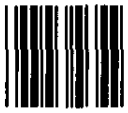

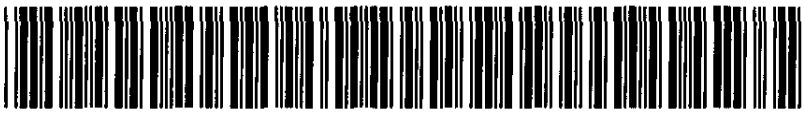

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3:02-CV-00129 UNITHER PHARMA INC V. REAL HEALTH

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ATTORNEYS FOR PLAINTIFFS UNITHER PHARMA, INC., THE BOARD OF TRUSTEES OF LELAND STANFORD JUNIOR UNIVERSITY, AND NEW YORK MEDICAL COLLEGE

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

UNITHER PHARMA, INC., THE BOARD OF TRUSTEES OF LELAND STANFORD JUNIOR UNIVERSITY, and NEW YORK MEDICAL COLLEGE Plaintiffs, v. REAL HEALTH LABORATORIES, INC., and JOHN F. DULLEA, Defendants.	Case No. '02 CV 0129 H (POR) **COMPLAINT FOR PATENT INFRINGEMENT AND DEMAND FOR JURY TRIAL**

Plaintiffs Unither Pharma, Inc., The Board of Trustees of Leland Stanford Junior University, and New York Medical College hereby allege as follows:

THE PARTIES

1. Unither Pharma, Inc. ("Unither Pharma") is incorporated under the laws of the State of California, and has a regular and established place of business at 1404 Old Country Road, Belmont, California 94002. Unither Pharma is the successor in interest of Cooke Pharma, Inc. ("Cooke Pharma"), which was incorporated under the laws of the State of California and located at the same address.

ORIGINAL

2. The Board of Trustees of Leland Stanford Junior University ("Stanford") is a body having corporate powers under the laws of the State of California with a location in Stanford, California.

3. New York Medical College ("NYMC") is a body having corporate powers under the laws of the State of New York with a location in Valhalla, New York.

4. Upon information and belief, Real Health Laboratories, Inc. ("Real Health") is a corporation incorporated under the laws of the State of California, and has a regular and established place of business at 1424 30th Street #B1, San Diego, California 92154.

5. Upon information and belief, John Dullea is presently, and has been at all times since 1999, the Chief Executive Officer of Real Health and has his office at 1424 30th Street #B1, San Diego, California 92154.

JURISDICTION AND VENUE

6. This Court has subject matter jurisdiction of this action under 28 U.S.C. § 1338(a) in which the district courts have original and exclusive jurisdiction of any civil action for patent infringement.

7. This Court has personal jurisdiction over defendants because defendant Real Health is located in and is doing business in this judicial district and defendant John Dullea works at Real Health in this judicial district.

8. Venue is proper in this Court under 28 U.S.C. §§ 1391 and 1400 because defendants reside in this judicial district.

AVERMENT OF FOUNDATION FACTS

9. Stanford is the assignee of named inventors of certain patents relating to the use of L-arginine in treating vascular disorders and improving vascular function and physical performance. These patents include U.S. Patent No. 6,117,872 issued September 12, 2000 ("the '872 patent" attached as Exhibit A hereto).

10. NYMC is the assignee of named inventors of certain patents relating, inter alia, to the use of L-arginine in treating high vascular resistance disorders. These patents include U.S. Patent No. 5,217,997 issued June 8, 1993 ("the '997 patent" attached as Exhibit B hereto).

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11. Stanford and Cooke Pharma entered into an agreement under which Stanford granted Cooke Pharma an exclusive license under the '872 patent in the manufacturing, use and sale of amino acid based dietary supplements, health supplements, other food products, and/or pharmaceuticals to enhance aerobic capacity by modulating the level of endogenous nitric oxide in the vascular system, excluding gene therapy. Under the same agreement, Stanford provided Cooke Pharma with a right to institute actions and recover damages for infringement of the '872 patent and the right to join Stanford as a plaintiff in any such action. Unither Pharma is the successor in interest to all rights granted by Stanford to Cooke Pharma under that agreement.

12. NYMC and Cooke Pharma entered into an agreement under which NYMC granted Cooke Pharma an exclusive worldwide license under the '997 patent in the manufacturing, use and sale of amino acid based functional foods, dietary supplements, medical foods or pharmaceuticals. Under the same agreement, NYMC provided Cooke Pharma with a right to institute actions and recover damages for infringement of the '997 patent and the right to join NYMC as a plaintiff in any such action. Unither Pharma is the successor in interest to all rights granted by NYMC to Cooke Pharma under that agreement.

13. Upon information and belief, the methods claimed in the '997 and '872 patents include treatment of human erectile dysfunction caused by high vascular resistance in the vasculature of the sex organs and enhancement of blood flow to improve physical performance, including physical performance of the sex organs.

14. Unither Pharma manufactures and sells products containing L-arginine designed to improve vascular health and to improve human sexual performance and treat erectile dysfunction covered by the '997 and '872 patents.

15. Upon information and belief, Defendant Real Health competes with Unither Pharma and sells at least six products, The CardioFitness Formula, The VasoRect Formula, The VasoRect SE Formula (recently renamed The VasoRect Plus Formula), The VasoRect Ultra Formula and The Sexual Health Formula that contain L-arginine as the primary active ingredient.

16. Defendant Real Health advertises, inter alia, that its L-Arginine-based products improve vascular health and support healthy erectile functioning and blood pressure.

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17. On information and belief, defendant John Dullea owns a significant fraction of the capital stock of Defendant Real Health .

18. Upon information and belief, Defendant John Dullea, in his capacity as Chief Executive Officer and as a major stockholder has substantial control over the actions of Defendant Real Health.

COUNT 1 - INFRINGEMENT OF THE '997 PATENT

19. Each allegation contained in paragraphs 1-18 above is incorporated herein by reference.

20. Defendant Real Health is currently and has been inducing and/or contributing to the infringement of the claims of the '997 patent by making, using, selling, offering to sell and/or importing its products containing L-arginine without the consent of Unither Pharma.

21. Defendant Real Health will continue to induce and/or contribute to the infringement of the claims of the '997 patent unless enjoined by this court.

22. Defendant Real Health has had actual notice that the use of its products are covered by the claims of the '997 patent since at least January 20, 2000 (Letter to Real Health attached hereto as Exhibit C).

23. Defendant Real Health has willfully and deliberately induced and/or contributed to the infringement of the claims of the '997 patent with full knowledge and wanton disregard of Plaintiffs' rights thereunder, rendering this an "exceptional" case within the meaning of 35 U.S.C. § 285.

COUNT 2 - INFRINGEMENT OF THE '872 PATENT

24. Each allegation contained in paragraphs 1-18 above is incorporated herein by reference.

25. Defendant Real Health is currently and has been inducing and/or contributing to the infringement of the claims of the '872 patent by making, using, selling, offering to sell and/or importing its products containing L-arginine without the consent of Unither Pharma.

26. Defendant Real Health will continue to induce and/or contribute to the infringement of the claims of the '872 patent unless enjoined by this court.

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COUNT 3 – INDUCEMENT OF INFRINGEMENT OF THE '997 PATENT

27. Each allegation contained in paragraphs 1-18 above is incorporated herein by reference.

28. Defendant John Dullea, with knowledge of the claims of the '997 patent, is currently and has been inducing Defendant Real Health to infringe and/or induce and/or contribute to the infringement of the claims of the '997 patent by actively and knowingly causing Defendant Real Health to make, use, sell, offer to sell and/or import products containing L-arginine without the consent of Unither Pharma.

29. Defendant John Dullea will continue to induce Real Health or others to infringe and/or induce or contribute to the infringement of the claims of the '997 patent unless enjoined by this court.

30. Defendant John Dullea has had actual notice that the use of Real Health products are covered by the claims of the '997 patent since at least January 20, 2000 (Letter to Real Health attached hereto as Exhibit C).

31. Defendant John Dullea has willfully and deliberately induced Defendant Real Health to induce and/or contribute to the infringement of the claims of the '997 patent with full knowledge and wanton disregard of Plaintiffs' rights thereunder, rendering this an "exceptional" case within the meaning of 35 U.S.C. § 285.

COUNT 4 – INDUCEMENT OF INFRINGEMENT OF THE '872 PATENT

32. Each allegation contained in paragraphs 1-18 above is incorporated herein by reference.

33. Defendant John Dullea, with knowledge of the claims of the '872 patent, is currently and has been inducing Defendant Real Health to infringe and/or induce and/or contribute to the infringement of the claims of the '872 patent by actively and knowingly causing Defendant Real Health to make, use, sell, offer to sell and/or import products containing L-arginine without the consent of Unither Pharma.

34. Defendant John Dullea will continue to induce Real Health or others to infringe and/or induce or contribute to the infringement of the claims of the '872 patent unless enjoined

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by this court.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs request the following relief:

a. That this Court adjudge and declare that defendants Real Health and John Dullea have induced and/or contributed to the infringement of the '997 patent;

b. That this Court preliminarily enjoin defendant Real Health, and its officers, directors, employees, agents, licensees, servants, successors and assigns, and any and all persons acting in privity or in concert with them, and John Dullea from infringing, actively inducing infringement or contributing to infringement of the '997 patent;

c. That this Court permanently enjoin defendant Real Health, and its officers, directors, employees, agents, licensees, servants, successors and assigns, and any and all persons acting in privity or in concert with them, and John Dullea from infringing, actively inducing infringement or contributing to infringement of the '997 patent;

d. That this Court award to plaintiffs damages adequate to compensate them for defendants Real Health's and John Dullea's acts of inducement of infringement of the '997 patent complained of herein, together with interest thereon;

e. That this Court award treble damages against defendants Real Health and John Dullea for their willful inducement of infringement of the '997 patent;

f. That this Court adjudge and declare that defendants Real Health and John Dullea have induced and/or contributed to the infringement of the '872 patent;

g. That this Court preliminarily enjoin defendant Real Health, and its officers, directors, employees, agents, licensees, servants, successors and assigns, and any and all persons acting in privity or in concert with them, and John Dullea from infringing, actively inducing infringement or contributing to infringement of the '872 patent;

h. That this Court permanently enjoin defendant Real Health, and its officers, directors, employees, agents, licensees, servants, successors and assigns, and any and all persons acting in privity or in concert with them, and John Dullea from infringing, actively inducing infringement or contributing to infringement of the '872 patent;

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i. That this Court award to plaintiffs damages adequate to compensate them for defendants Real Health's, and John Dullea's acts of inducement of infringement of the '872 patent complained of herein, together with interest thereon;

j. That this Court order defendants Real Health and John Dullea to pay plaintiffs their reasonable attorneys' fees of this action;

k. That this Court order defendants Real Health and John Dullea to pay plaintiffs any and all costs of this action; and,

l. That this Court grant to plaintiffs such other and further relief as it may deem just and equitable.

Date: January 18, 2002

Foley & Lardner

By: _____

Kenneth S. Klein
Attorneys For Plaintiffs Unither Pharma, Stanford, and NYMC

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TABLE OF CONTENTS FOR EXHIBITS

EXHIBIT A

US005217997A

United States Patent [19]

Levere et al.

[11] Patent Number: 5,217,997

[45] Date of Patent: Jun. 8, 1993

[54] **USE OF L-ARGININE IN THE TREATMENT OF HYPERTENSION AND OTHER VASCULAR DISORDERS**

[76] Inventors: **Richard D. Levere**, 5 Seymour Pl. W., Armonk, N.Y. 10504; **Nader G. Abraham**, 143 Charter Cir., Ossining, N.Y. 10562; **Michel L. Schwartzman**, 415 Old Country Rd., Elmsford, N.Y. 10523; **Pavel Martasek**, 60 Hillcrest Rd., Hartsdale, N.Y. 10530

[21] Appl. No.: **873,892**

[22] Filed: **Apr. 24, 1992**

Related U.S. Application Data

[63] Continuation of Ser. No. 513,895, Apr. 24, 1990, abandoned, which is a continuation-in-part of Ser. No. 462,638, Jan. 9, 1990, abandoned.

[51] **Int. Cl.**[5] **A61K 31/195**

[52] **U.S. Cl.** **514/565**

[58] **Field of Search** 514/565

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,542,929 11/1970 Roberts 514/565

OTHER PUBLICATIONS

Lais, et al., "Mechanism of Vascular Hyperresponsiveness in the Spontaneously Hypertensive Rat," *Cir. Res.*, 36/37: Suppl. 1, I-216, to I-222 (1975).

Pinto, et al., "Arachidonic Acid-Induced Endothelial-Dependent Relaxations of Canine Coronary Arteries: Contribution of a Cytochrome P-450-Dependent Pathway," *J. Pharmacol. Exp. Therap.*, vol. 240, No. 3, 856-863 (1987).

Luscher, et al., "Endothelium-Dependent Responses in Cartoid and Renal Arteries of Normotensive and Hypertensive Rats," *Hypertension*, vol. 11, No. 6, Part 2, 573-578 (1988).

Sacerdoti, et al., "Treatment with Tin Prevents the Development of Hypertension in Spontaneously Hypertensive Rats," *Science*, vol. 243, 388-390 (1989).

Martasek, et al., "Heme Arginate Lowers Blood Pressure in Spontaneous Hypertensive Rats," *Clin. Res.*, vol. 37, 553A (1989).

Merrick, et al., "Alternations in Hepatic Microsomal Drug Metabolism and Cytochrome P450 Proteins in Spontaneously Hypertensive Rats," *Pharmacol.* 30, 129-135 (1985).

Sacerdoti, et al., "Renal Cytochrome P450-Dependent Metabolism of Arachidonic Acid in Spontaneously Hypertensive Rats," *Biochem. Pharmacol.*, vol. 37, No. 3, 521-527 (1988).

Kappas, et al., "Control of Heme Metabolism with Synthetic Metalloprophyrins," *J. Clin. Invest.*, vol. 77, 335-339 (1986).

Simionatto, et al., "Studies of the Mechanism of Sn-Protoporphyrin Suppression and Hyperbilirubinemia: Inhibition of Heme Oxidation and Bilirubin Production," *J. Clin. Invest.*, vol. 75, 513-521 (1985).

Escalante, et al., "19(S)Hydroxyeicosatetraenoic Acid is a Potent Stimulator of Renal Na+-K+-ATPase," *Biochem. & Biophys. Res. Commun.*, vol. 152, No. 3, 1269-1273 (1988).

(List continued on next page.)

Primary Examiner—Allen J. Robinson

Attorney, Agent, or Firm—Burns, Doane, Swecker & Mathis

[57] **ABSTRACT**

A method for treating a high vascular resistance disorder in a mammal by administering to a mammalian organism in need of such treatment a sufficient amount of L-arginine or pharmaceutically acceptable salt thereof to treat a high vascular resistance disorder. The L-arginine is typically administered in the range of about 1 mg to 1500 mg per day. High vascular resistance disorders include hypertension, primary or secondary vasospasm, angina pectoris, cerebral ischemia and preeclampsia. Also disclosed is a method for preventing or treating bronchial asthma in a mammal by administering to a mammalian organism in need of such prevention or treatment a sufficient amount of L-arginine to prevent or treat bronchial asthma.

19 Claims, 3 Drawing Sheets

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OTHER PUBLICATIONS

Escalante, et al, "Vasoactivity of 20-Hydroxyeicosatetraenoic Acid is Dependent on Metabolism by Cyclooxygenase," *J. Pharmacol. Exp. Therap.*, vol. 248, No. 1, 229-232 (1989).

Furchgott, et al., "The Role of Endothelium in the Responses of Vascular Smooth Muscle to Drugs," *Ann. Rev. Pharmacol. Toxicol.*, 24, 175-197 (1984).

Palmer, et al., "Vascular Endothelial Cells Synthesize Nitric Oxide from L-Arginine," *Nature*, vol, 333, 664-666 (1988).

Ignarro, et al., "Endothelium-Derived Nitric Oxide: Actions and Properties," *FASEB J.*, vol. 3, 31-36 (1989).

Kordac, et al., "Changes of Myocardial Functions in Acute Hepatic Porphyrias, Role of Heme Arginate Administration," *Ann. Med.*, 21, 273-276 (1989).

Rees, et al., "Role of Endothelium-derived Nitric Oxide in the Regulation of Blood Pressure," *Proc. Natl. Acad. Sci. USA*, vol. 86, 3375-3378 (1989).

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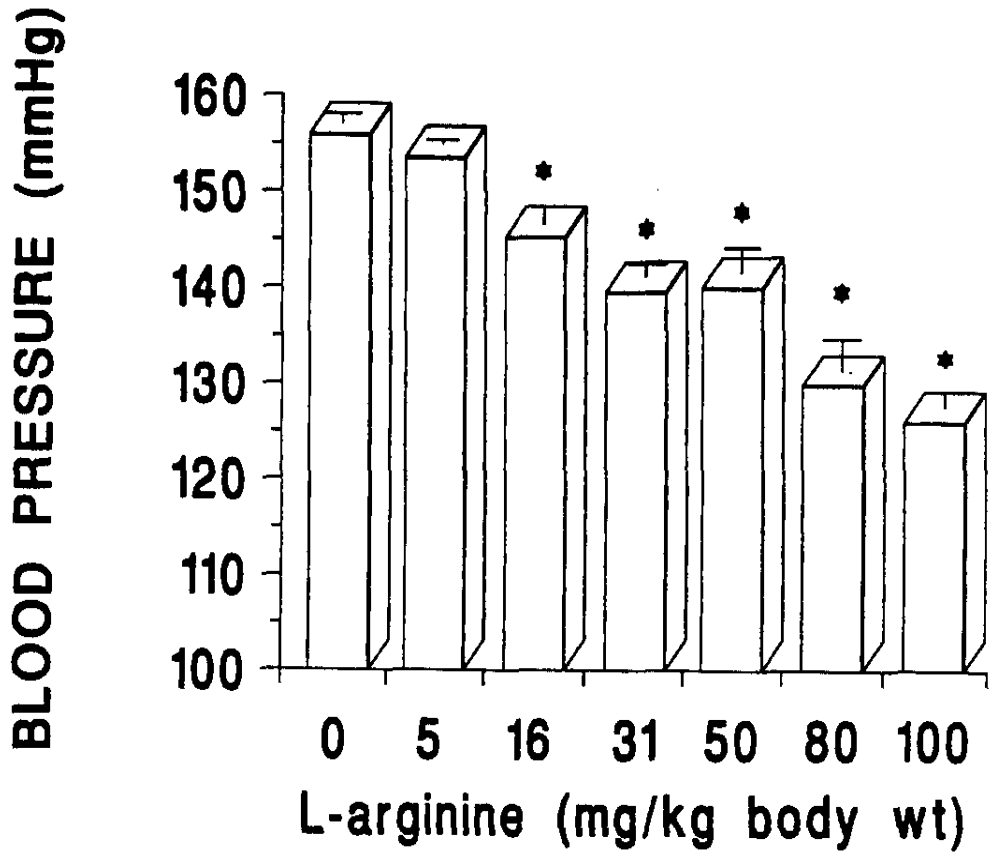

Fig. 1

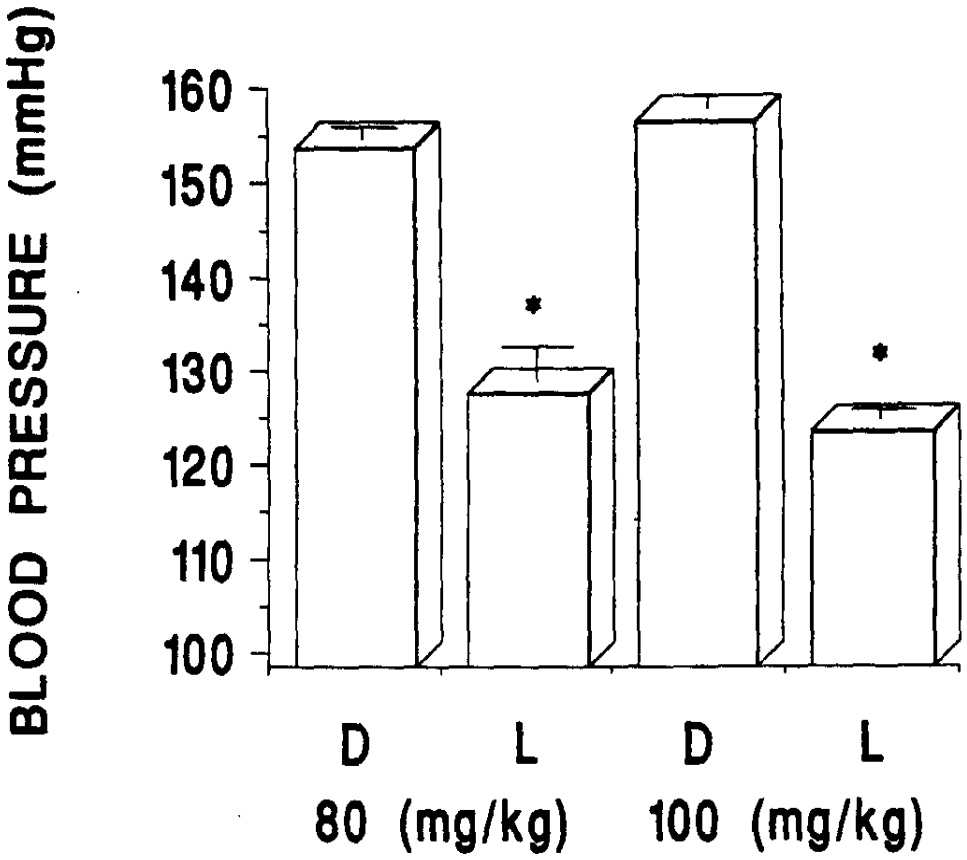

Fig. 2

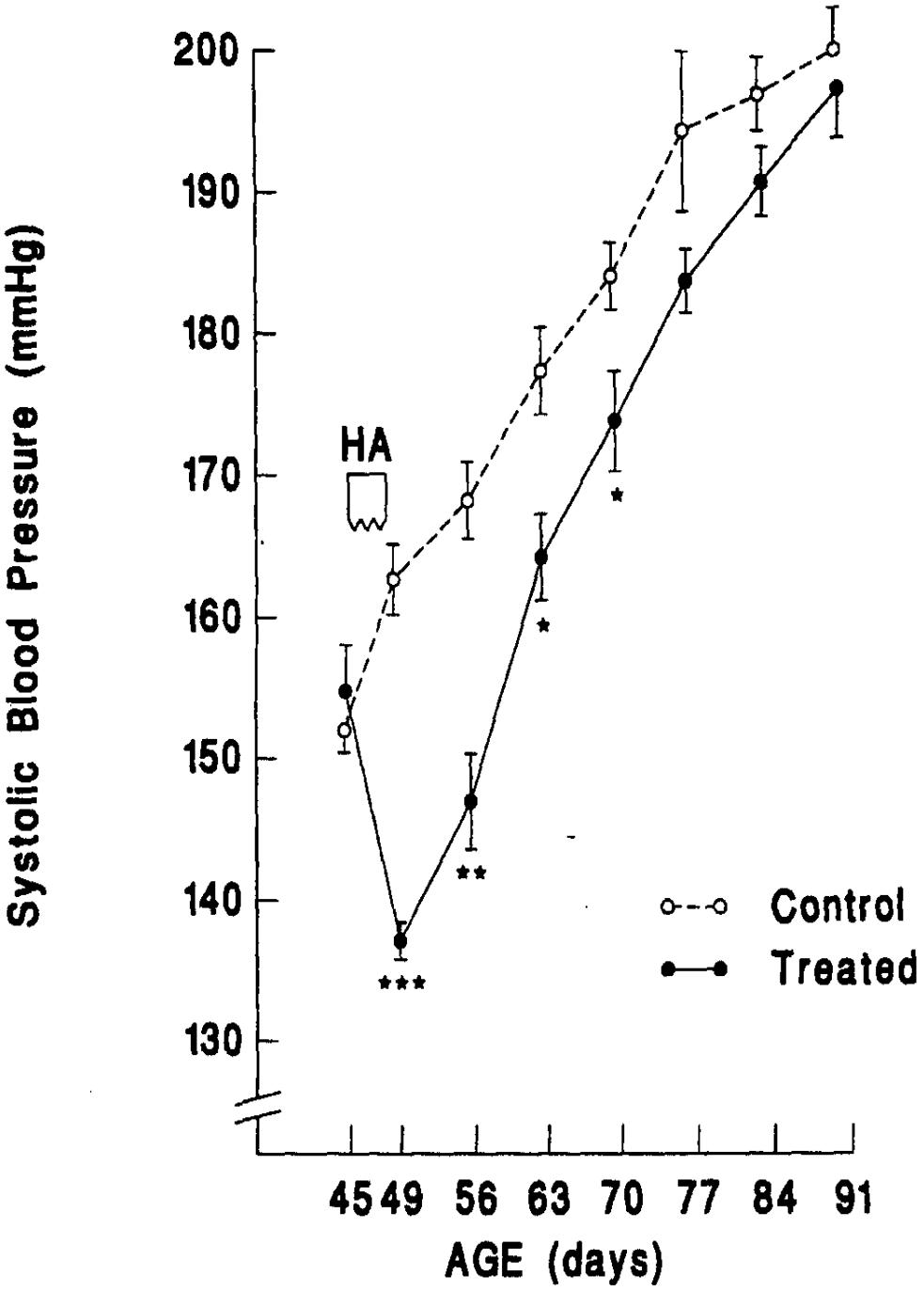

Fig. 3

USE OF L-ARGININE IN THE TREATMENT OF HYPERTENSION AND OTHER VASCULAR DISORDERS

This application is a continuation of application Ser. No. 07/513,895, filed Apr. 24, 1990 now abandoned, which is a continuation-in-part of U.S. Ser. No. 07/462,638, filed Jan. 9, 1990, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a method of using L-arginine or pharmaceutically acceptable salts thereof in the treatment of hypertension and other high vascular resistance disorders. High vascular resistance disorders include primary or secondary vasospasm, angina pectoris, cerebral ischemia and preeclampsia of pregnancy. The present invention is also concerned with a method of preventing or treating bronchial asthma using L-arginine or a pharmaceutically acceptable salt thereof.

2. Description of the Prior Art

The disposition of hypertensive patients to develop vascular disease which leads to increased mortality from stroke and myocardial infarction is a major problem in the western world. The development of hypertension can relate to abnormalities in the production or activities of vasoactive substances. Alteration in the responsiveness to both vasoconstrictors and vasodilators has been well documented in spontaneous hypertensive rats, the most frequently used animal model for the study of human essential hypertension. Thus, an increase in the contractile response of vascular smooth muscle of spontaneous hypertensive rats to vasoconstrictors such as norepinephrine as noted by Lais et al, "Mechanism of Vascular Hyperresponsiveness in the Spontaneously Hypertensive Rat", Cir. Res., 36/37: Suppl. 1, I-216 to I-222 (1975), and a decrease in the relaxant response to vasodilators such as acetylcholine, nitrovasodilators, prostaglandin and other arachidonic acid metabolites as noted by Pinto et al, "Arachidonic Acid-Induced Endothelial-Dependent Relaxations of Canine Coronary Arteries: Contribution of a Cytochrome P-450-Dependent Pathway", *J Pharmacol. Exp. Therap.*, 240, 856–863 (1987) and Luscher et al, "Endothelium-Dependent Responses in Carotid and Renal Arteries of Normotensive and Hypertensive Rats", *Hypertension*, 11, 573–578 (1988), may contribute overall to the development of high blood pressure.

It has also been demonstrated that cytochrome P-450-arachidonate metabolism is increased in the kidney of young spontaneous hypertensive rats and a selective reduction in the formation of these metabolites via induction of heme degradation with $SnCl_2$ caused a marked decrease in blood pressure as noted by Sacerdoti et al, "Treatment with Tin Prevents the Development of Hypertension in Spontaneously Hypertensive Rats", *Science*, 243, 388–390 (1989). Martasek et al, "Heme Arginate Lowers Blood Pressure in Spontaneous Hypertensive Rats", *Clin. Res.*, 37, 553A (1989) also noted that other heme oxygenase inducers such as heme arginate have been demonstrated to be an inducer of heme oxygenase causing reduction of renal P-450 and a decrease in blood pressure in young spontaneous hypertensive rats. The blood pressure lowering effect of heme arginate could be attributed to the heme component. The heme effect may be due to an induction of heme oxygenase, since it is blocked by an inhibitor of heme oxygenase.

The relaxation of vascular smooth muscle in response to many substances is typically endothelium-dependent and mediated by endothelium-derived relaxing factors as noted by Furchgott et al, "The Role of Endothelium in the Responses of Vascular Smooth Muscle to Drugs", *Ann. Rev. Pharmacol. Toxicol.*, 24, 175–197 (1984). One of the endothelium-derived relaxing factors has been recently identified as nitric oxide by Palmer et al, "Vascular Endothelial Cells Synthesize Nitric Oxide from L-arginine", *Nature*, 333, 664–666 (1988); and Ignarro et al, "Endothelium-Derived Nitric Oxide: Actions and Properties", *FASEB J.*, 3, 31–36 (1989). Nitric oxide elicits vasodilation by increasing the formation of c-GMP following direct interaction with the heme component of soluble guanylate cyclase (Ignarro et al, supra).

An increase in hepatic and renal cytochrome P-450 content and its related drug metabolizing enzyme systems has been demonstrated in spontaneous hypertensive rats as noted by Merrick et al, "Alterations in Hepatic Microsomal Drug Metabolism and Cytochrome P450 Proteins in Spontaneously Hypertensive Rats", *Pharmacol.*, 30, 129–135 (1985) and Sacerdoti et al, "Renal Cytochrome P450-Dependent Metabolism of Arachidonic Acid in Spontaneously Hypertensive Rats", Biochem. Pharmacol., 37, 521–527 (1988). More recently Sacerdoti et al demonstrated that abnormalities of renal function in young spontaneous hypertensive rats may be a functional expression of an alteration in renal cytochrome P-450-dependent metabolism of arachidonic acid. Cytochrome P-450 levels are regulated by the availability of cellular heme which in turn is controlled by the levels of heme oxygenase which is the controlling enzyme in the metabolism of heme to bilirubin. Induction of heme oxygenase by heavy metals such as $SnCl_2$ results in a depletion of renal cytochrome P-450 as described by Kappas et al, "Control of Heme Metabolism with Synthetic Metalloprophyrins", *J. Clin. Invest.*, 77, 335–339 (1986) and Simionatto et al, "Studies on the Mechanism of Sn-Protoporphyrin Suppression and Hyperbilirubinemia: Inhibition of Heme Oxidation and Bilirubin Production", *J. Clin. Invest.*, 75, 513–521 (1985).

Furthermore, it has recently been demonstrated by Escalante et al, "19(S)Hydroxyeicosatetraenoic Acid is a Potent Stimulatior of Renal Na^+-K^+-ATPase", *Biochem. Biophys. Res. Commun.*, 152, 1269–1273 (1988) and Escalante et al, "Vasoactivity of 20-Hydroxyeicosatetraenoic Acid is Dependent on Metabolism by Cyclooxygenase", *J. Pharmacol. Exp. Therap.*, 248, 229–232 (1989) that arachidonic acid metabolites of cytochrome P-450 ω/ω-1 hydroxylases, 19(S)-HETE (hydroxyeicosatetragenoic acid) and 20-HETE is a potent renal Na^+-K^+-ATPase stimulator and 20-HETE is a vasoconstrictor.

An acute attack of acute intermittent porphyria, a disease caused by inborn errors of porphyrin metabolism, is a life threatening condition, often characterized by agonizing abdominal pain, paresis and frequently accompanied by hypertension. The exact pathogenesis of hypertension in an acute porphyric attack is not well understood. Currently, hemin in the form of heme arginate is used in Europe in the treatment of acute attacks of acute intermittent porphyria so as to normalize the levels of "free" heme and thereby decrease the induced levels of delta-aminolevulinic acid synthetase, an en-

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zyme under negative feedback control by unbound or "free" heme. Kordac et al, "Changes of Myocardial Functions in Acute Hepatic Porphyrias. Role of Heme Arginate Administration", *Ann. Med.*, 21, 273–276 (1989) disclosed the use of heme arginate in the treatment of acute hepatic porphyria. Heme arginate was administered to those patients because it was speculated that acute hypoxia occurs in a porphyrin crisis due to lack of heme. The arginate was used as a way to solubilize the heme for administration to the patient.

It has long been thought that the source of nitric oxide and other nitroso species in animal tissues is L-arginine. Indeed, recent studies have demonstrated that cultured endothelial cells transform L-arginine to the nitroso species, thus supporting the suggestion that L-arginine is a physiological precursor of endothelium-derived nitric acid. Ignarro et al, supra.

Rees et al, "Role of Endothelium-derived Nitric Oxide in the Regulation of Blood Pressure", *Proc. Natl. Acad. Sci. USA*, 86, 3375–3378 (1989) used N-monomethyl-L-arginine to investigate the role of nitric oxide in the regulation of blood pressure in the anesthetized rabbit. The authors concluded that N-monomethyl-L-arginine caused a dose-dependent increase in mean arterial blood pressure. It was also determined that the administration of L-arginine abolished the inhibition of N-monomethyl-L-arginine within fifteen minutes. Their findings further suggested that there is a continuous utilization of L-arginine for the enzymatic formation of nitric oxide by resistance arteries and provides the first evidence that nitric oxide formation contributes to the regulation of blood pressure. No studies were undertaken to test whether the administration of L-arginine could affect blood pressure and the authors specifically stated that they did not believe that L-arginine directly affected blood pressure.

All investigations into the action of nitric oxide and L-arginine ended there. There nevertheless remained a long-felt need in the art for a way of treating a variety of vascular diseases including hypertension. In addition, there remains a long-felt need in the art for a way to prevent or treat bronchial asthma which involves the narrowing of large and small airways due to spasm of bronchial smooth muscle.

SUMMARY OF THE INVENTION

Surprisingly, the present inventors now find that the levorotatory form of arginine is useful in the treatment of high vascular resistance disorders including hypertension, primary or secondary vasospasm, angina pectoris, cerebral ischemia and preeclampsia of pregnancy in a mammalian organism, such as a human.

In one aspect, the present invention thus provides a method for treating hypertension in a mammalian organism by administering to a mammalian organism in need of such treatment a sufficient amount of L-arginine or pharmaceutically acceptable salt thereof to treat hypertension.

In another aspect, the invention thus provides a method for treating vasospasm in a mammalian organism in need of such treatment a sufficient amount of L-arginine or pharmaceutically acceptable salt thereof to treat vasospasm.

In a further aspect, the present invention provides a method for treating angina pectoris in a mammalian organism by administering to a mammalian organism in need of such treatment a sufficient amount of L-arginine or pharmaceutically acceptable salt thereof to treat angina pectoris.

In yet another aspect, the present invention provides a method for treating cerebral ischemia in a mammalian organism by administering to a mammalian organism in need of such treatment a sufficient amount of L-arginine or pharmaceutically acceptable salt thereof to treat cerebral ischemia.

In an additional aspect, the present invention provides a method for treating preeclampsia in a mammalian organism by administering to a mammalian organism in need of such treatment a sufficient amount of L-arginine or pharmaceutically acceptable salt thereof to treat preeclampsia.

A further embodiment of the present invention involves a method for treating bronchial asthma in a mammalian organism by administering to a mammalian organism in need of such treatment a sufficient amount of L-arginine or pharmaceutically acceptable salt thereof to treat bronchial asthma.

Other objects and advantages of the present invention will be apparent from the following description.

BRIEF DESCRIPTION OF THE FIGURES

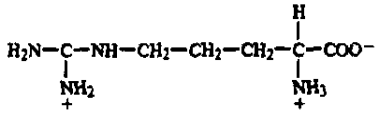
FIG. 1 is a bar chart showing that L-arginine caused a dose-dependent decrease in blood pressure of spontaneous hypotensive rats.

FIG. 2 is a bar chart showing that D-arginine at a dose of 80 and 100 mg/kg did not effect the blood pressure of spontaneous hypotensive rats.

FIG. 3 is a graph of the long-term effect of heme arginate on systolic blood pressure when 45-week-old spontaneous hypertensive rats were injected with heme arginate (15 mg/kg) for four consecutive days and the control spontaneous hypertensive rats were injected only with the vehicle.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Arginine, 2-amino-5-guanidinovaleric acid, is a basic amino acid with a positively charged guanidinium group. The IUPAC abbreviation is Arg. Arginine can be depicted as follows:



Arginine is considered to be a semi-essential amino acid. It can be synthesized in animal tissues at a rate sufficient for maintenance in the adult but not rapidly enough to support growth in the young animal. It is thus an essential amino acid for growth but not for maintenance.

In the mammalian body, arginine takes part in the formation of urea yielding ornithine. Arginine may be synthesized in the mammalian body from alpha-ketoglutaric acid, glutamic acid or proline.

L-arginine can be used in the treatment of a number of high vascular resistance disorders including hypertension, primary or secondary vasospasm, angina pectoris, cerebral ischemia and preeclampsia of pregnancy (toxemia). Each of those high vascular resistance disorders are well-known in the art.

Hypertension is characterized by persistently high arterial blood pressure. Various criteria for its threshold

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have been suggested ranging from 140 mm Hg systolic and 90 mm Hg diastolic to as high as 200 mm Hg systolic and 110 mm Hg diastolic. Hypertension may have no known cause (essential or idiopathic hypertension) or be associated with other primary diseases (secondary hypertension).

Vasospasm refers to a spasm of the blood vessels, resulting in a decrease in their caliber. Primary vasospasm can be described as a cold sensitivity of the Raynaud's type without an underlying disease. Secondary vasospasm generally refers to cold sensitivity of the Raynaud's type secondary to an associated disease such as lupus, scleroderma, certain medication, chronic arterial disease, dysprotenemias and the like.

Angina pectoris is a paroxysmal thoracic pain oftentimes accompanied by a feeling of suffocation and impending death, due, most often, to anoxia of the myocardium and precipitated by effort or excitement.

Cerebral ischemia is a deficiency of blood in the brain, due to functional constriction or actual obstruction of a blood vessel.

Preeclampsia is a toxemia of late pregnancy characterized by hypertension, edema and proteinuria.

Bronchial asthma is a reversible obstructive lung disorder characterized by increased responsiveness of the airways. Bronchial asthma attacks are characterized by narrowing of large and small airways due to spasm of bronchial smooth muscle, edema and inflammation of the bronchial mucosa and production of tenacious mucus. The role of inflammation in the perpetuation of the abnormal airway responses (late-phase reaction) is only now being appreciated. Airways obstruction causes hypoventilation in some lung areas, and continued blood flow to these area leads to a ventilation/perfusion imbalance resulting in hypoxema. Arterial hypoxemia is almost always present in attacks severe enough to require medical attention. Hyperventilation occurs early in the attack. As the attack progresses, the patient's capacity to compensate by hyperventilation of unobstructed areas of the lung is further impaired by more extensive airways narrowing and muscular fatigue. Arterial hypoxema worsens and can lead to respiratory acidosis.

In addition to L-arginine, any salt of L-arginine is suitable in the practice of the present invention. Such salts include 2,4-bisglyco-deuteroporphyrin L-arginate, 2,4-sulfonedeuteroporphyrin L-arginate, heme-L-arginate, L-arginine hydrochloride and the like. L-arginine hydrochloride is the preferred salt in the practice of the present invention.

Additional suitable anions for such a salt of L-arginine include bromide, fluoride, iodide, borate, hypobromite, hypochlorite, nitrite, nitrate, hyponitrite, sulfate, disulfate, sulfite, sulfonate, phosphate, diphosphate, phosphite, phosphonate, diphosphonate, perchlorate, perchlorite, oxalate, malonate, succinate, lactate, carbonate, bicarbonate, acetate, benzoate, citrate, 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-(methylthio)-butanoate and the like. The suitable cation for most salts is hydrogen, however, other cations such as sodium, potassium and the like would be acceptable in the preparation of such a salt. It would be advantageous if the specific salt form selected allowed a pH close to neutral.

Heme-L-arginate is a pharmacological agent with the ability to induce heme oxygenase. It is a stable compound bonding one molecule of heme to three molecules of arginine and forms a high spin-type compound. The half-life of heme arginate in humans is 10.8±0.6 hours with a volume of distribution of 33.7±0.34 liter.

The precise amount of L-arginine suitable for use in the practice of the present invention will vary depending on the condition for which the drug is administered, the size and kind of the mammal, as well as the specific form, i.e., salt, selected. Generally speaking, L-arginine is intended for administration to humans.

The typical effective amount of L-arginine or pharmaceutically acceptable salt thereof to reduce vascular resistance would be in the range of about 1 mg to about 1500 mg per day, more preferably, about 10 mg to about 400 mg. The preferred amount of L-arginine for use in the treatment of hypertension is about 1 mg to about 1500 mg per day, more preferably, about 10 mg to about 400 mg. Likewise, the typical effective amount of L-arginine or pharmaceutically acceptable salt thereof to prevent or treat bronchial asthma would be in the range of about 1 mg to about 1500 mg per day, more preferably about 10 mg to 400 mg per day. Pediatric compositions would typically contain proportionally less of the active ingredient.

L-arginine or a salt thereof may be administered to a mammalian organism by any route of administration. Suitable routes would, of course, include oral, parenteral, topical, and the like. The oral dosage form is preferred.

Preferably, the L-arginine is formulated with any suitable nontoxic pharmaceutically acceptable inert carrier material. Such carrier materials are well known to those skilled in the art of pharmaceutical formulations. For those not skilled in the art, reference is made to the text entitled, "REMINGTON'S PHARMACEUTICAL SCIENCES".

In a typical preparation for oral administration, e.g., tablet or capsule, the active ingredient, i.e., L-arginine, may be combined with any oral nontoxic pharmaceutically acceptable inert carrier such as lactose, starch (pharmaceutical grade), dicalcium phosphate, calcium sulfate, kaolin, mannitol and powdered sugar. Additionally, when required, suitable binders, lubricants, disintegrating agents and coloring agents may be included. Typical binders include starch, gelatin, sugars such as sucrose, molasses and lactose, natural and synthetic gums such as acacia, sodium alginate, extract of Irish moss, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone, polyethylene glycol, ethylcellulose and waxes. Typical lubricants for use in these dosage forms can include, without limitation, boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine and polyethylene glycol. Suitable disintegrators can include, without limitation, starch, methylcellulose, agar, bentonite, cellulose, wood products, alginic acid, guar gum, citrus pulp, carboxymethylcellulose and sodium lauryl sulfate.

If desired, a conventional pharmaceutically acceptable dye can be incorporated into the dosage unit form, i.e., any of the standard FD&C dyes. Sweetening and flavoring agents and preservatives can also be included, particularly when a liquid dosage form is formulated, e.g., an elixir, suspension or syrup. Also, when the dosage form is a capsule, it may contain, in addition to

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materials of the above type, a liquid carrier such as a fatty oil. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. Such compositions should preferably contain at least 0.1% of active components; generally, the active ingredients will be between about 2% to about 60% of the weight of the unit.

Likewise, injectable (injectable, intramuscular, intraperitoneal and the like) and topical, especially the newer topical "patch" forms of L-arginine, may be prepared by any method known in the art.

The L-arginine may also be formulated as a long-acting preparation to minimize the intervals between administration. Such long-acting formulations would be applicable with virtually all known routes of administration and such preparations may be formulated by techniques known in the art.

The mechanism of the blood pressure lowering effect of L-arginine is still unclear. However, while not wishing to be bound by any theory, the observation that spontaneous hypertensive rats have a diminished endothelial-dependent relaxation response and that L-arginine may be the physiological precursor of the most powerful endothelial-derived releasing factor, nitric oxide, may suggest that administration of L-arginine to spontaneous hypertensive rats increases the formation of nitric oxide and contributes to an overall decrease in peripheral vascular resistance, and therefore causes a reduction in blood pressure. It is also possible that there is a direct effect on renal hemodynamics. Likewise, with respect to bronchial asthma, it is speculated that the administration of L-arginine results in the relaxation of bronchial smooth muscle.

While the present invention is described above in connection with preferred or illustrative embodiments, those embodiments are not intended to be exhaustive or limiting of the invention. Rather, the invention is intended to cover all alternatives, modifications and equivalents included within its spirit and scope, as defined by the appended claims.

EXAMPLE 1

Administration of L-arginine Hydrochloride

Male spontaneous hypertensive rats and Wistar Kyoto rats were purchased from Charles River (Wilmington, Mass.) and were fed and housed under identical conditions for at least five days before use. The rats were caged in groups of three with food and water ad libitum under an artificial light-dark cycle of twelve hours.

L- and D-arginine hydrochloride were obtained from Aldrich (Milwaukee, Wis.). Forty-five-day-old spontaneous hypertensive rats were injected with L-arginine hydrochloride, 5, 16, 50, 80, 100 and 500 mg/kg body weight, or D-arginine-hydrochloride, 80 and 100 mg/kg body weight, intraperitoneally, in a final volume of 1.0 ml buffered saline for four consecutive days. The control spontaneous hypertensive rats and Wistar Kyoto rats were injected with saline. Forty-five day old Wistar Kyoto strain rats were injected with 50 mg/kg body weight of L-arginine hydrochloride for four days. Each group consisted of four or five experimental animals. Blood pressure from the tail was measured before the first injection and twenty-four hours after the last arginine administration. Blood pressure was measured with anesthesia, using a pletysmograph.

Results are expressed as the means ±SEM. A two-way analysis of variance was performed to compare blood values between control, L-arginine and D-arginine treated spontaneous hypertensive rats. Specific differences between groups were tested by the Newman-Keuls test. The null hypothesis was rejected when the p value was less than 0.05.

The blood pressure of 7-week-old spontaneous hypertensive rats and Wistar Kyoto rats was monitored before and after administration of L- and D-arginine. As seen in FIG. 1, L-arginine caused a dose-dependent decrease in blood pressure of 7-week-old spontaneous hypertensive rats. At a dose as low as 16 mg/kg body weight, L-arginine lowered blood pressure by 10 mm Hg ($p=7$). The maximal effect of L-arginine, a decrease of 30 mm Hg, was achieved at 100 mg/kg body weight. Higher doses of L-arginine did not lower blood pressure further.

The effect of D-arginine on blood pressure of 7-week-old spontaneous hypertensive rats is shown in FIG. 2. D-arginine administered at a dose of 80 and 100 mg/kg body weight for four days had no effect on blood pressure of spontaneous hypertensive rats. Furthermore, D-arginine did not alter blood pressure at concentrations lower than 80 mg/kg body weight. Neither L-arginine nor D-arginine had any effect on blood pressure of the age-matched Wistar Kyoto rats. For example, blood pressure of 7-week-old Wistar Kyoto rats remained unchanged following four days of treatment with L-arginine (50 mg/kg body weight), 122.3±1.3 mm Hg vs. 124.5±1.7 mm Hg for control and treated, respectively, $n=3$.

The results described in this study clearly demonstrate that L-arginine is a potent remedy in reducing blood pressure of young spontaneous hypertensive rats. Overall, a twenty percent (20%) reduction (30 mmHg) of blood pressure was achieved at the maximal dose, whereas no effect was documented in normotensive rats. The L-arginine effect may be mediated via generation of nitric oxide which elicits vasodilation and consequently lowers blood pressure.

EXAMPLE 2

Administration of Heme-L-arginate

Five-week-old male spontaneous hypertensive rats and normotensive Wister Kyoto rats were purchases from Charles River (Wilmington, Mass.) and were fed and housed under identical conditions. Both spontaneous hypertensive rats and Wister Kyoto rats weighed the same at the beginning of the study, 116.3±12.5 g and 120.6±12.0 g, respectively. Forty-five-day-old spontaneous hypertensive rats and Wister Kyoto rats were injected with heme-L-arginate obtained from Leiras-Medica, Finland at 15 or 30 mg/kg body weight intraperitoneally in a final volume of 1.0 ml saline for four consecutive days. The dilution was made just before injection. The control spontaneous hypertensive rats and Wister Kyoto rats were injected with saline. Blood pressure from the tail was measured without anesthesia using a plethysmograph before and 23 hours after the last heme arginate administration.

Control and treated animals were sacrificed in pairs at intervals of 5, 7 and 24 hours after the last heme-L-arginate administration. One kidney from each rat and parts of the liver were immediately frozen in liquid nitrogen

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for RNA extraction. The remaining control and treated animals were killed 24 hours after the heme-L-arginate treatment. The livers and kidney were perfused with cold saline. Groups of control and treated animals were retained for study of the long-term effect on blood pressure after heme-L-arginate treatment at age 45 to 48 days; blood pressure was measured once a week from 7 to 13 weeks of age.

As seen in Table 1, administration of heme-L-arginate resulted in a marked decrease in blood pressure in 7-week-old spontaneous hypertensive rats (SHR), whereas no significant changes in blood pressure were monitored in age-matched normotensive Wistar Kyoto (WKY). The effect of heme-L-arginate could be detected following the first day of its administration. The maximal effect was achieved by the fourth day of treatment, but started to increase after cessation of heme-L-arginate administration as noted in FIG. 3. Systolic blood pressure was measured by tail cuff plethysmograph before and 24 hours after the last ingestion and then every other week. Results are the means ±SD, n=3 in each group; ***$p<0.001$, **$p<0.01$, and *$p<0.05$. Furthermore, the heme-L-arginate effect on blood pressure was also evident in 22-week-old spontaneous hypertensive rats. As seen in Table 2, administration of heme-L-arginate at 15 mg and 30 mg/kg body weight for 4 days decreased blood pressure by 7 and 12 mmHg, respectively. Although the heme-L-arginate effect on blood pressure in older spontaneous hypertensive rats was much lower than in younger spontaneous hypertensive rats, i.e., a decrease of blood pressure of 7 mm Hg vs. 26 mmHg for 20- and 7-week-old spontaneous hypertensive rats, respectively, it was significantly different from the controls as noted in Table 2.

Applicants further examined whether the effect is due to heme or to the arginine component of heme arginate. In separate experiments, applicants treated 7-week-old spontaneous hypertensive rats with heme-L-arginate, hemin alone and L-arginine alone at the same dose (15 mg/kg body weight). As seen in Table 3, both heme and L-arginine significantly reduced blood pressure in 7-week-old spontaneous hypertensive rats by 14.3 and 9.7 mmHg, respectively. At the same period, blood pressure in control spontaneous hypertensive rats increased by 7.7 mmHg. However, the effect of heme-L-arginate on blood pressure had a much greater decrease of 21.8 mmHg, which is the sum of the heme and arginine effects as noted in Table 3. Interestingly, when 7-week-old spontaneous hypertensive rats were treated with heme-L-arginate and an inhibitor of heme oxygenase, ZnDPBG (Zn-2,4-deuteropophyrin IX bis glycol), blood pressure decreased by only 14 mmHg. The heme oxygenase inhibitor alone did not have any effect on blood pressure as seen in Table 3.

Administration of heme-L-arginate (15 mg/kg body weight for 4 days) resulted in a marked decrease in blood pressure from 156.3±4.7 to 129.8±4.5 mm Hg ($p<0.001$), whereas blood pressure in spontaneous hypertensive rats receiving the vehicle control was not affected. In contrast, administration of heme-L-arginate or its vehicle to age-matched Wister Kyoto rats did not influence blood pressure, 119.5±3.3 mm Hg vs. 121.0±2.1 mmHg, respectively.

Applicants also studied the effect of heme-L-arginate, a potent inducer of heme oxygenase, on microsomal cytochrome P-450 levels in spontaneous hypertensive rats at 7 weeks of age.

Heme oxygenase activity was increased in both hepatic and renal microsomes of spontaneous hypertensive rats and Wister Kyoto rats by two to four fold following treatment with heme-L-arginate. The increase in heme oxygenase activity was associated with a parallel decrease in cytochrome P-450 content and in the activity of cytochrome P-450 ω/ω-1 arachidonate hydroxylases in kidneys of spontaneous hypertensive rats. Expression of the heme oxygenase gene following administration of heme-L-arginate was examined by Northern blot hybridization. Maximal increase of heme oxygenase mRNA occurred 5 to 7 hours after the last injection of heme-L-arginate and returned to control levels after 24 hours.

While not wishing to be bound by any theory, Applicants postulate that heme-L-arginate treatment resulted in induction of heme oxygenase which consequently led to a diminution of cytochrome P-450 especially the arachidonate ω/ω-1 hydroxylases leading to a marked decrease in 19-HETE and 20-HETE. The effect of heme-L-arginate on blood pressure may be mediated via these biochemical events as both 19-HETE and 20-HETE produced by the kidney may promote hypertension by causing vasoconstriction and sodium retention.

TABLE 1

Effect of Heme-L-Arginate on Systolic Blood Pressure in 7-week-old SHR and WKY

	Systolic blood pressure	
	Control (mmHg)	Treated (mmHg)
SHR (n = 8)	156.3 ± 4.7	129.8 ± 4.5*
WKY (n = 8)	119.5 ± 3.3	121.0 ± 2.1

Systolic blood pressure was measured by tail cuff pletysmograph 23 hours after the last heme-L-arginate administration (15 mg/kg body weight/day for 4 consecutive days, i.p.); results are means ±SD; * indicates significance from control, $p<0.001$.

TABLE 2

Systolic Blood Pressure in 22-Week-Old SHR

Heme-L-arginate dose	Before treatment (mmHg)	After treatment (mmHg)
15 mg/kg (n = 4)	193.3 ± 2.1	187.5 ± 1.3**
30 mg/kg (n = 3)	198.1 ± 4.8	185.7 ± 4.2*

Heme-L-arginate was given to 22-week-old spontaneous hypertensive rats (SHR) for 4 consecutive days. Systolic blood pressure was measured by tail cuff pletysmograph before and 20 hours after the last injection. Results are the means ±SD; * indicates significance from control, $p<0.005$ and ** indicates significance from control, $p<0.05$.

TABLE 3

Systolic Blood Pressure in 7-Week-Old SHR

	Before treatment (mmHg)	After treatment (mmHg)
Control	158.4 ± 4.1	166.0 ± 2.7
Heme-L-arginate (15 mg hemin, 16 mg L-arginine per kg)	154.3 ± 1.9	132.5 ± 7.3*
Hemin (15 mg/kg)	156.5 ± 3.3	142.2 ± 2.4*
L-arginine (16 mg/kg)	154.1 ± 4.5	144.4 ± 3.0*
Heme-L-arginate +	160.4 ± 3.8	146.5 ± 2.6*

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TABLE 3-continued

Systolic Blood Pressure in 7-Week-Old SHR		
	Before treatment (mmHg)	After treatment (mmHg)
ZnDPBG (7 mg/kg)		
ZnDPBG (7 mg/kg)	154.0 ± 4.0	169.1 ± 2.5

Systolic blood pressure was measured by tail cuff pletysmograph before and 23 hours after the last injection. Results are the same ±SD, n=5 in each group; significance from control spontaneous hypertensive rats after 4 days of treatment with the vehicle (166.0±2.7), *p<0.005.

While the invention has now been described with reference to several preferred embodiments, those skilled in the art will appreciate that various substitutions, omissions, modifications, and changes may be made without departing from the scope or spirit thereof. Accordingly, it is intended that the foregoing description be considered merely exemplary of the invention and not a limitation thereof.

We claim:

1. A method for treating a high vascular resistance disorder in a mammal, said method comprising administering to a mammalian organism in need of such treatment a sufficient amount of L-arginine or pharmaceutically acceptable salt thereof to treat a high vascular resistance disorder.

2. The method as claimed in claim 1, wherein the high vascular resistance disorder is hypertension.

3. The method as claimed in claim 1, wherein the high vascular resistance disorder is primary or secondary vasospasm.

4. The method as claimed in claim 1, wherein the high vascular resistance disorder is angina pectoris.

5. The method as claimed in claim 1, wherein the high vascular resistance disorder is cerebral ischemia.

6. The method as claimed in claim 1, wherein the high vascular resistance disorder is preeclampsia.

7. The method as claimed in claim 1, wherein L-arginine is present in an amount from about 1 mg to 1500 mg per day.

8. The method as claimed in claim 7, wherein L-arginine is present in an amount from about 10 mg to 400 mg per day.

9. The method as claimed in claim 1, wherein L-arginine is present along with a pharmaceutically acceptable carrier.

10. The method as claimed in claim 1, wherein L-arginine is in the form of L-arginine hydrochloride.

11. The method as claimed in claim 1, wherein L-arginine is adapted for oral administration.

12. The method as claimed in claim 1, wherein L-arginine is formulated as a tablet or capsule.

13. The method as claimed in claim 11, wherein L-arginine is in sustained release form.

14. The method as claimed in claim 1, wherein L-arginine is in parenteral form.

15. The method as claimed in claim 1, wherein L-arginine is suitable for intraperitoneal administration.

16. A method for treating hypertension in a mammal, said method comprising administering to a mammalian organism in need of such treatment about 1 mg to 1500 mg of L-arginine or pharmaceutically acceptable salt thereof.

17. The method as claimed in claim 16, wherein L-arginine is suitable for oral administration.

18. The method as claimed in claim 16, wherein L-arginine is suitable for parenteral administration.

19. The method as claimed in claim 16, wherein L-arginine is in the form of L-arginine hydrochloride.

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US006117872A

United States Patent [19]

Maxwell et al.

[11] Patent Number: 6,117,872

[45] Date of Patent: Sep. 12, 2000

[54] **ENHANCEMENT OF EXERCISE PERFORMANCE BY AUGMENTING ENDOGENOUS NITRIC OXIDE PRODUCTION OR ACTIVITY**

[75] Inventors: **Andrew J. Maxwell**, Fremont; **John P. Cooke**, Palo Alto, both of Calif.

[73] Assignee: **The Board of Trustees of the Leland Stanford Junior University**, Stanford, Calif.

[21] Appl. No.: **09/103,340**

[22] Filed: **Jun. 23, 1998**

[51] **Int. Cl.**[7] **A61K 31/205**; A61K 31/195; A61K 31/16; A61K 31/13; A61K 31/015; A61K 33/04; A61K 31/495; A61K 31/50; A61K 31/55; A61K 31/34

[52] **U.S. Cl.** **514/249**; 424/702; 514/458; 514/474; 514/556; 514/564; 514/565; 514/625; 514/665; 514/763

[58] **Field of Search** 514/249, 458, 514/474, 556, 564, 565, 665, 625, 763; 424/702

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Primary Examiner—Kimberly Jordan

Attorney, Agent, or Firm—Bertram I. Rowland; Rae-Venter Law Group, P.C.

[57] **ABSTRACT**

NO precursors are administered at elevated levels in addition to the diet of the individual to enhance exercise performance. Particularly, L-arginine and L-lysine by enhancing endothelial NO production can provide for greater aerobic capacity and improved exercise performance.

14 Claims, 7 Drawing Sheets

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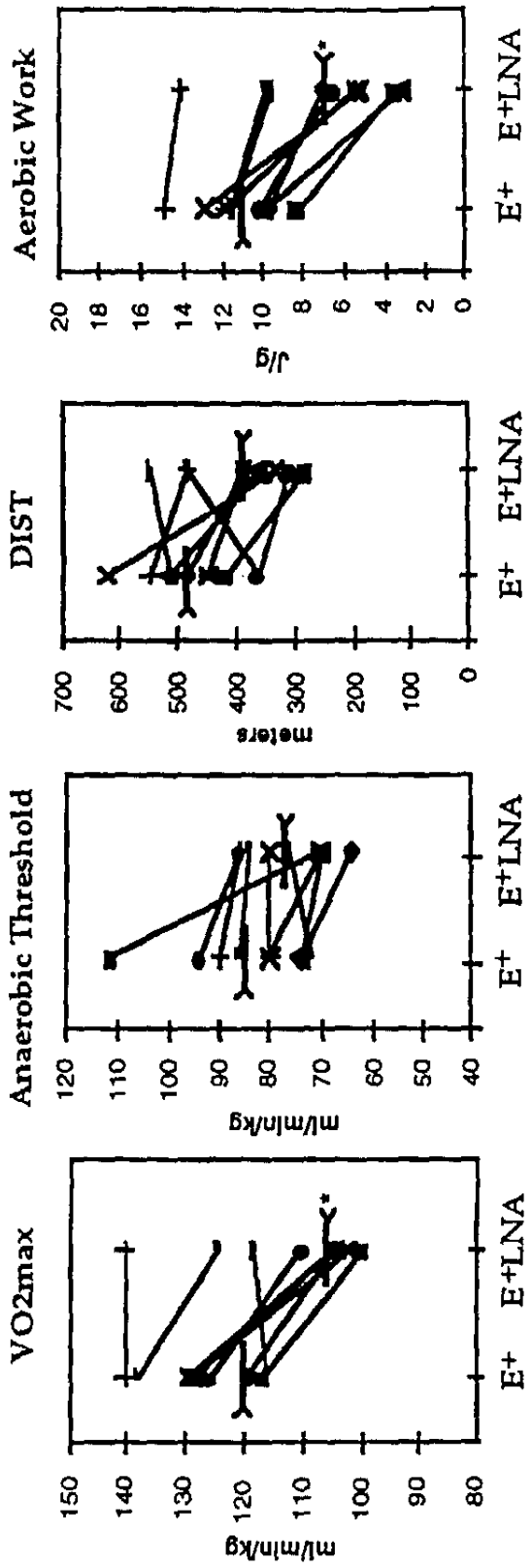

Figure 1. Effect of inhibition of EDNO on aerobic capacity.

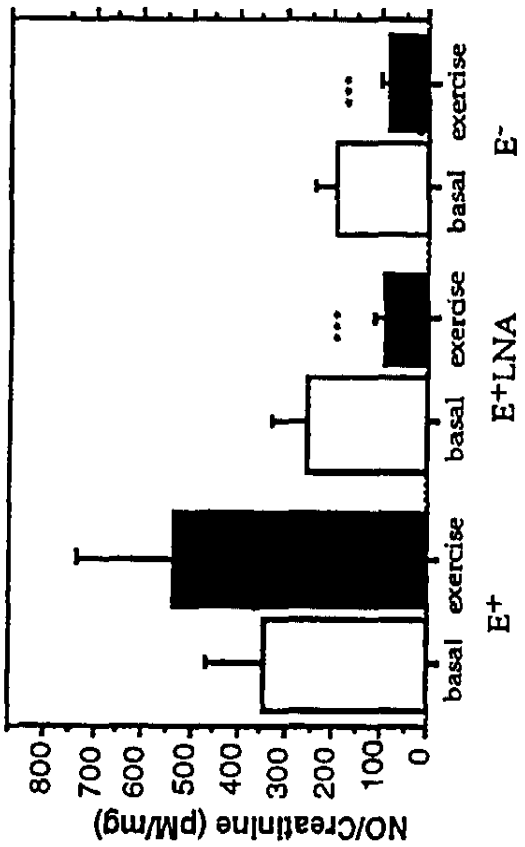

Figure 2. Systemic Production of Nitric Oxide Before and After Inhibition of EDNO.

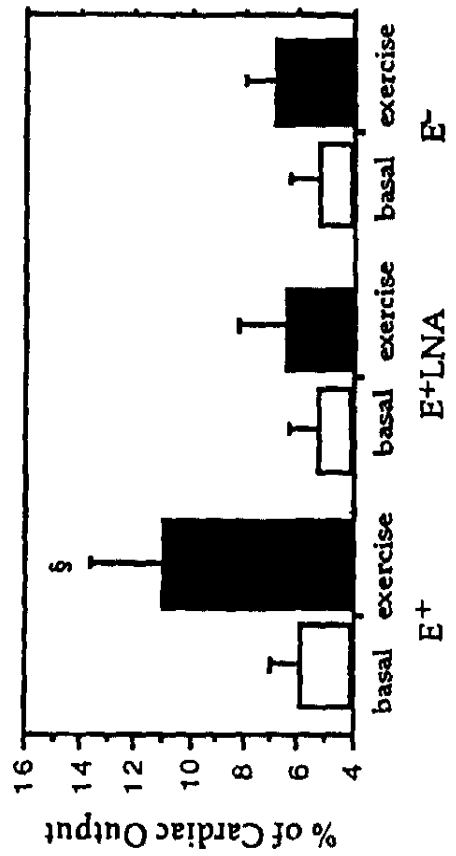

Figure 3. Blood Flow to the Hind limbs of Mice Before and After Inhibition of EDNO.

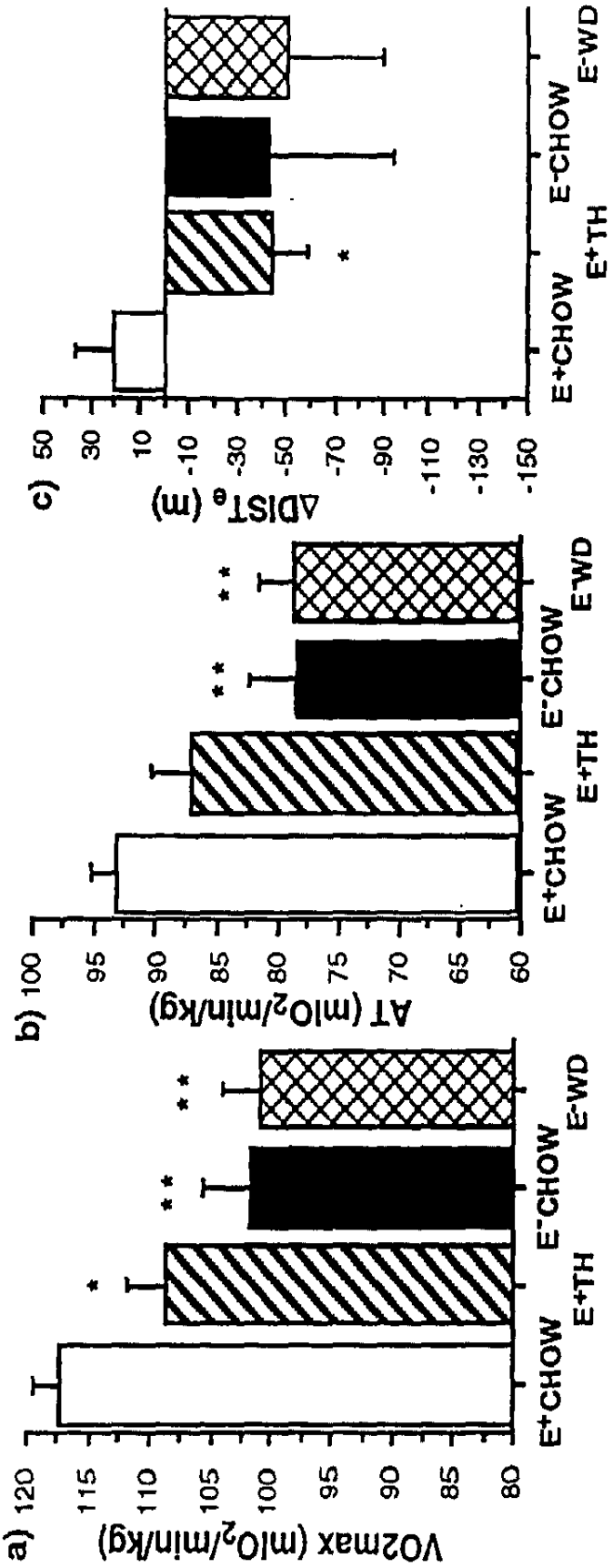

Figure 4. Effect of Cholesterol on Aerobic Capacity.

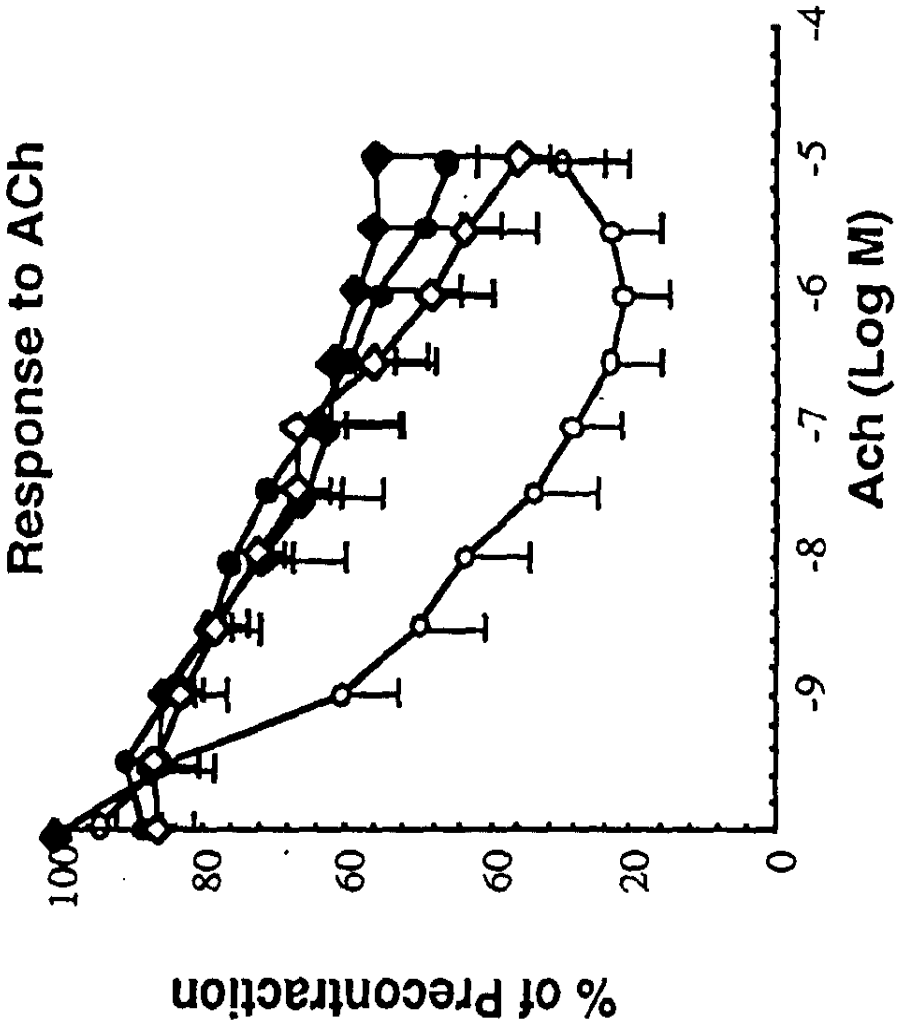

Figure 5. Vascular Function in Normal and Hypercholesterolemic Mice.

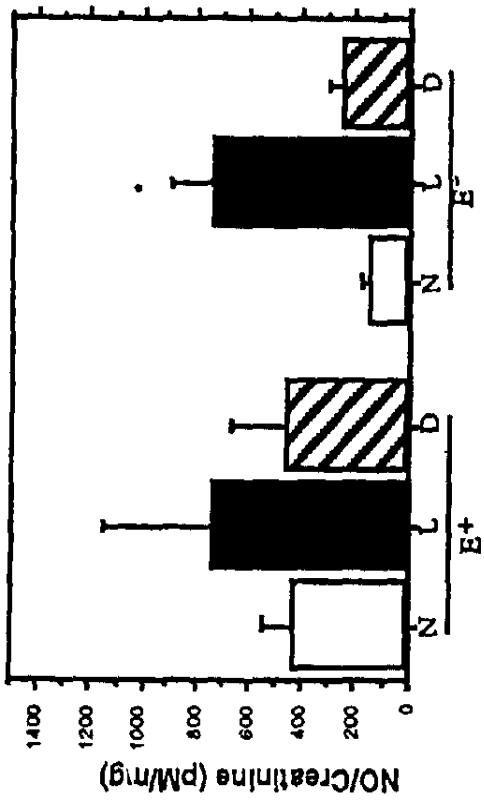

Figure 6. Systemic Nitric Oxide Production Following Exercise after L-arginine.

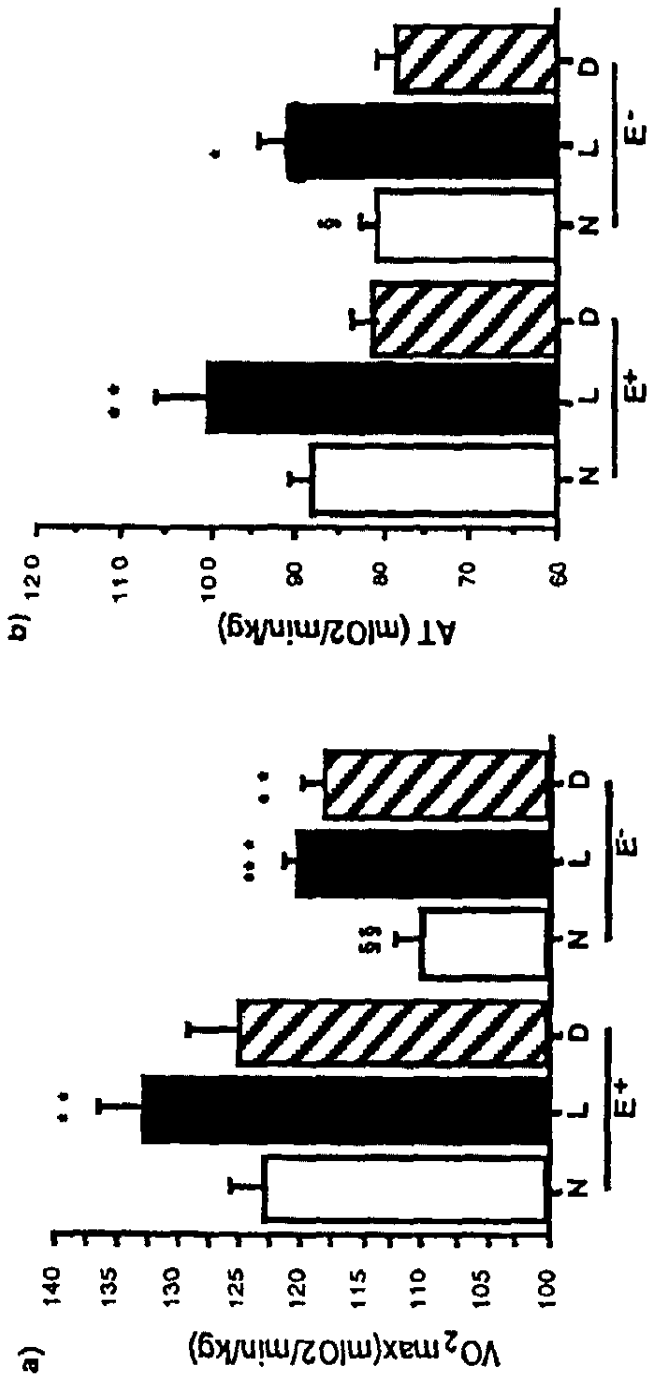

Figure 7. Aerobic Capacity of Mice on L-arginine.

ENHANCEMENT OF EXERCISE PERFORMANCE BY AUGMENTING ENDOGENOUS NITRIC OXIDE PRODUCTION OR ACTIVITY

This invention was made with Government support under contracts HI.58638 and HI.02660 awarded by the National Institutes of Health. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Aerobic exercise capacity is partly limited by vascular transport of oxygen and nutrients to end organs such as the heart and skeletal muscles. Vascular transport is, in turn, partly regulated by the elaboration of endothelial-derived nitric oxide (EDNO). Administration of physiologically acceptable compounds which enhance the elaboration of endogenous nitric oxide by the host allow for greater vascular transport and enhanced aerobic performance. Alternatively, compounds, or combinations of compounds, may be administered to enhance nitric oxide production, particularly in conjunction with the administration of a nitric oxide precursor to enhance aerobic performance.

Exercise capacity is limited by the rate by which oxygen can be taken up by a host (Schaible T F, Scheuer J: Cardiac adaptations to chronic exercise. *Progress in Cardiovascular Disease* 1985; 27:297–324; Wasserman K: Coupling of external to cellular respiration during exercise: the wisdom of the body revisited. *American Journal of Physiology* 1994; 266:E519–E539). In a generally healthy host, the rate of oxygen uptake, termed maximal velocity of oxygen uptake (VO_2max), is mostly limited by the oxygen transport capacity which is determined by the vascular conduction and distribution of blood flow (Barclay J K, Stainsby W N: The role of blood flow in limiting maximal metabolic rate in muscle. *Medicine and Science in Sports and Exercise* 1975; 7:116–119; di Prampero P E: An analysis of the factors limiting maximal oxygen consumption in healthy subjects. *Chest* 1992; 101:188S–191S). Therefore, the normal mechanisms which regulate blood flow during exercise can be limiting to aerobic exercise capacity. Furthermore, when these mechanisms are deranged, aerobic capacity may be further limited.

The production of nitric oxide by the endothelium (EDNO) contributes significantly to blood flow regulation and aerobic capacity during exercise (Maxwell A J, Schauble E, Bernstein D, Cooke J P: Limb blood flow during exercise is dependent upon nitric oxide. *Circulation* 1998;, Accepted for publication). This has been shown by the following series of experiments in the animal model. Administration of an inhibitor of the synthesis of EDNO acutely reduces aerobic capacity as measured by the VO_2max, the anaerobic threshold, running distance before exhaustion and aerobic work, as shown herein.

There are a significant number of cardiovascular disorders, where the individuals' ambulatory abilities are extensively impaired. These include individuals who suffer severe fatigue with exercise, which condition frequently is associated with heart failure. These disorders also include atherosclerosis affecting the coronary or limb arteries which can be manifested by angina (chest pain) or intermittent claudication (leg pain) with walking. Enhancing aerobic capacity to enhance performance would be of great advantage to these patients.

The use of L-arginine for prophylaxis and therapy in the case of atherosclerosis is taught in U.S. Pat. No. 5,5,428,070.

SUMMARY OF THE INVENTION

Physical capacity of individuals involved in muscular exertion is improved by administration of high levels of basic amino acids in addition to the diet normal for the individual. The basic amino acids are administered prior to the anticipated muscular exertion, particularly in association with substances which are antioxidants or other substances which enhance vascular nitric oxide synthesis or activity to cause vasodilation of vessels supplying exercising skeletal muscles and thereby enhance aerobic capacity.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** has a series of graphs of various aspects of aerobic capacity showing the effect of inhibition of endothelial-derived nitric oxide (EDNO) on aerobic capacity;

FIG. **2** is a bar graph comparing NO/creatinine production with different mice under different conditions after exercise;

FIG. **3** is a bar graph showing the changes in blood flow to the hind limbs of different mice before and after inhibition of EDNO;

FIG. **4** is a series of bar graphs showing the effect of cholesterol levels in animal models on aerobic capacity;

FIG. **5** is a graph of the change in vascular function in normal and hypercholesterolemic mice;

FIG. **6** is a bar graph showing systemic nitric oxide production following exercise after L-arginine administration; and

FIG. **7** has two bar graphs comparing aerobic capacity of mice on L-arginine.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

In accordance with the subject invention, exercise and athletic performance, aerobic capacity and muscular output are improved by administering high levels of the basic amino acids, L-arginine and L-lysine, individually or combined, to individuals, including humans and race animals, prior to physical exertion. The individuals may be hypocholesterolemic, normocholesterolemic or hypercholesterolemic, where normocholesterolemic falls for total plasma cholesterol level approximately between about 120–240 mg/dL cholesterol.

The physical exertion will usually involve the expenditure rate of at least about 100 Watts, usually at least about 200 Watts, during the course of the activity, which may be as short as a few seconds, as in a 100 meter race, or as long as a few hours, as in a marathon. Thus, the subject invention when involving performance in athletic prowess or physical effort, will require a minimum expenditure of energy in order to warrant the intake of the NO precursor amino acid.

The normal individual in the normal diet ingests about 1–6 grams of arginine per day and about 1.5–7 grams of lysine per day. For the purpose of this invention, within 48 hours prior to the physical exertion, preferably within about 24 hours prior to the physical exertion, and more preferably within about 6 hours of the physical exertion, at least about a total of 2 g, more usually at least about 3 g, preferably at least about 4 g, more preferably about a total of 4–9 g of basic amino acid, usually not more than about 12 g, will be administered orally as a bolus or in multiple doses, usually not more than about 6 doses, preferably not more than about 4 doses. By comparison, for race animals, the basic amino acids will be administered at at least about 60 mg/kg/day. The ratio of arginine to lysine would generally be in the

range of about 0–1:1–0, more usually in the range of about 0.2–1:0.8–0. While there is no maximum amount of the basic amino acids which may be employed, normally the total dosage per day will be under about 16 g, more usually under about 12 g, with individual dosages usually being in the range of 2–6 g.

While for the most part, the amino acids will be administered as monomers, they may also be administered as oligomers, generally having fewer than 10 units, more usually fewer than 8 units, and preferably having from about 2–6 units.

The administration of the basic amino acids may be a single administration, a few administrations, generally not more than about 8 over a period of 1–2 days, or may be administered on a daily basis. The particular regimen will depend upon the individual, the purpose for taking the basic amino acids for exercise performance enhancement, and whatever other aspects are involved. Since the basic amino acid will be taken to improve aerobic performance, it will generally be taken within one day of the activity and may be taken within 6 h of the activity, particularly within 3 h of the activity.

Desirably, the formulation which is employed for the basic amino acids will include other additives, particularly antioxidants, which prolong the half-life of EDNO, such as vitamins A, C and E; cysteine, glutathione or plant-based antioxidants; or other factors which may enhance EDNO synthesis or activity, including folic acid; biopterins, such as tetrahydrobiopterin, methyltetrahydrobiopterin, sepiapterin; B complex vitamins, specifically, B_6 and B_{12}, flavinoids, e.g. resveratrol, and carotenoids, e.g. lycopene, phytoestrogens, where these agents may be used individually or in combination, generally not more than about 5 of the members being used in combination, more usually not more than about 3. In addition, agents which may improve skeletal muscle metabolism may be employed, including L-carnitine (0–500 mg), L-creatine (0–20 g) and L-taurine (0–8 g).

The amounts of the individual components described above will generally be at or about the levels normally described for these compositions as the required daily dose, usually in the range of about 0.001 g to 2 g, where additives such as vitamin C or vitamin A may be at the upper level, while other additives will generally be below about 0.5 g, more usually below about 0.1 g. The formulation may be in solid or liquid form and may include tablets, capsules, powders, and the like. These particular formulations will usually include various excipients, as well as other conventional additives for improving disintegration, slow release, absorption, stability, and the like. Desirably, the subject compositions will be included in a food substance, which may be either liquid or solid. Thus, the subject composition may be included in a drink, particularly a soft drink which may comprise electrolytes, flavorings, sweeteners, or other components to enhance the organoleptic properties of the drink, such as evidenced by Gatorade®. Alternatively, the subject formulations may be introduced into various solid foods, particularly health foods which are low in cholesterol, such as cereals, health bars, including fruit bars, and the like. The dosages would depend upon the desired dosage and frequency with which the particular dietary supplement or food would be taken. Generally, there would be at least 2 g of the amino acids, preferably at least about 3 g, and not more than about 12 g, usually not more than about 6 g in the food supplement for a single administration. Of particular interest in solid foods are health bars, including fruit bars such as a date bar, fig bar, apricot bar, etc., and granola bars, grains, such as granola, cornflakes, wheat flakes, etc.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

The production of nitric oxide by the endothelium (EDNO) contributes significantly to blood flow regulation and aerobic capacity during exercise. This has been shown by the following series of experiments in an animal model. Administration of an inhibitor of the synthesis of EDNO acutely reduces aerobic capacity as measured by the VO_2max, the anaerobic threshold, running distance before exhaustion and aerobic work

FIG. 1. Effect of inhibition of EDNO on acrobic capacity. Individual and average maximal oxygen uptake (VO_2max), anaerobic threshold, running distance to exhaustion, and aerobic work capacity (area under VO_2-time curve) of healthy mice (E^+) and the same mice after three days of L-nitroarginine administered in the drinking water (E^+LNA), * $p<0.05$).

Eight week old female wild type and apoE deficient (E^-) C57BL/6J mice (Jackson Laboratories, Bar Harbor, Me. and DCM) were entered into experimental protocols after a 1 week period of acclimation. In order to determine the effects of EDNO inhibition on aerobic capacity, a set of mice underwent the following treadmill studies. Eight week old wild type (E^+; n=9) and E^- mice (n=9) were kept sedentary for 4 weeks. At twelve weeks of age, each mouse was treadmill-tested to measure indices defining exercise capacity. The wild type mice were then administered L-ω-nitroarginine (LNA, Sigma Chemical Co., St. Louis) in the drinking water (6 mg/100 cc; E^+LNA). This dose of LNA is similar to that shown to attenuate basal urinary nitrate excretion (Böger, et al.: Long-term administration of L-arginine, L-NAME, and the exogenous NO donor molsidomine modulates urinary nitrate and cGMP excretion in rats. *Cardiovasc Res* 1994; 28:494–9) and similar molar doses of L-nitroarginine methyl ester have been shown to suppress the release of EDNO and the excretion of cGMP and nitrate in other animal models (Torok and Gerova: Vascular responses after long-term inhibition of nitric oxide synthesis in newborn dogs. *Physiol Res* 1996; 45:323–8). After 4 days, the mice underwent a second treadmill testing. Mice were sacrificed in random order following treadmill testing by overdose of methoxyflurane (Pitman-Moore, Mundelein, Ill.) inhalation anesthesia.

Indices of Exercise Capacity

Maximal oxygen uptake (VO_2max) is defined as the plateau in VO_2 despite increasing work intensity.

The anaerobic threshold (AT) is an independent measure of aerobic capacity expressed in units of VO_2. For each mouse the AT was determined from computer analysis (confirmed by blinded observer) of VCO_2/VO_2 plots by the V-slope method of Beaver (Beaver, et al.: A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol* 1986; 60:2020–7). In situations when the slope of VCO_2/VO_2 did not increase at higher work rates, the VO_2max was taken as the AT.

The distance run to exhaustion ($DIST_e$) is taken as an approximate measure of overall work performance and is the total distance run.

Aerobic work capacity (AWC) was determined by the summation of minute oxygen uptake above basal rate over the course of treadmill running until exhaustion. This was multiplied by the constant 20 $J/ml O_2$ to convert oxygen uptake to aerobic work (Harpur: The rat as a model for physical fitness. *Comp. Biochem. Physiol.* 1980; 66A:553–574; Wasserman, et al.: Principles of Exercise Testing and Interpretation. 1994; 479).

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Treadmill Testing

At the time of treadmill testing, each mouse was placed on a treadmill at a constant 8° angle enclosed by a metabolic chamber capable of measuring oxygen and carbon dioxide outflow once every minute (Model CT-2, Columbus Instruments). After a 15 minute period of acclimation, basal measurements were obtained over 7 minutes. The treadmill was then started at 10 m/min and the speed was incrementally increased 1 m/min every minute until the mouse reached exhaustion. Exhaustion was defined as spending time on the shocker plate without attempting to re-engage the treadmill. Data on oxygen uptake (VO_2), carbon dioxide output (VCO_2), respiratory quotient (RQ), and distance run to exhaustion ($DIST_e$) were collected and stored on hard disk (Oxymax software, Columbus Instruments).

EXAMPLE 2

In this study the reduction of EDNO production was confirmed by a significantly reduced urinary excretion of nitrates following exercise. Measurement of urinary nitrate excretion normalized to creatinine is used as a measure of systemic nitric oxide production during exercise (Bode-Böger S M, Böger R H, Schröder P E, Frölich J C: Exercise increases systemic nitric oxide production in men. *Journal of Cardiovascular Risk* 1994; 1:173–178). The significance of this observation was extended to the regulation of limb blood flow using a fluorescent microsphere experiment.

FIG. 2. Systemic Production of Nitric Oxide Before and After Inhibition of EDNO. Healthy mice (E+) increase nitric oxide production from basal levels following treadmill exercise as measured by increased urinary excretion of nitrates. This increase is completely suppressed by administration of L-nitroarginine for 3 days (E+LNA), Hypercholesterolemic mice (E−) demonstrate a reduced nitrate excretion as well. * $p<0.005$ vs. E+.

Mice were placed in metabolic chambers for basal and post-exercise urinary nitrate collection (Bode-Böger, et al.: L-arginine infusion decreases peripheral arterial resistance and inhibits platelet aggregation in healthy subjects. *Clin Sci (Colch)* 1994; 87:303–10). For the basal state, mice were confined to cages for greater than 24 hours and for the post-exercise state, mice were treadmill exercised over 22 minutes to a final treadmill speed of 32 m/min. Metabolic chambers were constructed as described previously (Maxwell, et al.: Hypercholesterolemia impairs exercise capacity: Role of nitric oxide. *American Journal of Physiology* 1998; submitted for publication:). Urine was collected in test tubes containing 100 μl of isopropyl alcohol submerged in ice water for the duration of the 5 hour collection period. Urine was centrifuged at 4,000 rpm for 5 min and the supernatant was collected, diluted 1:9 in distilled water and stored at −80° C. for measurement of nitrogen oxides (NO_x) and creatinine.

NO_x in the urine was measured with a commercially available chemiluminescence apparatus (model 2108, Dasibi Corp., Glendale, Calif.) as previously described (Tsao, et al.: Enhanced endothelial adhesiveness in hypercholesterolemia is attenuated by L-arginine. *Circulation* 1994; 89:2176–82). The samples (50 μl) were injected into boiling acidic vanadium (III) chloride. This technique utilizes acidic vanadium (III) chloride at 98° C. to reduce both NO_2^- and NO_3^- to NO, which is then detected by the chemiluminescence apparatus after reacting with ozone. Signals from the detector were analyzed by computerized integration of curve areas. Standard curves for $NaNO_2$/$NaNO_3$ were linear over the range of 50 pM to 10 nM. Urine creatinine was measured by the modified method of Slot developed by Sigma Diagnostics (Heinegard and Tiderstrom: Determination of serum creatinine by a direct colorimetric method. *Clin Chim Acta* 1973; 43:305).

EXAMPLE 3

Animals administered EDNO inhibitor demonstrate reduced blood flow to the exercising limbs as measured by fluorescent microspheres injected into the ascending aorta during maximal exertion. These data indicate that in the normal host EDNO production is critical to limb blood flow and aerobic capacity during exercise.

FIG. 3. Blood Flow to the Hind limbs of Mice Before and After Inhibition of EDNO. Healthy mice (E+) increase blood flow to the hind limbs from basal levels during treadmill exercise as measured by percent of total cardiac output. This increase in blood flow during exercise is suppressed by administration of L-nitroarginine for 3 days (E+LNA). Hypercholesterolemic mice demonstrate reduced limb blood flow during exercise as well § $p<0.05$ vs. basal.

In order to determine blood flow distribution during exercise, an additional set of mice underwent a microsphere study. Eight week old wild type mice and E^- mice (n=8) were kept sedentary for 4 weeks. At twelve weeks of age, the wild type mice were divided into 2 groups; one receiving regular water (E^+; n=8) and one administered LNA in the drinking water (6 mg/100 cc) for 4 days (E^+LNA; n=8). Each mouse underwent treadmill testing to determine VO_{2max}. The aorta was then surgically cannulated and, after overnight recovery, the mice underwent a microsphere delivery study (described below). Mice were sacrificed following microsphere delivery by overdose of methoxyflurane inhalation anesthesia. The hindlimb muscles were collected and weighed for determination of microsphere density.

Regional blood flow to hindlimb muscles was determined as a percentage of cardiac output using a modification of previously described techniques (Musch, et al.: Effects of high-intensity sprint training on skeletal muscle blood flow in rats. *Journal of Applied Physiology* 1991; 71:1387–1395; Barbee, et al.: Microsphere and dilution techniques for the determination of blood flows and volumes in concious mice. *American Journal of Physiology* 1992; 263:R728–R733).

Surgical Preparation;

Mice were anesthetized using isofluorane (Ohmeda Caribe, Guayama, PR) inhalation. An incision was made in the ventral midline of the neck. After the carotid sheath was exposed, the carotid artery was separated from the neurovascular bundle and secured by two 4–0 silk sutures. An incision was made in the carotid and a 30 cm length of PE10 tubing (Beckton Dickinson, Sparks Md.) tapered at one end by gentle stretch was filled with heparin (100 U/ml, Elkins-Sinn, Cherry Hill, N.J.), introduced into the carotid artery and advanced to the ascending aorta just distal to the aortic valve. The incision was oversewn and the tubing was tunneled subcutaneously to a pouch under the skin on the back. The mice were then given a single dose of ampicillin (100 mg/kg diluted in saline 10 mg/ml, i.p.). After overnight recovery, the mice were assessed for running ability. Mice that could not attain 80% VO_2max as determined previously were eliminated from the study.

Treadmill exercise protocol;

The instrumented mice were placed on a treadmill in a metabolic chamber in random order. The tubing was fed through a hole in the chamber and the carotid artery tubing was connected to a pressure transducer for continuous heart rate and blood pressure measurement. After 20 minutes of acclimation and oxygen uptake analysis, blue-green micro-

spheres were injected into the carotid tubing and infused with normal saline for regional blood flow determination at rest. An equal volume of blue-green microspheres was injected into a reference vial for a "100% of flow" control. The treadmill was then started at 10 m/min and increased 1 m/min/min while following the oxygen uptake curve. As the oxygen uptake curve began to plateau, yellow-green microspheres were injected into the carotid cannula and infused with normal saline. An equal volume of yellow-green microspheres was injected into a reference vial. Following the completion of the yellow-green microsphere infusion mice were sacrificed by methoxyflurane overdose. The gastrocnemius, quadriceps and both kidneys were removed, were dissected free of fat and connective tissue, blotted dry, weighed and placed in sample vials for fluorimetric determination.

Microsphere protocol;

Blue-green and yellow-green fluorescently labeled microspheres (15 μm dia., Molecular Probes, OR) were diluted to 20,000 microspheres per 20 μl of saline. At the time of injection, the microsphere solution was vortexed for 5 minutes. Microspheres (20 μl) were drawn into a microinjector syringe and transferred to the carotid artery tubing. The cannula was connected to a glass syringe mounted in an injector pump and filled with saline which was used to flush the carotid cannula (100 μl over 30 sec).

Fluorimetric determination of microsphere number;

Tissues and reference samples were digested in 2N KOH in methanol overnight in a shaker bath at 40° C. The samples were centrifuged at 3,000 g and the supernatant was removed to the level of the tissue plug. The tissue plug was resuspended twice in distilled water with 0.5% Tween80 (Fisher Scientific, NJ), centrifuged and the supernatant was decanted. The plug was then resuspended in methanol, centrifuged and decanted. The remaining methanol was removed by evaporative drying. The remaining microsphere residue was dissolved in 2 ml of 2-ethoxyethyl acetate (Arcon Organics, NJ) and measured by fluorimetery (Model LS50B; Perkin-Elmer, Norwalk, Conn.) using the recommended extinction and emission frequencies for microsphere fluorescence (λ_{ex} of 425 and λ_{em} of 468 for blue-green and λ_{ex} of 490 and λ_{em} of 505 for yellow-green).

Regional blood flow for resting and exercise states was calculated from fluorescent intensity as the percent of cardiac output to the tissue (%COt);

$$\%Cot_{s,i}=ft_{s,i}(WTt_{average}/WTt_i)/frs_{s,i}$$

where $ft_{s,i}$ is the fluorescent intensity of the tissue sample residue from mouse i during state s (resting or exercise) and $frs_{s,i}$ is the fluorescent intensity of the reference sample residue. Variability in fluorescent intensity due to variation in tissue weight collected is removed by normalizing the tissue weight WTt_i to the average tissue weight of all animals $WTt_{average}$.

EXAMPLE 4

The significance of the above findings is demonstrated in the hypercholesterolemic paradigm whereby EDNO synthesis and activity is deranged (Freiman P C, Mitchell G G, Heistad D D, Armstrong M L, Harrison D G: Atherosclerosis impairs endothelium-dependent vascular relaxation to acetylcholine and thrombin in primates. *Circ Res* 1986; 58:783–9; Jayakody R L, Senarate M P, Thomson A B R, Kappagoda C T: Cholesterol feeding impairs endothelium-dependent relaxation in rabbit aorta. *Canadian Journal of Pharmacology* 1985; 63:1206–1209). In hypercholesterolemic animals (both diet-induced and genetically prone caused by an apoE deficiency), aerobic exercise capacity has been shown to be inversely related to serum cholesterol level (Maxwell A J, Niebauer J, Lin P S, Tsao P S, Bernstein D, Cooke J P: Hypercholesterolemia impairs exercise capacity: Role of nitric oxide. *American Journal of Physiology* 1998; submitted for publication).

FIG. 4. Effect of Cholesterol on Aerobic Capacity. Four groups of mice differing in average total serum cholesterol (TSC) level; (E+CHOW: normal mice fed a chow diet; TSC=153 mg/dl, E+TH: normal mice fed a high fat diet; TSC=306, E−CHOW: apoE deficient mice fed a chow diet; TSC=1325 and E−WD: apoE deficient mice fed a high fat diet; TSC=2154). Mice were treadmill tested to determine indices of aerobic capacity (maximal oxygen uptake; VO_2max, anaerobic threshold; AT, and change in distance run to exhaustion from study start (AEDISTe). *p<0.05,** p<0.01 vs. E+CHOW.

Wild type mice (n=25) and apoE mice (n=25) were randomly selected at 8 weeks of age to undergo treadmill testing and urinary nitrate measurement. Mice from both colonies were then randomized into 2 dietary groups. One group of wild type mice (E^+CHOW, n=49) were fed regular mouse chow (0.022% cholesterol {29 ppm}, 11% total fat by weight {4.3% monounsaturated fatty acids, 3.7% saturated fatty acids, 2.5% linoleic acid, 0.2% linolinic acid, 0.2% omega-3-fatty acid and 0.03% arachidonic acid}, Purina, Richmond, Ind.) and a second group (E^+TH, n=22) received a high cholesterol/high fat modified Thomas-Hartroft diet (1.3% cholesterol, 15% fat from cocoa butter, Dyets, Bethlehem, Pa.) (Paigen, et al.: Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. *Arteriosclerosis* 1990; 10:316–323). One group of apoE mice (E^-CHOW, n=23) received regular mouse chow whereas the other apoE group (E^- WD, n=10) received a Western-type diet (0.15% cholesterol, 21% fat from butterfat, Dyets) (Nakashima, et al.: ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arteriosclerosis and Thrombosis* 1994; 14:133–140). The high cholesterol diets were selected based on the ability of the mice to tolerate dietary cholesterol loads as previously reported (Paigen, et al.: Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. *Arteriosclerosis* 1990; 10:316–323). Following either 4 weeks (E^+CHOW; n=32, E^+TH; n=22, E^-CHOW; n=14, E^-WD; n=10) or 12 weeks (E^+CHOW; n=17, E^-CHOW; n=9) of dietary intervention, selected mice underwent treadmill-testing and urinary nitrate measurement. Mice were sacrificed in random order following treadmill testing by overdose of methoxyflurane (Pitman-Moore, Mundelein, Ill.) inhalation anesthesia. The thoracic aorta was harvested for studies of vascular reactivity and the infrarenal abdominal aorta was harvested for studies of stimulated nitric oxide production.

EXAMPLE 5

The observed reduction in exercise capacity in hypercholesterolemic animals is associated with an endothelium-dependent vasodilator dysfunction as demonstrated by a reduced vasodilatory response to acetylcholine of aortae from hypercholesterolemic mice (FIG. 5). Furthermore, the post-exercise urinary nitrate excretion and limb blood flow of hypercholesterolemic animals is reduced (FIGS. 2 and 3). These data support a strong relationship of cholesterol level with endothelial function, EDNO activity, limb blood flow and aerobic capacity.

FIG. 5. Vascular Function in Normal and Hypercholesterolemic Mice. Response of murine aortae to increasing doses

of acetylcholine. Groups of mice differ in total serum cholesterol level as described in FIG. 4. open circles; E+CHOW, closed circles; E+TH, open diamonds; E−CHOW, closed diamonds; E−WD.

One 7 mm segment of thoracic aorta (measured proximal from the diaphragm) was dissected free of connective tissue and immediately placed in cold physiologic saline solution (PSS) that was composed of the following (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2, $NaHCO_3$, 25; Na_2EDTA, 0.026; dextrose, 11.1; L-arginine, 0.1. Aortic segments were quickly mounted on wire stirrups, hung from force transducers and submerged in oxygenated PSS at 37° C. Over the course of 60 minutes, the segments were progressively stretched to the optimum point of their length-tension relationship (determined previously to be 3 g). Subsequently, the concentration of norepinephrine inducing half-maximal response (EC_{50}) was determined by exposing the segments to increasing concentrations of norepinephrine (in half-log increments from 10^{-9} to 10^{-4} M). Once a maximal response was obtained, the segments were washed repeatedly with fresh PSS for 60 minutes until the tension returned to the previous baseline value. Responses to the vasodilating agents, nitroglycerine (TNG) and acetylcholine (ACh), were studied after precontracting the segments with the EC_{50} concentration of norepinephrine. After a stable contraction was obtained, the segments were exposed to increasing doses of vasodilator.

EXAMPLE 6

Supplementation of healthy animals with L-arginine (6% in drinking water) for 4 to 8 weeks was shown to increase urinary nitrate excretion and aerobic capacity as measured by VO_2max, anaerobic threshold, and running distance before exhaustion (FIGS. 6 and 7). L-arginine increased VO_2max 9% over control, increased anaerobic threshold 12% over control, and increased running distance to exhaustion 61% over controls. Supplementation with the optical isomer of L-arginine, D-arginine, which cannot be directly converted to nitric oxide, did not result in this improvement.

In the hypercholesterolemic animal model, L-arginine supplementation restored to normal post-exercise urinary nitrate excretion and aerobic capacity (FIGS. 6 and 7). Supplementation of L-arginine reversed the 11% decline in VO_2max and the 23% reduction in running distance. D-arginine was able to partly reverse this dysfunction perhaps through the hepatic conversion to L-arginine.

FIG. 6. Systemic Nitric Oxide Production Following Exercise after L-arginine. Normal (E+) and hypercholesterolemic mice (E−) demonstrate an increase in urinary nitrate excretion following L-arginine supplementation (L) but not following D-arginine administration (D). * $p<0.05$ vs. controls (N).

Eight week old E^+ and E^- mice divided into 6 groups (Table 1); two supplemented with L-arginine (6 g/100 ml drinking water, LE^+; n=16 and LE^-; n=16); two administered D-arginine (the optical isomer of L-arginine which is not a substrate for nitric oxide synthase, 6 g/100 ml drinking water, DE^+; n=8 and DE^-; n=8); and two received regular drinking water (NE^+; n=27 and NE^-; n=24). The mice were kept sedentary for 4 to 8 weeks. At 12 to 16 weeks of age, the mice were treadmill-tested in random order by an investigator blinded to the identity of its group to measure indices defining exercise capacity. Because this study was designed to determine the effect of chronic enhancement of EDNO production rather than an acute effect of arginine, all water bottles containing arginine were replaced with regular water 48 hours before treadmill-testing. Urine was collected after treadmill exercise for determination of vascular nitric oxide production. Mice were sacrificed following treadmill testing by overdose of methoxyflurane (Pitman-Moore, Mundelein, Ill.) inhalation anesthesia.

EXAMPLE 7

FIG. 7. Aerobic Capacity of Mice on L-arginine. Normal (E+) and hypercholesterolemic mice (E−) demonstrate an increase in oxygen uptake (VO_2max) and anaerobic threshold (AT) following L-arginine supplementation (L) but not following D-arginine administration (D). * $p<0.05$ vs. controls (N).

Another method of enhancing nitric oxide activity and performance is by pharmacologically enhancing consitutive nitric oxide synthase (cNOS) expansion. One way to do this is to provide a cNOS inhibitor during training to upregulate cNOS expression, followed by withdrawal of the antagonist prior to the exercise test. This has been shown in the following experiment. Twelve week old wild type mice were administered either L-nitroarginine in the drinking water (6 mg/100 ml) or regular water over the course of a 4 week period. During this time some of the mice from both groups were trained (1 hour twice daily, 5 days/week×4 weeks). At the end of the training period the drinking water with L-nitroarginine was replaced with regular drinking water. Three days later, all mice were exercise tested to obtain measures of aerobic capacity. Mice receiving regular drinking water increased their VO2max to 124±12 mlO2/min/kg (sedentary controls were 109 mO2/min/kg). The mice that received L-nitroarginine during training increased their VO2max to 134±12 mlO2/min/kg ($p<0.05$). This enhanced effect from chronic NOS inhibition may be due to an upregulation of NOS enzyme level. In order to demonstrate this, the gastrocnemius muscles of the mice were analysed for cNOS content. Muscle tissue homogenates were prepared for Western Blot analysis using mouse anti-human cNOS IgG1 monoclonal antibody. Using this method cNOS protein concentration was found to be elevated in muscle tissue of the exercising mice. However, the message was significantly more elevated in the mice treated with L-nitroarginine.

Therefore, chronic use of NOS inhibitors during training, followed by withdrawal during the exercise test, is another method to enhance EDNO production. Several compounds exist which block NOS activity including L-nitroarginine, L-NG-nitroarginine methyl ester (L-NAME), and asymmetric dimethylarginine (ADMA) or other L-arginine derivative. During training a pharmacologic agent is administered in amounts to inhibit nitric oxide production. This is followed by a period free of inhibitor, during which period a compound to enhance nitric oxide production is administered. An example of this would be the chronic use of an inhibitory L-arginine derivative during athletic training for an athletic event. Three to 7 days before the athletic event, the inhibitor would be discontinued and the EDNO enhancing agent administration begun.

In summary, augmentation of the EDNO production has been shown to enhance aerobic capacity in healthy animals and in an animal model of hypecholesterolemia.

Perhaps the best documented method of enhancing aerobic exercise performance is by exercise training (Blomqvist C G, Saltin B: Cardiovascular adaptations to physical training. *Annual Review of Physiology* 1983; 45:169–89). Exercise training in the form of running and marching daily for 2 months has been shown to increase VO_2max by 6% in healthy young men (Frick M H, Sjogren A -L, Perasalo J: Cardiovascular dimensions and moderate physical training in young men. *J Appl Physiol* 1970; 29:452–455). Fourteen

weeks of ergometer training 34 min/day, 3 d/wk increased VO_2max by 31% (Stein R A, Michielle D, Diamond J, al. e: The cardiac response to exercise traininig: echocardiographic analysis at rest and during exercise. *Am J Cardiol* 1980; 46:219–225). In animals, administration of L-arginine to healtby mice for 4 weeks had an affect on aerobic capacity that was equal to that of 4 weeks of treadmill exercise training (2 hours/day, 6 days/week) in these animals (Niebauer J, Maxwell A J, Lin P S, Wang D, Hydari S, Tsao P S, Cooke J P: Chronic exercise training attenuates atherogenesis in hypercholesterolemic mice. *Circulation* 1998; in preparation.).

Few studies have been reported demonstrating enhancement of aerobic performance by nutrient or pharmacologic manipulation. Oral supplementation with creatine has been shown to increase the running time to exhaustion by 13% (Bosco C, Tihanyi J, Pucspk J, Kovacs I, Gabossy A, Colli R, Pulvirenti G, Tranquilli C, Foti C, Viru M, Viru A: Effect of oral creatine supplementation on jumping and running performance. *Int J Sports Med* 1997; 18:369–72). Biochemical studies in rats suggest that this effect is the result of improved buffering capacity within skeletal muscle which results in enhancement of both aerobic and anaerobic metabolism. Inhaled albuterol has been shown to increase riding time in competitive cyclists (Bedi J F, Gong H, Jr., Horvath S M: Enhancement of exercise performance with inhaled albuterol. *Can J Sport Sci* 1988; 13:144–8). VO_2max also increased with albuterol use although this was not statistically significant. There is no data available comparing manipulation of the nitric oxide system with these other methods.

It is evident from the above results that by using a nitric oxide precursor, as exemplified by L-arginine, in the absence of atherosclerosis, but even in the presence of elevated cholesterol levels, substantially enhanced exercise capacity can be achieved. The results support the conclusion that elevated levels of NO precursors administered to normocholesterolemic and hypercholesterolemic individuals provide a positive benefit for the exercise capacity of the individual. By providing for easy means to administer the NO precursors, individuals can have substantially enhanced exercise performance.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

1. A method for enhancing physical performance of a mammal prior to said physical performance, said method comprising:

administering to said mammal prior to said physical performance as the active ingredient an amino acid composition consisting of at least one amino acid selected the group consisting of arginine and lysine of at least about 60 mg/kg/day within 24 h of said physical performance.

2. A method according to claim **1**, wherein said mammal is normocholesterolemic.

3. A method according to claim **1**, wherein said mammal is hypercholesterolemic.

4. A method according to claim **1**, wherein an antioxidant is administered to said mammal in an amount to enhance the endothelial NO.

5. A method according to claim **1**, wherein said administering comprises the inclusion of an agent further enhancing EDNO synthesis.

6. A method according to claim **1**, wherein said administering is as a health bar, grain or drink.

7. A method for enhancing human physical performance prior to said physical performance, said method comprising:

administering to said human within 6 h prior to said physical performance as the active ingredient an amino acid composition consisting of at least one amino acid selected from the group consisting of arginine and lysine of at least about 2 g per day in combination with an agent enhancing ENDO synthesis, as a health bar, grain or drink.

8. A method according to claim **7**, wherein said agent comprises at least one of Vitamin A, Vitamin C, Vitamin E, selenium, carotenoid, flavanoid, L-carnitine, L-creatine, and L-taurine.

9. A method according to claim **7**, wherein said agent is at least one of folic acid and biopterin.

10. A method according to claim **7**, wherein said administering of said arginine and lysine is from about 2 to 12 g per day within 24 hours of said physical exertion.

11. A method for enhancing physical performance of a mammal prior to physical exertion, said method comprising:

at least about 3 days prior to said physical exertion, administering to said mammal an NOS inhibitor for inhibiting NOS; and

within about 3 days prior to said physical exertion, discontinuing the NOS inhibitor, and administering to said mammal an agent enhancing EDNO synthesis.

12. A method enhancing human physical performance prior to said physical performance, said method comprising:

administering to said human prior to said physical performance as the active ingredient an amino acid composition consisting of at least one amino acid selected from the group consisting of arginine and lysine of at least about 2 g per day within 24 h of said physical performance.

13. A method according to claim **12**, wherein said human is normocholesterolemic.

14. A method according to claim **12**, wherein said human is hypercholesterolemic.

* * * * *

EXHIBIT C

RAE-VENTER LAW GROUP, P.C.

intellectual property law

January 20, 2000

VIA CERTIFIED MAIL – RETURN RECEIPT REQUESTED

Real Health Laboratories, Inc.
1424 30th Street, #B1
San Diego, CA 92154

Gentlemen:

I am intellectual property counsel for Cooke Pharma. Your advertisement concerning the use of arginine for improving cardiovascular activity was given to me by my client.

As you are probably aware, John Cooke, M.D., Ph.D., the founder of Cooke Pharma, has been and continues to be a pioneer in the use of enhanced levels of arginine for a wide variety of indications associated with vascular function. Cooke Pharma is presently selling its "Heart Healthy Bar®" containing arginine for uses associated with vascular function in a broad spectrum of applications. Cooke Pharma and Dr. John Cooke have completed a number of clinical studies to demonstrate the effectiveness of enhanced levels of arginine for its effect in a number of situations associated with vascular function. Dr. Cooke and Dr. Andrew Maxwell, a principal in Cooke Pharma, and their associates, have been the authors of numerous articles on the mechanism and efficacy of arginine to improve vascular function.

Cooke Pharma is the exclusive worldwide licensee of Letters Patent from The Leland Stanford Jr. University and the Medical College of New York and has filed in its own name applications for Letters Patent, all concerned with products containing arginine and uses for arginine. In view of your activity in the field in the United States, we are providing you with copies of the issued United States Letters Patent for which Cooke Pharma has exclusive rights, so that you may monitor your activities in the sale and advertising of your arginine-containing products, both as to your products and the manner in which you encourage your users to use your products.

Sincerely yours,

Bertram I. Rowland, Ph.D.
Attorney at Law

BIR:mef
Encls: U.S. Patent Nos.: 5,217,997
5,428,070
5,852,058
5,861,168

cc: Barbara Wagner

200 Sheridan Ave., Suite 440, Palo Alto, CA 94306 USA P.O. Box 60039, Palo Alto, CA 94306 USA tel: 650.328.4400 • fax: 650.328.4477
email: [illegible]

C
26

TO: **Commissioner of Patents and Trademarks Washington, D.C. 20231**	**REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT**

In compliance with the Act of July 19, 1952 (66 Stat. 814; 35 U.S.C. 290) you are hereby advised that a court action has been filed on the following patent(s) in the U.S. District Court:

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
02cv129H (POR)	1/18/02	United States District Court, Southern District of California
PLAINTIFF		DEFENDANT
Unither Pharma Inc		Real Health Laboratories
PATENT NO.	**DATE OF PATENT**	**PATENTEE**
1.5,217,997	Jun 8, 1993	Richard D Levere
2. 6,117,872	Sep 12 2000	Andrew J Maxwell
3.		
4.		
5		

In the above-entitled case, the following patent(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT NO.	**DATE OF PATENT**	**PATENTEE**
1		
2		
3		
4		
5		

In the above-entitled case, the following decision has been rendered or judgment issued:

DECISION/JUDGMENT		
CLERK	(BY) DEPUTY CLERK	DATE

Copy 1 - Upon initiation of action, mail this copy to Commissioner Copy 3 - Upon termination of action, mail this copy to Commissioner
Copy 2 - Upon filing document adding patent(s), mail this copy to Commissioner Copy 4 - Case file copy

(Rev. 3/99)

CIVIL COVER SHEET

The JS-44 civil cover sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet. (SEE INSTRUCTIONS ON THE REVERSE OF THE FORM.)

I. (a) PLAINTIFFS

UNITHER PHARMA, INC., THE BOARD OF TRUSTEES OF LELAND STANFORD JUNIOR UNIVERSITY, and NEW YORK MEDICAL COLLEGE

02 JAN 18 PM 4:02

CLERK, U.S. DISTRICT COURT SOUTHERN DISTRICT OF CALIFORNIA

DEPUTY

DEFENDANTS

REAL HEALTH LABORATORIES, INC., and JOHN F. DULLEA

'02 CV 0129 H (POR)

(b) COUNTY OF RESIDENCE OF FIRST LISTED PLAINTIFF San Mateo
(EXCEPT IN U.S. PLAINTIFF CASES)

COUNTY OF RESIDENCE OF FIRST LISTED DEFENDANT San Diego
(IN U.S. PLAINTIFF CASES ONLY)

NOTE: IN LAND CONDEMNATION CASES, USE THE LOCATION OF THE TRACT OF LAND INVOLVED.

(c) ATTORNEYS (FIRM NAME, ADDRESS, AND TELEPHONE NUMBER)

Kenneth S. Klein
Foley & Lardner
402 West Broadway, 23rd Floor
San Diego, CA 92101
(619) 234-6655

ATTORNEYS (IF KNOWN)

II. BASIS OF JURISDICTION (PLACE AN 'X' IN ONE BOX ONLY)

- [ ] 1 U.S. Government Plaintiff
- [X] 3 Federal Question (U.S. Government Not a Party)
- [ ] 2 U.S. Government Defendant
- [ ] 4 Diversity (Indicate Citizenship of Parties in Item III)

III. CITIZENSHIP OF PRINCIPAL PARTIES (PLACE AN 'X' IN ONE BOX FOR PLAINTIFF AND ONE BOX FOR DEFENDANT)
(For Diversity Cases Only)

	PTF	DEF		PTF	DEF
Citizen of This State	[ ] 1	[ ] 1	Incorporated *or* Principal Place of Business In This State	[ ] 4	[ ] 4
Citizen of Another State	[ ] 2	[ ] 2	Incorporated *and* Principal Place of Business In Another State	[ ] 5	[ ] 5
Citizen or Subject of a Foreign Country	[ ] 3	[ ] 3	Foreign Nation	[ ] 6	[ ] 6

IV. NATURE OF SUIT *(PLACE AN "X" IN ONE BOX ONLY)*

CONTRACT
- [ ] 110 Insurance
- [ ] 120 Marine
- [ ] 130 Miller Act
- [ ] 140 Negotiable Instrument
- [ ] 150 Recovery of Overpayment & Enforcement of Judgment
- [ ] 151 Medicare Act
- [ ] 152 Recovery of Defaulted Student Loans (Excl. Veterans)
- [ ] 153 Recovery of Overpayment of Veteran's Benefits
- [ ] 160 Stockholders' Suits
- [ ] 190 Other Contract
- [ ] 195 Contract Product Liability

REAL PROPERTY
- [ ] 210 Land Condemnation
- [ ] 220 Foreclosure
- [ ] 230 Rent Lease & Ejectment
- [ ] 240 Torts to Land
- [ ] 245 Tort Product Liability
- [ ] 290 All Other Real Property

TORTS

PERSONAL INJURY
- [ ] 310 Airplane
- [ ] 315 Airplane Product Liability
- [ ] 320 Assault, Libel & Slander
- [ ] 330 Federal Employers' Liability
- [ ] 340 Marine
- [ ] 345 Marine Product Liability
- [ ] 350 Motor Vehicle
- [ ] 355 Motor Vehicle Product Liability
- [ ] 360 Other Personal Injury

PERSONAL INJURY
- [ ] 362 Personal Injury - Med. Malpractice
- [ ] 365 Personal Injury - Product Liability
- [ ] 368 Asbestos Personal Injury Product Liability

PERSONAL PROPERTY
- [ ] 370 Other Fraud
- [ ] 371 Truth in Lending
- [ ] 380 Other Personal Property Damage
- [ ] 385 Property Damage Product Liability

CIVIL RIGHTS
- [ ] 441 Voting
- [ ] 442 Employment
- [ ] 443 Housing/ Accommodations
- [ ] 444 Welfare
- [ ] 440 Other Civil Rights

PRISONER PETITIONS
- [ ] 510 Motion to Vacate Sentence

HABEAS CORPUS:
- [ ] 530 General
- [ ] 535 Death Penalty
- [ ] 540 Mandamus & Other
- [ ] 550 Civil Rights
- [ ] 555 Prison Condition

FORFEITURE/PENALTY
- [ ] 610 Agriculture
- [ ] 620 Other Food & Drug
- [ ] 625 Drug Related Seizure of Property 21 USC 881
- [ ] 630 Liquor Laws
- [ ] 640 R.R. & Truck
- [ ] 650 Airline Regs.
- [ ] 660 Occupational Safety/Health
- [ ] 690 Other

LABOR
- [ ] 710 Fair Labor Standards Act
- [ ] 720 Labor/Mgmt. Relations
- [ ] 730 Labor/Mgmt. Reporting & Disclosure Act
- [ ] 740 Railway Labor Act
- [ ] 790 Other Labor Litigation
- [ ] 791 Empl. Ret. Inc. Security Act

BANKRUPTCY
- [ ] 422 Appeal 28 USC 158
- [ ] 423 Withdrawal 28 USC 157

PROPERTY RIGHTS
- [ ] 820 Copyrights
- [X] 830 Patent
- [ ] 840 Trademark

SOCIAL SECURITY
- [ ] 861 HIA (1395ff)
- [ ] 862 Black Lung (923)
- [ ] 863 DIWC/DIWW (405(g))
- [ ] 864 SSID Title XVI
- [ ] 865 RSI (405(g))

FEDERAL TAX SUITS
- [ ] 870 Taxes (U.S. Plaintiff or Defendant)
- [ ] 871 IRS - Third Party 26 USC 7609

OTHER STATUTES
- [ ] 400 State Reapportionment
- [ ] 410 Antitrust
- [ ] 430 Banks and Banking
- [ ] 450 Commerce/ICC Rates/etc.
- [ ] 460 Deportation
- [ ] 470 Racketeer Influenced and Corrupt Organizations
- [ ] 810 Selective Service
- [ ] 850 Securities/Commodities/ Exchange
- [ ] 875 Customer Challenge 12 USC 3410
- [ ] 891 Agricultural Acts
- [ ] 892 Economic Stabilization Act
- [ ] 893 Environmental Matters
- [ ] 894 Energy Allocation Act
- [ ] 895 Freedom of Information Act
- [ ] 900 Appeal of Fee Determination Under Equal Access to Justice
- [ ] 950 Constitutionality of State Statutes
- [ ] 890 Other Statutory Actions

V. ORIGIN *(PLACE AN "X" IN ONE BOX ONLY)*

- [X] 1 Original Proceeding
- [ ] 2 Removed from State Court
- [ ] 3 Remanded from Appellate Court
- [ ] 4 Reinstated or Reopened
- [ ] 5 Transferred from Another district (specify)
- [ ] 6 Multidistrict Litigation
- [ ] 7 Appeal to District Judge from Magistrate Judgment

VI. CAUSE OF ACTION (CITE THE U.S. CIVIL STATUTE UNDER WHICH YOU ARE FILING AND WRITE A BRIEF STATEMENT OF CAUSE. DO NOT CITE JURISDICTIONAL STATUTES UNLESS DIVERSITY.)

28 U.S.C. Section 1338(a), patent infringement

VII. REQUESTED IN COMPLAINT: [ ] CHECK IF THIS IS A **CLASS ACTION** **DEMAND $** UNDER F.R.C.P. 23

CHECK YES only if demanded in complaint:
JURY DEMAND: [X] YES [ ] NO

VIII. RELATED CASE(S) IF ANY (See instructions): JUDGE Nita L. Stormes DOCKET NUMBER 01CV0854W(NLS)

DATE January 18, 2002

ORIGINAL

SIGNATURE OF ATTORNEY OF RECORD Kenneth S. Klein

Kenneth S. Klein

FOR OFFICE USE ONLY

RECEIPT # 078672 AMOUNT $150.00 APPLYING IFP _____ JUDGE _____ MAG. JUDGE _____