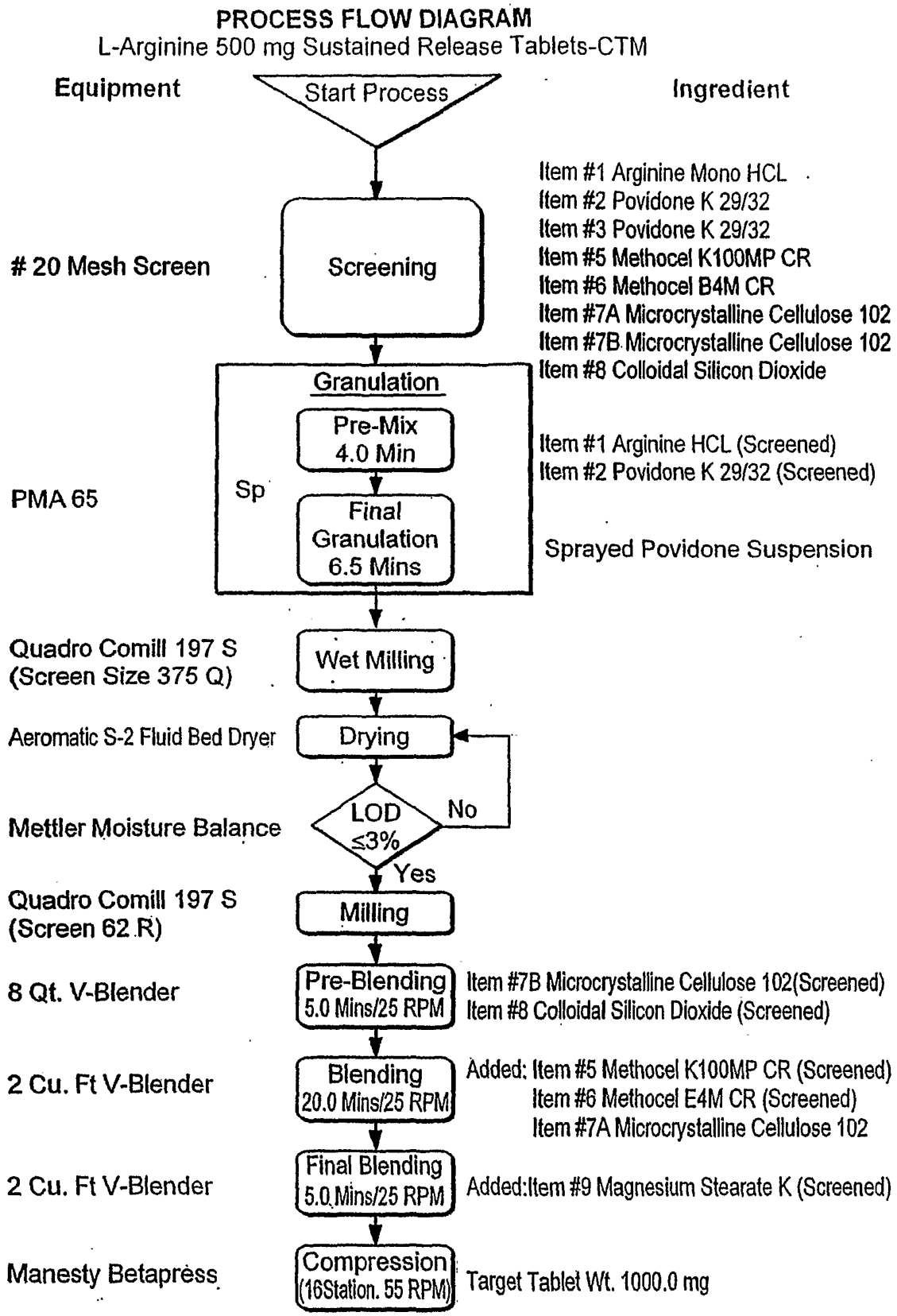

Start Process
Screening
Step 105
Granulation
Step 110
Pre-Mix
Step 115
Final Granulation
Step 120
Wet Milling
Step 125
Drying
Step 130
Step 135
LOD
No
Yes
Milling
Step 140
Pre-Blending
Step 145
Blending
Step 150
Final Blending
Step 155
Compression
Step 160

FIG. 5


PROCESS FLOW DIAGRAM
L-Arginine 500 mg Sustained Release Tablets-CTM
Equipment
Ingredient
Start Process
20 Mesh Screen
Screening
Item #1 Arginine Mono HCL
Item #2 Povidone K 29/32
Item #3 Povidone K 29/32
Item #5 Methocel K100MP CR
Item #6 Methocel B4M CR
Item #7A Microcrystalline Cellulose 102
Item #7B Microcrystalline Cellulose 102
Item #8 Colloidal Silicon Dioxide
Granulation
PMA 65
Sp
Pre-Mix 4.0 Min
Final Granulation 6.5 Mins
Item #1 Arginine HCL (Screened)
Item #2 Povidone K 29/32 (Screened)
Sprayed Povidone Suspension
Quadro Comill 197 S (Screen Size 375 Q)
Wet Milling
Aeromatic S-2 Fluid Bed Dryer
Drying
Mettler Moisture Balance
LOD ≤3%
No
Yes
Quadro Comill 197 S (Screen 62 R)
Milling
8 Qt. V-Blender
Pre-Blending 5.0 Mins/25 RPM
Item #7B Microcrystalline Cellulose 102(Screened)
Item #8 Colloidal Silicon Dioxide (Screened)
2 Cu. Ft V-Blender
Blending 20.0 Mins/25 RPM
Added: Item #5 Methocel K100MP CR (Screened)
Item #6 Methocel E4M CR (Screened)
Item #7A Microcrystalline Cellulose 102
2 Cu. Ft V-Blender
Final Blending 5.0 Mins/25 RPM
Added:Item #9 Magnesium Stearate K (Screened)
Manesty Betapress
Compression (16Station. 55 RPM)
Target Tablet Wt. 1000.0 mg

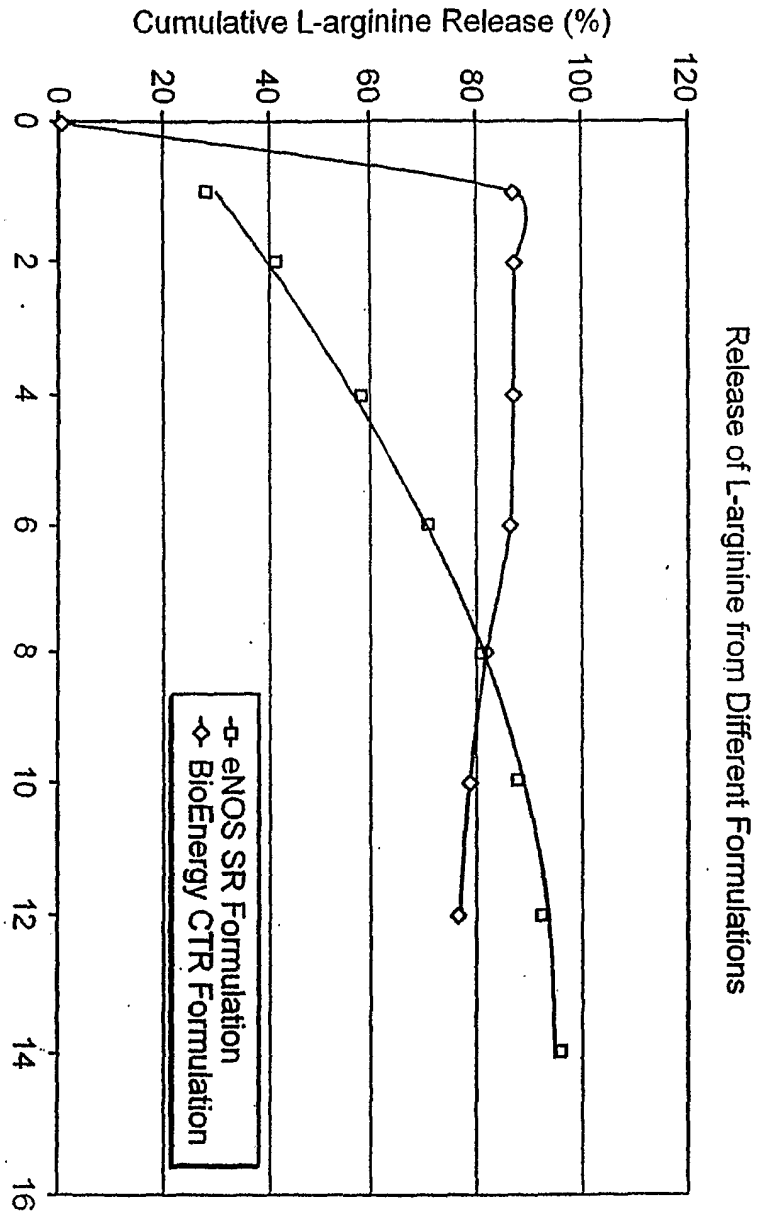

Release of L-arginine from Different Formulations
Cumulative L-arginine Release (%)
0
20
40
60
80
100
120
Time (hours)
0
2
4
6
8
10
12
14
16
eNOS SR Formulation
BioEnergy CTR Formulation

FIG. 7

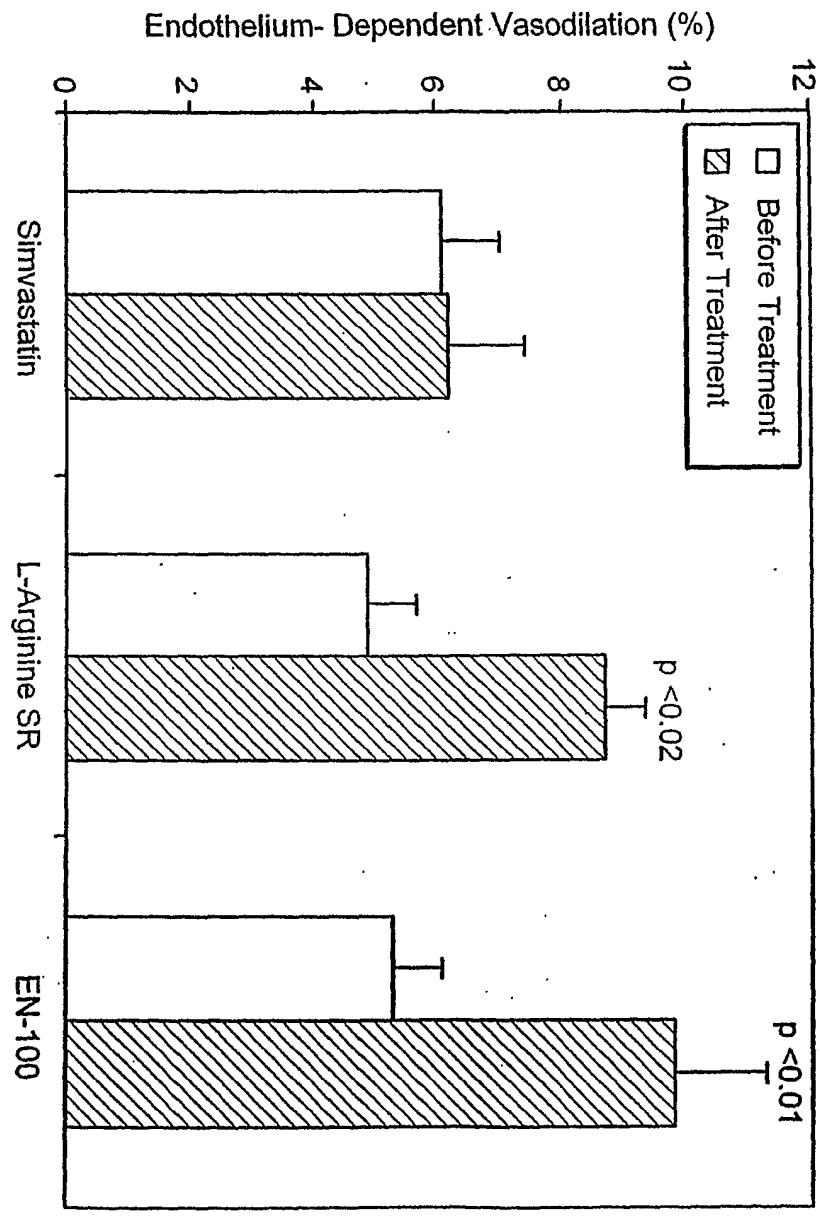

Endothelium- Dependent Vasodilation (%)
0
2
4
6
8
10
12
Before Treatment
After Treatment
Simvastatin
L-Arginine SR
EN-100
p <0.02
p <0.01
Treatments

FIG. 8

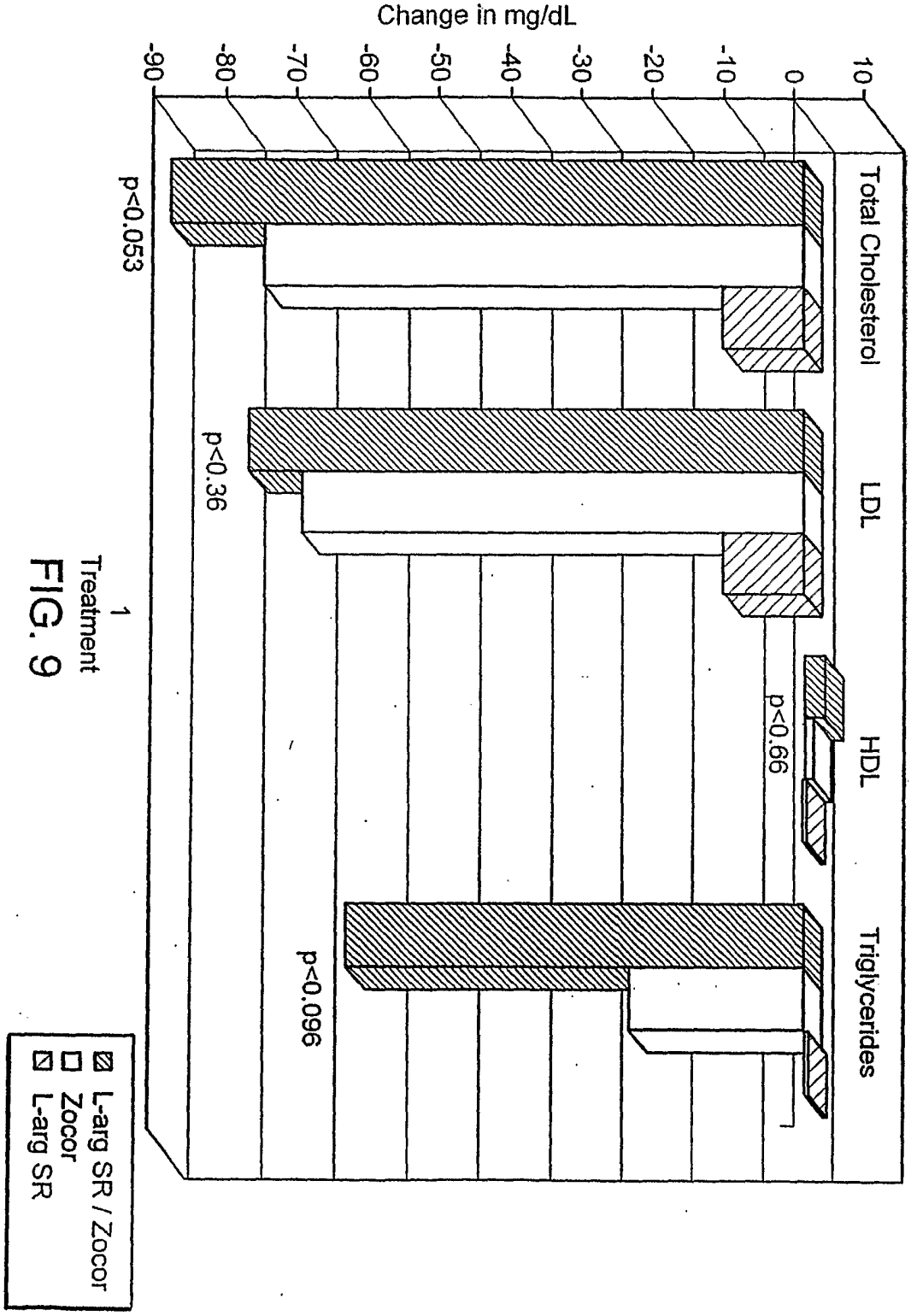

Change in mg/dL
-90
-80
-70
-60
-50
-40
-30
-20
-10
0
10
Total Cholesterol
LDL
HDL
Triglycerides
p<0.053
p<0.36
p<0.66
p<0.096
1
Treatment
L-arg SR / Zocor
Zocor
L-arg SR

FIG. 9

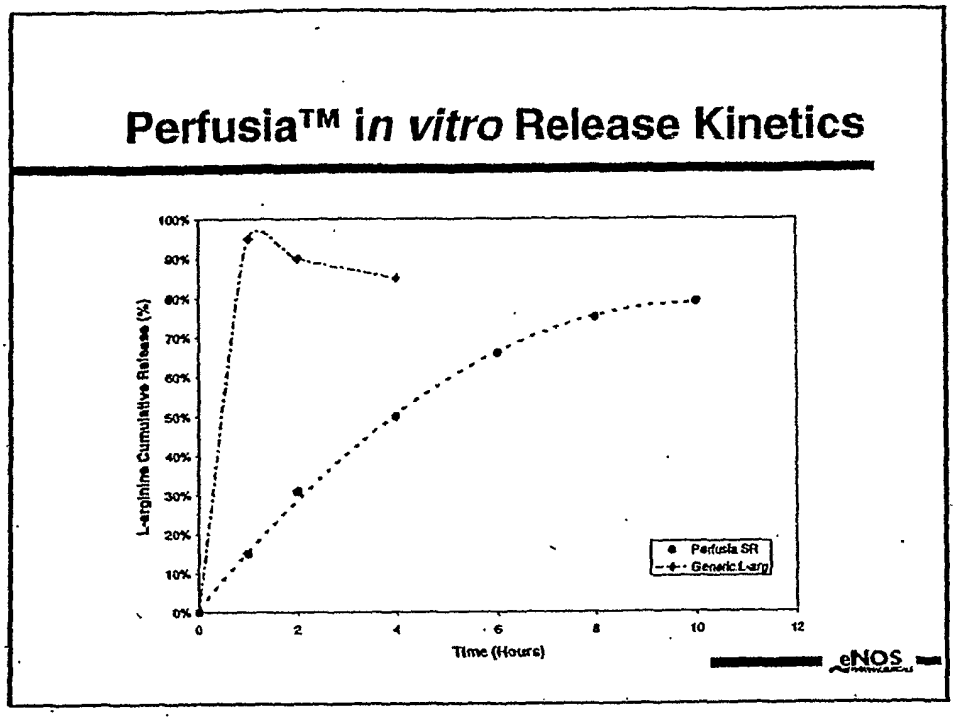

Perfusia™ in vitro Release Kinetics
L-arginine Cumulative Release (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
10
12
Time (Hours)
Perfusia SR
Generic L-arg
eNOS

FIGURE 10

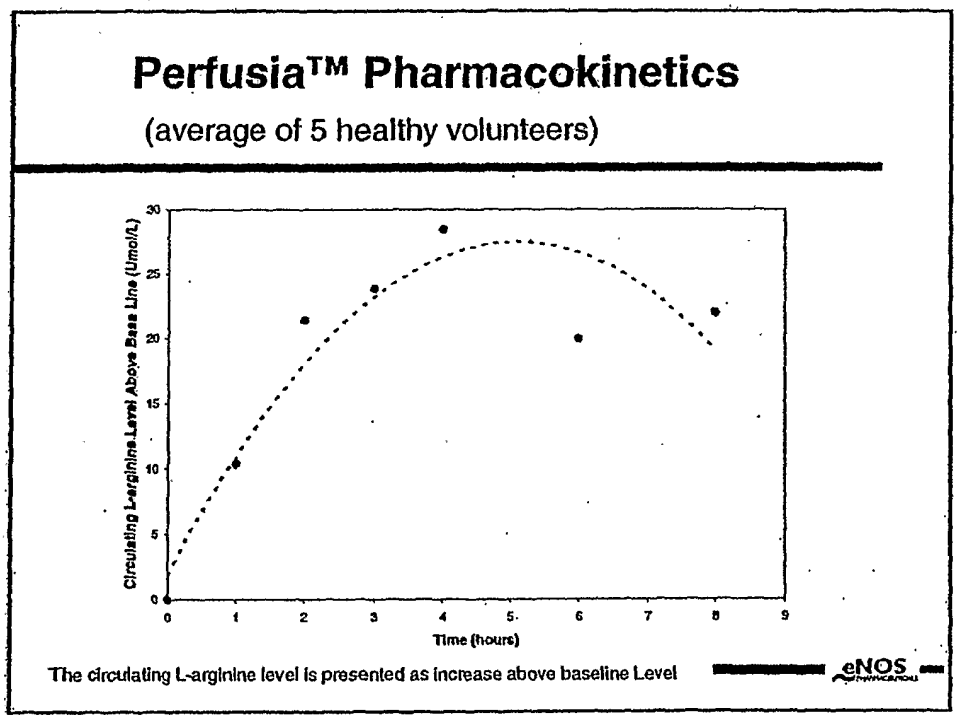

Perfusia™ Pharmacokinetics
(average of 5 healthy volunteers)
Circulating L-arginine Level Above Base Line (Umol/L)
0
5
10
15
20
25
30
0
1
2
3
4
5
6
7
8
9
Time (hours)
The circulating L-arginine level is presented as increase above baseline Level
eNOS

FIGURE 11

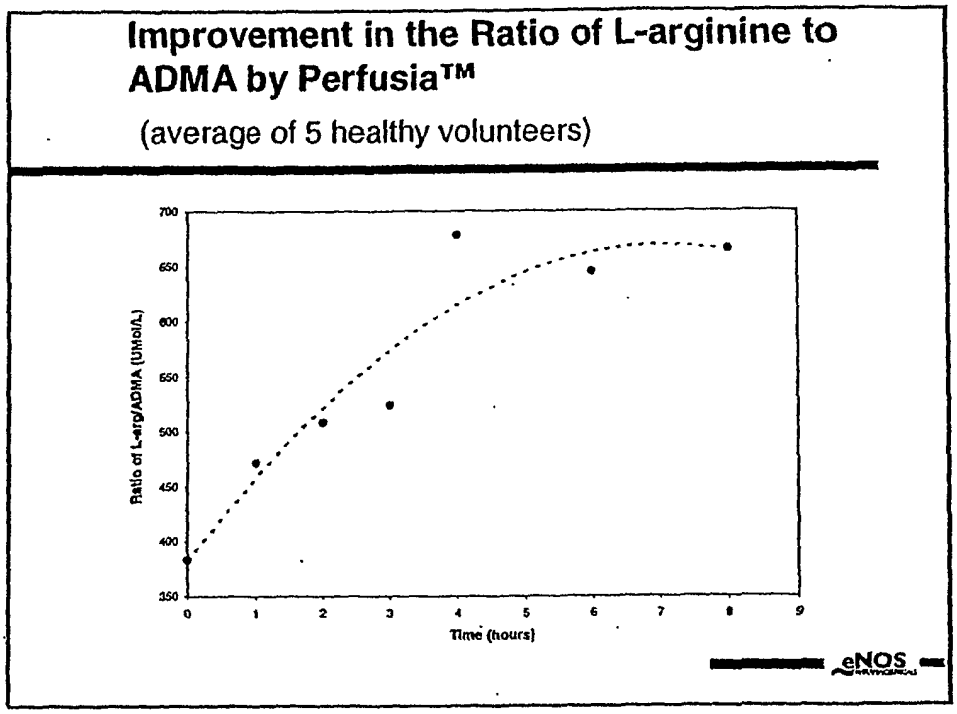

Improvement in the Ratio of L-arginine to ADMA by Perfusia™
(average of 5 healthy volunteers)
Ratio of L-arg/ADMA (Umol/L)
700
650
600
550
500
450
400
350
0
1
2
3
4
5
6
7
8
9
Time (hours)
eNOS

FIGURE 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US06/02127

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **A61K 47/32**(2006.01),**9/52**(2006.01),**9/22**(2006.01)

USPC: 514/772.4;424/457,468
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)
U.S. : 514/772.4; 424/457, 468

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,217,997 A (LEVERE et al) 08 June 1993 (08.06.1993), abstract; col. 6, lines 15-61; col. 5.	1-5 and 14-22 --- 6-13, 23 and 24
X --- Y	US 6,174,548 B1 (CHEN et al) 16 January 2001 (16.01.2001), abstract; col. 2, lines 33-39, 41-44, 62.	1-5 and 14-22 --- 6-13, 23 and 24
A	US 4,920,098 A (COTTER et al) 24 April 1990 (24.04.1990), see he whole document.	1-24
A	US 6,365,184 B1 (DEPUI et al) 02 April 2002 (02.04.2002), see the whole document.	1-24
A	US 6,531,507 B1 (PFLAUM et al) 11 March 2003 (11.03.2003), see the whole document.	1-24

☐ Further documents are listed in the continuation of Box C. ☐ 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 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)

"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 the actual completion of the international search
10 May 2006 (10.05.2006)

Date of mailing of the international search report
26 MAY 2006

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