UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

IOVATE HEALTH SCIENCES, INC., IOVATE T & P, INC., MTOR US TRADEMARK LTD., HHC US TRADEMARK LTD., LEUKIC US TRADEMARK LTD., and MULTI FORMULATIONS LTD., **Plaintiffs,** **v.** **ALLMAX NUTRITION, INC., HEALTHY BODY SERVICES, LLC, and HEALTHY BODY SERVICES, INC.,** **Defendants.**	Civil Action No. 07-CV-12334-NMG **JURY TRIAL DEMANDED**

SECOND AMENDED COMPLAINT
LEAVE TO FILE GRANTED JUNE 24, 2008

Plaintiffs Iovate Health Sciences, Inc., Iovate T & P, Inc., MTOR US Trademark Ltd., HHC US Trademark Ltd., Leukic US Trademark Ltd., and Multi Formulations Ltd. (together with their respective predecessors and affiliates, "Iovate" or "Plaintiffs"), by and through undersigned counsel, bring this action for patent infringement, false designation of origin in violation of the Lanham Act, and unfair competition in violation of Massachusetts common law against Defendants Allmax Nutrition, Inc., Healthy Body Services, LLC, and Healthy Body Services, Inc. ("Defendants"), as follows:

PARTIES

1. Plaintiff Iovate Health Sciences, Inc. ("Iovate HS") is a corporation organized and existing under the laws of Canada and has a principal place of business at 381 North Service Road West, Oakville, Ontario, L6M 0H4.

2. Plaintiff Iovate T & P, Inc. ("Iovate T & P") is a corporation organized and existing under the laws of Canada and has a principal place of business at 381 North Service Road West, Oakville, Ontario, L6M 0H4.

3. Plaintiff MTOR US Trademark Ltd. is a corporation organized and existing under the laws of Canada and has a principal place of business at 381 North Service Road West, Oakville, Ontario, L6M 0H4.

4. Plaintiff HHC US Trademark Ltd. is a corporation organized and existing under the laws of Canada and has a principal place of business at 381 North Service Road West, Oakville, Ontario, L6M 0H4.

5. Plaintiff Leukic US Trademark Ltd. is a corporation organized and existing under the laws of Canada and has a principal place of business at 381 North Service Road West, Oakville, Ontario, L6M 0H4.

6. Plaintiff Multi Formulations Ltd. is a corporation organized and existing under the laws of Canada and has a principal place of business at 381 North Service Road West, Oakville, Ontario, L6M 0H4.

7. Upon information and belief, Defendant, Allmax Nutrition, Inc. ("Allmax") is a corporation organized and existing under the laws of Canada and has a principal place of business at 4576 Yonge Street, Suite 509, North York, Ontario, M2N-6N4.

8. Upon information and belief, Defendant Healthy Body Services, LLC ("HBSL") is a limited-liability company organized and existing under the laws of the State of Nevada, that conducts business in the State of Nevada, and has a resident agent in the State of Nevada, Resident Agents of Nevada Inc., with a principal place of business at 711 South Carson Street,

Carson City, Nevada 89701. Upon information and belief, HBSL is the distributor of infringing products made, used and/or sold by Defendants.

9. Upon information and belief, Defendant Healthy Body Services, Inc. ("HBSI") is a corporation organized and existing under the laws Canada and has a principal place of business at 4576 Yonge Street, Suite 509, North York, Ontario, M2N-6N4. Upon information and belief, Defendant HBSI is the manufacturer of the infringing products made, used, and/or sold by or for the Defendants. Upon information and belief, HBSI is the owner and operator of the domain www.allmaxnutrition.com, which markets and sells the infringing products made, used, and/or sold by or for Defendants.

JURISDICTION AND VENUE

10. This is an action for patent infringement arising under the patent laws of the United States, Title 35 of the United States Code; for violations of the Trademark Act of 1946 as amended (the "Lanham Act"), 15 U.S.C. § 1051 *et seq.*; and for other violations of applicable Massachusetts common and statutory law.

11. The Court has jurisdiction over the subject matter of this action pursuant to (i) 28 U.S.C. §§ 1331, 1332 and 1338(a) for claims arising from the Lanham Act and the patent laws of the United States, (ii) § 1338(b) for claims arising from the common law, and (iii) principles of supplemental jurisdiction.

12. The Court has personal jurisdiction over the Defendants because: (i) the Defendants knowingly transact business in this Commonwealth and district; (ii) the Defendants advertise and market their infringing goods within this jurisdiction; and (iii) the Defendants' conduct outside this jurisdiction is causing injury in this forum.

13. Venue is proper in this Court, pursuant to 28 U.S.C. §§ 1331, 1391(b), 1391(d) and 1400, in that a substantial part of the events giving rise to the claims occurred in this district, and the Defendants transact business in this district.

GENERAL ALLEGATIONS

14. For more than a decade, Iovate has marketed and sold the highest quality food supplements and nutritional products throughout the United States under, among others, its "Muscle-Tech" brand.

15. Iovate revolutionized the industry by bringing to the widespread public products originally formulated and designed for professional body builders and other elite athletes. In particular, Iovate's trademarked "Hydroxycut," "Anator-P70," and "Leukic" products, as well as Iovate's "Hydroxycut Hardcore" product, have been spectacularly popular with recreational athletes and health-conscious individuals alike. With market leading sales and name recognition, Iovate's "Muscle-Tech" brand of products, which includes Iovate's trademarked "Anator-P70" product and Iovate's "Hydroxycut Hardcore" product, as well as Iovate's trademarked "Hydroxycut" product and Iovate's "Leukic" product, have become the choice of consumers seeking the highest quality health supplements available.

16. Long after Plaintiffs had established their valuable marks, and long after Plaintiffs had established themselves as the leading choice of health-conscious consumers, Defendants began a campaign to unlawfully appropriate Plaintiffs' goodwill and valuable intellectual property assets.

17. Defendants perpetrated this campaign by, *inter alia*, infringing Plaintiffs' patents, marketing and packaging products which are confusingly similar to Plaintiffs' products, and by hiring at least one former employee of Plaintiffs. That former employee, Ryan Foley ("Foley"),

was employed by Plaintiffs and possessed intimate knowledge of Plaintiffs' products, product formulations, branding and marketing efforts and strategies and other valuable and proprietary business information. Shortly after Defendants hired Foley, on information and belief, they, with Foley's assistance, began the campaign referenced above. Foley is even pictured on the packaging material of Defendants' infringing "Rapidcuts Hardcore" product.

18. Further, on information and belief, Defendants, or those acting under Defendants' direction or control, have made various injurious and untruthful statements in Defendants' product labeling, advertisements, and marketing efforts about Plaintiffs' products, including, *inter alia*, that Plaintiffs' products contain predominantly "filler" instead of active ingredients, or are otherwise inferior to products marketed and sold by Defendants.

19. To protect the goodwill that Plaintiffs have established in their product name and branding, and their "Hydroxycut," "Hydroxycut Hardcore," "Anator-P70," and "Leukic" marks, Plaintiffs bring this action for trademark infringement and false designation of origin under the Federal Trademark (Lanham) Act, and unfair competition under Massachusetts common law.

20. Plaintiffs seek also to protect their valuable inventions by bringing this action under the patent laws of the United States. Plaintiffs seek injunctive relief, an accounting, damages and recovery of their costs and attorneys' fees.

THE IOVATE PATENTS

21. On October 26, 1999, United States Patent No. 5,973,199 ("the '199 Patent"), titled "Hydrosoluble Organic Salts of Creatine," was duly and legally issued by the United States Patent and Trademark Office. A true and correct copy of the '199 Patent is attached as Exhibit A of this Complaint.

22. Iovate HS is the owner of all rights, title and interest in and to the '199 Patent.

23. On October 19, 1999, United States Patent No. 5,968,900 ("the '900 Patent"), titled "Increasing Creatine and Glycogen Concentration in Muscle," was duly issued by the United States Patent and Trademark Office. A true and correct copy of the '900 Patent is attached as Exhibit B of this Complaint.

24. Iovate T & P is the owner of all rights, title and interest in and to the '900 Patent.

25. On August 21, 2001, United States Patent No. 6,277,396 ("the '396 Patent"), titled "Dietary Supplement Containing a Thermogenic Substance and an Adrenal Support Substance," was duly issued by the United States Patent and Trademark Office. A true and correct copy of the '396 Patent is attached as Exhibit C of this Complaint.

26. Iovate T & P is the owner of all rights, title and interest in and to the '396 Patent.

27. On February 17, 1998, United States Patent No. 5,719,119 ("the '119 Patent"), titled "Parenteral Nutrition Therapy with Amino Acids," was duly issued by the United States Patent and Trademark Office. A true and correct copy of the '119 Patent is attached as Exhibit D of this Complaint.

28. Multi Formulations Ltd. is the owner of all rights, title and interest in and to the '119 Patent.

29. On November 9, 2004, United States Patent No. 6,814,986 ("the '986 Patent"), titled "Composition for Treating Obesity and Esthetic Treatment Process," was duly issued by the United States Patent and Trademark Office. A true and correct copy of the '986 Patent is attached as Exhibit E of this Complaint.

30. Multi Formulations Ltd. is the owner of all rights, title and interest in and to the '986 Patent.

31. On December 14, 2004, United States Patent No. 6,830,765 ("the '765 Patent"), titled "Green Tea Extract for Treating Obesity," was duly issued by the United States Patent and Trademark Office. A true and correct copy of the '765 Patent is attached as Exhibit F of this Complaint.

32. Multi Formulations Ltd. is the owner of all rights, title and interest in and to the '765 Patent.

33. Upon information and belief, Defendants have made, used, offered for sale, sold and/or imported certain nutritional supplements products, including, without limitation, the products distributed by Allmax under the trade names "Rapidcuts Hardcore," "Rapidcuts Femme," "Krush 4," "NOK 2," "Quickmass," "Leutor 70," "R-ALA," and "Razor8" throughout the United States and in this judicial district.

FIRST CAUSE OF ACTION
(Infringement of the '199 Patent)

34. Plaintiffs reallege and incorporate the allegations of paragraphs 1-33 of this Complaint as if set forth at length and in full herein.

35. Upon information and belief, certain products made, used, sold and offered for sale by Defendants, including, without limitation, the product(s) distributed by the Defendants under the trade names "NOK 2" and "Quickmass" incorporate or embody the inventions claimed in the '199 Patent.

36. By their actions, Defendants have infringed, and are infringing, one or more of the claims of the '199 Patent.

37. Upon information and belief, Defendants have actively induced others to infringe the claims of the '199 Patent.

38. Defendants' infringing conduct has been and continues to be unlawful and willful.

39. As a result of Defendants' acts of infringement, Plaintiffs have suffered and will continue to suffer damages in an amount to be proved at trial.

SECOND CAUSE OF ACTION
(Infringement of the '900 Patent)

40. Plaintiffs reallege and incorporate the allegations of paragraphs 1-39 of this Complaint as if set forth at length and in full herein.

41. Upon information and belief, certain products made, used, sold and offered for sale by Defendants, including, without limitation, the products distributed by Defendants under the trade names "Krush 4" and "Quickmass" incorporate or embody the inventions claimed in the '900 Patent.

42. By their actions, Defendants have infringed, and are infringing, one or more of the claims of the '900 Patent.

43. Upon information and belief, Defendants have actively induced others to infringe the claims of the '900 Patent.

44. Defendants' infringing conduct has been and continues to be unlawful and willful.

45. As a result of Defendants' acts of infringement, Plaintiffs have suffered and will continue to suffer damages in an amount to be proved at trial.

THIRD CAUSE OF ACTION
(Infringement of the '396 Patent)

46. Plaintiffs reallege and incorporate the allegations of paragraphs 1-45 of this Complaint as if set forth at length and in full herein.

47. Upon information and belief, certain products made, used, sold and offered for sale by Defendants, including, without limitation, the products distributed by Defendants under

the trade names “Rapidcuts Hardcore” and “Rapidcuts Femme,” incorporate or embody the inventions claimed in the '396 Patent.

48. By their actions, Defendants have infringed, and are infringing, one or more of the claims of the '396 Patent.

49. Upon information and belief, Defendants have actively induced others to infringe the claims of the '396 Patent.

50. Defendants’ infringing conduct has been and continues to be unlawful and willful.

51. As a result of Defendants’ acts of infringement, Plaintiffs have suffered and will continue to suffer damages in an amount to be proved at trial.

FOURTH CAUSE OF ACTION
(Infringement of the '119 Patent)

52. Plaintiffs reallege and incorporate the allegations of paragraphs 1-51 of this Complaint as if set forth at length and in full herein.

53. Upon information and belief, certain products made, used, sold and offered for sale by Defendants, including, without limitation, the products distributed by Defendants under the trade name “NOK 2,” incorporate or embody the inventions claimed in the '119 Patent.

54. By their actions, Defendants have infringed, and are infringing, one or more of the claims of the '119 Patent.

55. Upon information and belief, Defendants have actively induced others to infringe the claims of the '119 Patent.

56. Defendants’ infringing conduct has been and continues to be unlawful and willful.

57. As a result of Defendants’ acts of infringement, Plaintiffs have suffered and will continue to suffer damages in an amount to be proved at trial.

FIFTH CAUSE OF ACTION
(Infringement of the '986 Patent)

58. Plaintiffs reallege and incorporate the allegations of paragraphs 1-57 of this Complaint as if set forth at length and in full herein.

59. Upon information and belief, certain products made, used, sold and offered for sale by Defendants, including, without limitation, the products distributed by Defendants under the trade names "Rapidcuts Hardcore," "Rapidcuts Femme," and "Razor8," incorporate or embody the inventions claimed in the '986 Patent.

60. By their actions, upon information and belief, Defendants have infringed, and are infringing, one or more of the claims of the '986 Patent.

61. Upon information and belief, Defendants have actively induced others to infringe the claims of the '986 Patent.

62. Defendants' infringing conduct has been and continues to be unlawful and willful.

63. As a result of Defendants' acts of infringement, Plaintiffs have suffered and will continue to suffer damages in an amount to be proved at trial.

SIXTH CAUSE OF ACTION
(Inducement of Infringement of the '765 Patent)

64. Plaintiffs reallege and incorporate the allegations of paragraphs 1-63 of this Complaint as if set forth at length and in full herein.

65. Upon information and belief, Defendants have actively induced others to infringe the claims of the '765 Patent through their promotion and marketing of "Rapidcuts Hardcore," "Rapidcuts Femme," and "Razor8."

66. Defendants' conduct has been and continues to be unlawful and willful.

67. As a result of Defendants' acts of inducement of infringement, Plaintiffs have suffered and will continue to suffer damages in an amount to be proved at trial.

SEVENTH CAUSE OF ACTION
(False Designation of Origin in Violation of 15 U.S.C. § 1125(a)(1)(A))

68. Plaintiffs reallege and incorporate the allegations of paragraphs 1-67 of this Complaint as if set forth at length and in full herein.

69. This claim is for false designation of origin that is likely to cause confusion, mistake, and deception as to the affiliation, connection, or association of Defendants' products with Plaintiffs' products in violation of Section 43(a) of the Lanham Act, 15 U.S.C. § 1125(a)(1)(A).

70. Plaintiffs manufacture, market and sell a range of nutritional supplement products, including, but not limited to, products known by the trade-names "Hydroxycut," "Hydroxycut Hardcore," "Anator-P70," and "Leukic."

71. Plaintiffs have used the "Hydroxycut" trade-name and -mark since at least 1998, and obtained Federal trademark registration for "Hydroxycut" on August 4, 1998 (registration number 2178046). Plaintiffs have used the "Anator-P70" trade-name and -mark since at least 2006, and obtained Federal trademark registration for "Anator-P70" on October 30, 2007 (registration number 3326676). Plaintiffs have used the "Leukic" trade-name and -mark since at least 2005, and obtained Federal trademark registration for "Leukic" on November 13, 2007 (registration number 3336514).

72. Plaintiffs' products generally, and "Hydroxycut," "Hydroxycut Hardcore," "Anator-P70," and "Leukic" in particular, are widely-recognized for their superior quality, and enjoy substantial popularity with professional body builders, elite athletes, and fitness-conscious consumers alike.

73. As a result of Plaintiffs' exclusive and extensive use of "Hydroxycut," "Hydroxycut Hardcore," "Anator-P70," and "Leukic" in connection with its nutritional supplements products and merchandise, those marks have become exclusively associated with Plaintiffs by consumers. Plaintiffs have developed extensive goodwill in their brand, and in their "Hydroxycut," "Hydroxycut Hardcore," "Anator-P70," and "Leukic" products through years of substantial effort and investment, including expending significant resources to develop, advertise and promote their products' distinctive look and feel.

74. Plaintiffs advertise and promote their products in national fitness publications such as *Flex*, *MuscleMag*, and *Muscular Development*. Plaintiffs also advertise their products nationally via the Internet, and expend considerable effort and expense in graphic art associated with product packaging and point-of-purchase display.

75. Plaintiffs' efforts have generated goodwill and have created a legally cognizable interest in the products, including the unique and distinctive name of their products, as well as the distinctive and well-known presentation of the products in the marketplace, through product packaging, marketing, and advertising.

76. Plaintiffs have defended this goodwill and interest in the distinctive look and feel of their products.

77. Plaintiffs' mark, when used on or in connection with nutritional supplements products, is identified and associated in consumers' minds exclusively with Plaintiffs. Plaintiffs' mark is strong, highly distinctive of Plaintiffs, and entitled to the broadest scope of protection.

78. Defendants market and sell a range of nutritional supplements targeted to health conscious consumers. On information and belief, Defendants' products, generally, are targeted

to the precise class of consumers as Plaintiffs' products, and are sold through virtually the same channels of sale, and are advertised in the same or similar fitness publications.

79. Defendants are not affiliated with Plaintiffs, and have never been licensed or otherwise authorized by Plaintiffs to use Plaintiffs' marks, or to appropriate the goodwill associated with Plaintiffs' product trade-names, or the distinctive look and feel of Plaintiffs' products, product packaging or advertising.

80. The Defendants market and sell two products called "Rapidcuts Hardcore" and "Leutor 70." Upon information and belief, Defendants intended to cause customer confusion and are causing customer confusion by, among other things: (1) naming one of their products "Rapidcuts Hardcore," which is substantially similar in name, look and feel to Plaintiffs' leading "Hydroxycut Hardcore" product and which purports to have the same or similar benefits; (2) naming one of their products "Leutor 70," which is substantially similar in name, look and feel to Plaintiffs' leading "Leukic" and "Anator-P70" products and which purports to have the same or similar benefits; and (3) packaging and marketing their "Rapidcuts Hardcore" product with an insignia nearly indistinguishable from that utilized by Plaintiffs in their "MD" product line.

81. The Plaintiffs have been and will continue to suffer damage to the extent that consumers mistakenly associate Defendants' products with those of the Plaintiffs. For example, consumers who are dissatisfied with Defendants' products, sold under the confusingly similar packaging and product names "Rapidcuts Hardcore" and "Leutor 70," likely will displace that dissatisfaction on the Plaintiffs. The potential for Defendants' infringing "Rapidcuts Hardcore" and "Leutor 70" products to reflect negatively on Plaintiffs' "Hydroxycut Hardcore," "Leukic,"

and "Anator-P70" products threatens to irreparably harm the valuable goodwill Plaintiffs have built in their trade names over the years.

82. The goodwill that Plaintiffs have built up in their product names, branding, and distinctive look and feel through years of substantial investment and effort is put at risk by virtue of Defendants' commercial misappropriation of Plaintiffs' mark for goods over which Plaintiffs have no control. As a result, Plaintiffs' valuable reputation and the reputation of their "Hydroxycut Hardcore," "Anator-P70," and "Leukic" products, and the valuable reputation of their brand may be permanently damaged.

83. On information and belief, Defendants, by using a similar look and feel in their product packaging, and graphics and marketing materials, intended to cause customer confusion and are causing customer confusion.

84. On information and belief, Defendants' use of the confusingly similar "Rapidcuts Hardcore" and "Leutor 70" product names and packaging is a deliberate attempt to: (1) trade on the goodwill that Plaintiffs have established in their "Hydroxycut Hardcore," "Leukic," and "Anator-P70" products and brand, and (2) create a false impression as to the source and sponsorship of Defendants' products, or (3) otherwise pass off their products as being authorized or endorsed by Plaintiffs.

85. Defendants' activities complained of herein constitute false designation of origin or false description or representation that Defendants' products originate from, or are offered, sponsored, authorized, licensed by or otherwise somehow connected with Plaintiffs. Defendants' conduct is thereby likely to cause confusion, mistake, and deception as to the affiliation, connection, or association under Section 43(a) of the Lanham Act, 15 U.S.C. § 1125(a)(1)(A).

86. Upon information and belief, Defendants have engaged in this illegal conduct willfully, and deliberately, with the intent to appropriate the goodwill developed by Plaintiffs in their products.

87. Plaintiffs have been and will continue to be damaged by Defendants' acts.

EIGHTH CAUSE OF ACTION
(Unfair Competition in Violation of Massachusetts Common Law)

88. Plaintiffs reallege and incorporate the allegations of paragraphs 1-87 of this Complaint as if set forth at length and in full herein.

89. Defendants' activities complained of herein constitute unfair competition under Massachusetts Common Law.

90. Defendants engaged in this illegal conduct in bad faith, willfully, deliberately and intentionally.

91. Defendants' actions have caused and will continue to cause substantial damage to Plaintiffs and/or irreparable harm for which there is no adequate remedy at law.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs pray for entry of judgment against Defendant as follows:

A. that Defendants infringe the '199, '900, '396, '119, and '986 Patents;

B. that Defendants have actively induced others to infringe the '199, '900, '396, '119, '986, and '765 Patents;

C. that Defendants' infringement and inducement of infringement of the '199, '900, '396, '119, '986, and '765 Patents is willful;

D. that Defendants, their officers, directors, affiliates, agents, servants, employees and attorneys, and all those persons in privity or in concert with any of them, be preliminarily and permanently enjoined from infringement of the '199, '900, '396, '119, '986, and '765 Patents;

E. that Plaintiffs be awarded their damages for infringement of the '199, '900, '396, '119, '986, and '765 Patents, and that the damages be trebled;

F. that this case be declared to be exceptional in favor of Plaintiffs under 35 U.S.C. § 285, and that Plaintiffs be awarded their costs, attorneys' fees, and other expenses incurred in connection with this action;

G. that Defendants have violated 15 U.S.C. § 1125(a);

H. that Defendants' violation of 15 U.S.C. § 1125(a) is willful;

I. that Defendants, their officers, directors, affiliates, agents, servants, employees and attorneys, and all those persons in privity or in concert with any of them, be preliminarily and permanently enjoined from further violation of 15 U.S.C. § 1125(a);

J. that the injunction expressly enjoins Defendants, their officers, directors, affiliates, agents, servants, employees and attorneys, and all those persons in privity or in concert with any of them from using any false designation or origin or false description (including, without limitation, any colors, letters or symbols), or performing any act, which can, or is likely to, lead members of the trade or public to believe that any product or service rendered, offered for sale, advertised or promoted by Defendants is in any way produced, sponsored, licensed or approved by, or connected or affiliated or associated with Plaintiffs;

K. that the injunction orders Defendants and their successors and assigns to deliver up to the Court for destruction all merchandise, labels, signs, prints, packages, receptacles, advertisements and promotional materials bearing Plaintiffs' distinctive mark, or any mark

confusingly similar to that mark, and all plates, molds, matrices and other means of making the same;

L. that the injunction orders that Defendants, in accordance with 15 U.S.C. § 1116, file with the Court and serve on the Plaintiffs, within thirty days after service on Defendants of any injunction issued in this action, a report in writing, setting forth in detail the manner and form in which Defendants have complied with the injunction;

M. that Plaintiffs be awarded their damages for Defendants' violation of 15 U.S.C. § 1125(a) and that the damages be trebled;

N. that Plaintiffs be awarded costs, attorneys' fees and other expenses incurred in connection with such violation of 15 U.S.C. § 1125(a);

O. that Defendants be directed to account for and pay over to Plaintiffs the profits, gains, and advantages obtained or derived by Defendants from their acts of infringement of Plaintiffs' mark, and awarding Plaintiffs treble that amount pursuant to 15 U.S.C. § 1117;

P. that Defendants have violated Massachusetts' common and statutory law by engaging in unfair competition;

Q. that Plaintiffs be awarded compensatory, actual, punitive and exemplary damages and attorneys' fees and costs of suit stemming from Defendants' violations of Massachusetts law; and

R. that Plaintiffs be awarded such other and further relief as may be appropriate.

DEMAND FOR JURY TRIAL

Plaintiffs demand a trial by jury.

Dated: June 25, 2008

Respectfully submitted,

/s/ Brian C. Barry

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Attorneys for Plaintiffs

CERTIFICATE OF SERVICE

I hereby certify that on the date indicated below I caused a copy of the foregoing motion to be filed with the Court's ECF filing system, which will cause an electronic notice to be sent to counsel of record.

Dated: June 25, 2008 */s/ Brian C. Barry*

Exhibit A

US005973199A

United States Patent [19]

Negrisoli et al.

[11] **Patent Number: 5,973,199**

[45] **Date of Patent: *Oct. 26, 1999**

[54] **HYDROSOLUBLE ORGANIC SALTS OF CREATINE**

[75] Inventors: **Gianpaolo Negrisoli**; **Lucno Del Corona**, both of Bergamo, Italy

[73] Assignee: **Flamma S.p.A.**, Bergamo, Italy

[*] Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

[21] Appl. No.: **08/649,620**

[22] PCT Filed: **Jul. 21, 1995**

[86] PCT No.: **PCT/EP95/02897**

§ 371 Date: **May 22, 1996**

§ 102(e) Date: **May 22, 1996**

[87] PCT Pub. No.: **WO96/04240**

PCT Pub. Date: **Feb. 15, 1996**

[30] **Foreign Application Priority Data**

Aug. 4, 1994 [IT] Italy MI94A001693

[51] **Int. Cl.**[6] **C07C 241/00**

[52] **U.S. Cl.** **562/560**

[58] **Field of Search** 562/560

[56] **References Cited**

U.S. PATENT DOCUMENTS

1,967,400	7/1934	Fischl	562/560
4,420,432	12/1983	Chibata	562/560
5,091,171	2/1992	Yu	424/642
5,387,696	2/1995	Kottenhahn	548/533
5,489,589	2/1996	Wittman	514/232.8
5,627,172	5/1997	Almada	514/120

FOREIGN PATENT DOCUMENTS

0 669 083	8/1995	European Pat. Off. .	
53-6204	3/1978	Japan	562/560
94/02127	2/1994	WIPO .	

OTHER PUBLICATIONS

Chemical Abstracts, vol. 84, No. 1, 1976 Columbus, Ohio, US; p. 13433.

ACTA Physiol. Scand. (1995) , 153 (2), 207–9 Coden: APSCAX: ISSN: 0001–6772, 1995 Earnest, C.P. et al.

Primary Examiner—Michael L. Shippen

Attorney, Agent, or Firm—Griffin, Butler, Whisenhunt & Szipl,

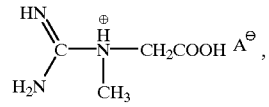
[57] **ABSTRACT**

Hydrosoluble organic salts of creatine are disclosed. The salts are useful in the dietetic and food industry.

3 Claims, No Drawings

HYDROSOLUBLE ORGANIC SALTS OF CREATINE

The present invention refers to hydrosoluble organic salts of creatine of general formula I:



(I)

wherein $A^{\ominus}$ represents the anion of a mono, bi- or tricarboxylic acid. Preferred anions are the citrate, maleate, fumarate, tartrate or malate.

Creatine or N-(aminoiminomethyl)-N-methylglycine is a sarcosine derivative present in the muscle tissue of many vertebrates, man included, mainly combined with phosphoric acid in form of phosphorylcreatine and it is involved in the energy transfer from mitochondria to the ATP utilization sites.

Several studies indicate that there is a relationship between the creatine (phosphoryl creatine) concentration in the muscles having the function of keeping an high intracellular ATP/ADP ratio and maximum sustainable physical effort (Annu. Rev. Biochem. 54: 831–862, 1985; Science 24: 448–452, 1981; BESSMAN S. P., and F. SAVABI. The role of the phosphocreatine energy shuttle in exercise and muscle hypertrophy. In: Biochemistry of Exercise VII. A. W. Taylow, P. D. Gollnick, H. J. Green, C. D. Ianuzzo, E. G. Noble, G. Metivier, and J. R. Sutton., Intl. Series Sports Sciences 21: 167–178, 1990).

The creatine increase in diets may therefore be useful to bring the plasma creatine concentrations at levels providing significant values of creative itself in the muscle. The short creatine half-life in plasma (1–1.5 hours) makes however necessary to reach rapidly said levels and this, in view of the bioavailability degree of creatine, is obtainable only by the administration of high doses of 5–10 g (for mean body weights of 70 kg), amounts well tolerated because of the lack of toxicity of the compound.

The low solubility of creatine in water (1 g in 75 ml) is therefore a practical limitation to the possibility of marking immediately available in the specific diet the necessary amounts of creatine.

WO 94/02127, published on Feb. 3, 1994, discloses the use of creatine, optional combined with aminoacids or other components, in order to increase the muscle performance in mammals.

The present invention provides hydrosoluble stable organic salts of creatine of formula I characterized by high water solubility (from 3 to 15 times higher them that of creatine itself) and a process for their preparation. The salts of formula I are prepared by salifying creatine with the corresponding acids in aqueous or hydroalcoholic concentrated solution or in a water-immiscible solvent, at temperatures ranging from the room temperature to 50° C., optionally concentrating the solutions and filtering the crystallized salts. According to a preferred embodiment the salts of formula I are prepared by reacting creatine with an excess organic acid in ethyl acetate until the salt is completely formed, detectable with the IR analysis, cooling and filtering. The filtrated solvent, containing the excess acid is recycled and, after filling up of the components, is used for a further reaction.

The salts are characterized by IR, melting point, potentiometric and HPLC assay.

Table 1 reports the solubility of the salts I of the invention.

TABLE 1

Creatine salt	Water solubility % (g/100 ml)
Citrate	10
Maleate	19
Fumarate	3
Tartarate	8,5
Malate	4,5

EXAMPLE 1

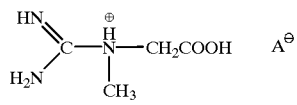
39.45 g (0.18 mol) of monohydrate citric acid are suspended in 100 ml of ethyl acetate. 20 g (0.134 mol) of monohydrate creatine are added to the stirred suspension at 20–25° C. and the mixture is stirred 4 hours at 25° C. After IR control, the product is filtered and washed with ethyl acetate, then dried in oven at 50–55° C., obtaining 90% of salts, m.p. 112–114° C., 99.2% titer.

EXAMPLE 2

14.9 g (0.1 mol) of monohydrate creatine are added to a solution of 11.6 g (0,1 mol) of maleic acid in 20 ml of water. The so obtained solution is concentrated, cooled to 5° C. and the product filtered and dried under vacuum at 50° C., obtaining 87% of salt, m.p. 128–129° C., 99.8% titer.

We claim:

1. An isolated hydrosoluble salt of creatine of the formula:



wherein A^- represents the anion of citric, maleic, fumaric, or malic acid.

2. The hydrosoluble salt of claim **1**, wherein A^- is a citrate anion, said salt having a melting point of 112–114° C.

3. The hydrosoluble salt of claim **1**, wherein A^- is a maleate anion, said salt having a melting point of 128–129° C.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,973,199

DATED : October 26, 1999

INVENTOR(S) : NEGRISOLI et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page item [75], change "Gianpaolo" to --Giampaolo-- and change "Lucno" to --Lucio--.

Signed and Sealed this

Twenty-eighth Day of March, 2000

Attest:

Q. TODD DICKINSON

Attesting Officer

Commissioner of Patents and Trademarks

Exhibit B

US005968900A

United States Patent [19]

Greenhaff et al.

[11] **Patent Number: 5,968,900**

[45] **Date of Patent: Oct. 19, 1999**

[54] **INCREASING CREATINE AND GLYCOGEN CONCENTRATION IN MUSCLE**

[75] Inventors: **Paul Leonard Greenhaff**, Wollaton; **Allison Lesley Green**, The Park; **Ian Andrew MacDonald**, Beeston, all of United Kingdom; **Eric Hultman**, Stockholm, Sweden

[73] Assignee: **The University of Nottingham**, Nottingham, United Kingdom

[21] Appl. No.: **08/875,326**

[22] PCT Filed: **Dec. 15, 1995**

[86] PCT No.: **PCT/GB95/02933**

§ 371 Date: **Sep. 29, 1997**

§ 102(e) Date: **Sep. 29, 1997**

[87] PCT Pub. No.: **WO96/18313**

PCT Pub. Date: **Jun. 20, 1996**

[30] **Foreign Application Priority Data**

Aug. 25, 1995 [GB] United Kingdom 9517443

[51] **Int. Cl.**[6] **A61K 38/28**; A61K 31/70; A61K 31/715; A61K 31/195

[52] **U.S. Cl.** **514/3**; 514/4; 514/23; 514/53; 514/54; 514/565

[58] **Field of Search** 514/3, 4, 23, 53, 514/54, 565

[56] **References Cited**

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Primary Examiner—Kimberly Jordan
Attorney, Agent, or Firm—Watts, Hoffmann, Fisher & Heinke Co., L.P.A.

[57] **ABSTRACT**

Compositions herein increased creatine retention and/or glycogen storage in muscle. A composition comprises creatine or its derivative and a carbohydrate or its derivative. The carbohydrate is in an amount by weight which is greater than the amount of creatine. The amount of carbohydrate and the amount of creatine are effective for increasing creatine retention and/or glycogen storage in muscle. The compositions may be in the form of a pharmaceutical or a dietary supplement and are intended for use in the human or animal body. Other compositions comprise creatine or an active derivative together with insulin or an active derivative. The amount of creatine and the amount of insulin are effective for increasing creatine retention and/or glycogen storage in muscle. The compositions including creatine and insulin may further contain a carbohydrate or its derivative. A method of increasing creatine retention in a human or animal body comprises causing an increase in blood plasma creatine concentration and causing a substantially simultaneous increase in blood plasma insulin concentration. A method of increasing glycogen storage in a human or animal body comprises causing an increase in blood plasma creatine carbohydrate concentration and causing a substantially simultaneous increase in blood plasma creatine concentration. The compositions to increase the creatine retention and/or glycogen storage in the muscle are administered by injection or ingestion.

53 Claims, 3 Drawing Sheets

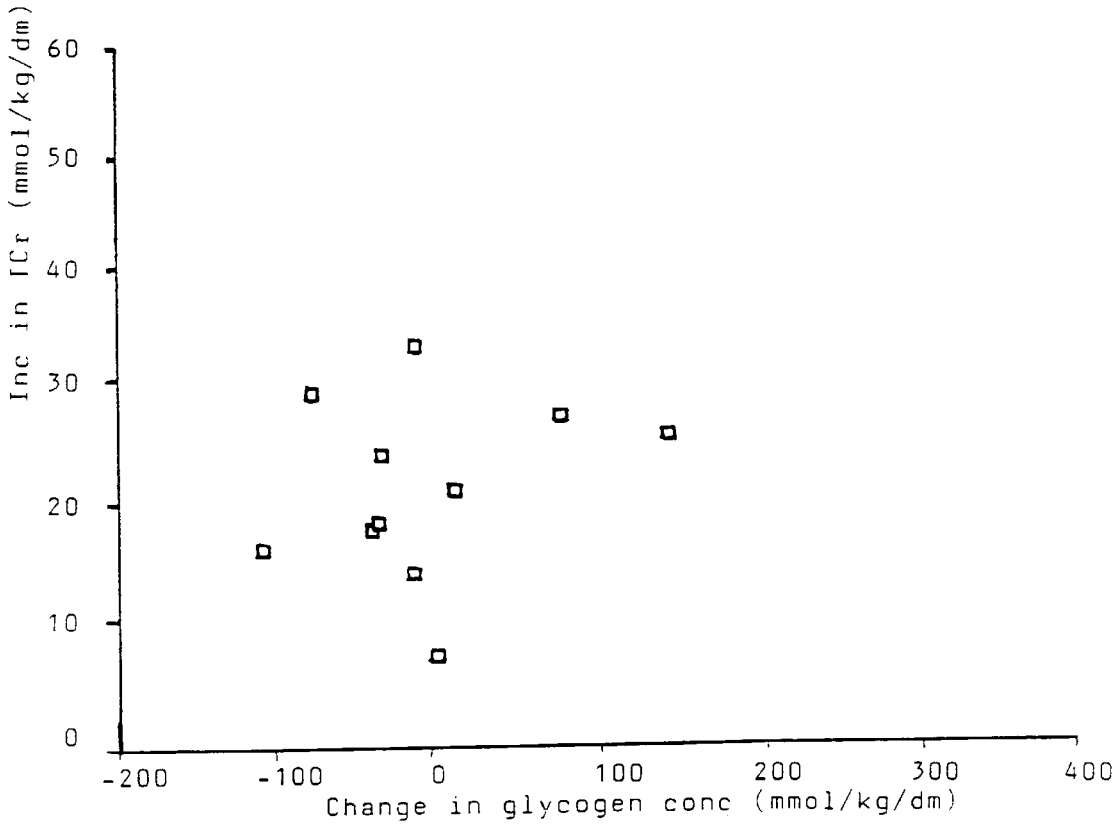

FIGURE 1

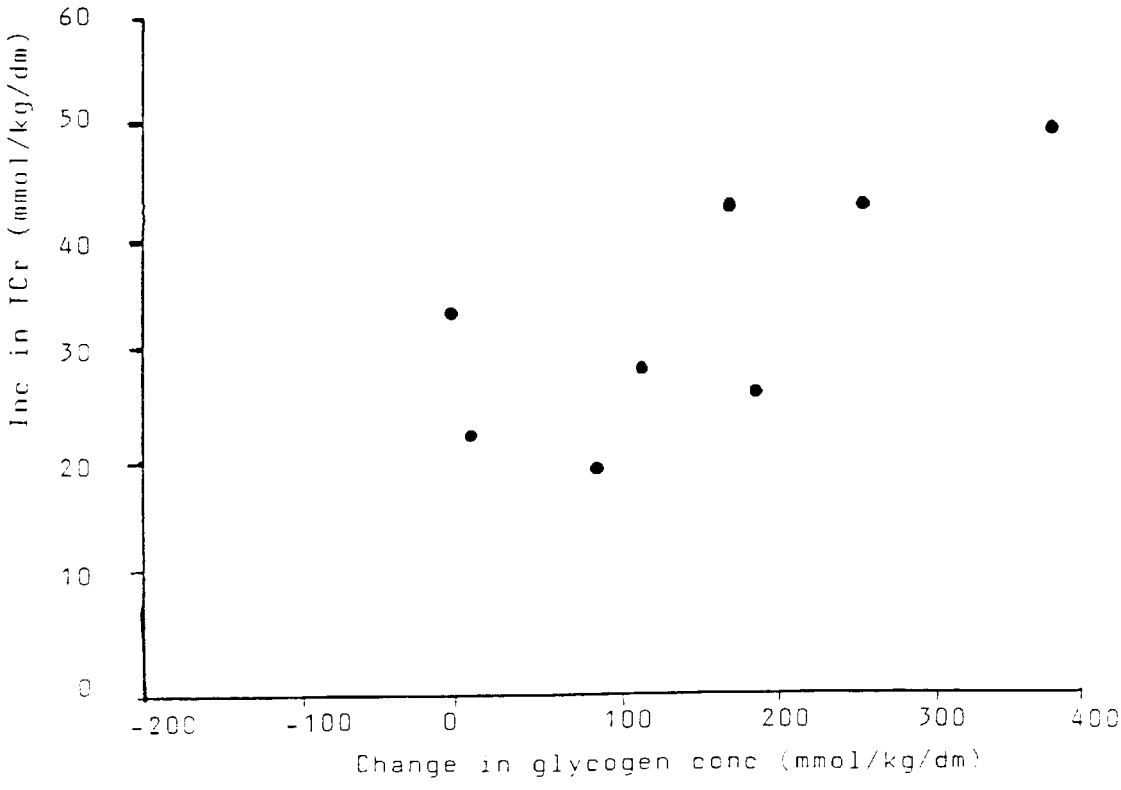

FIGURE 2

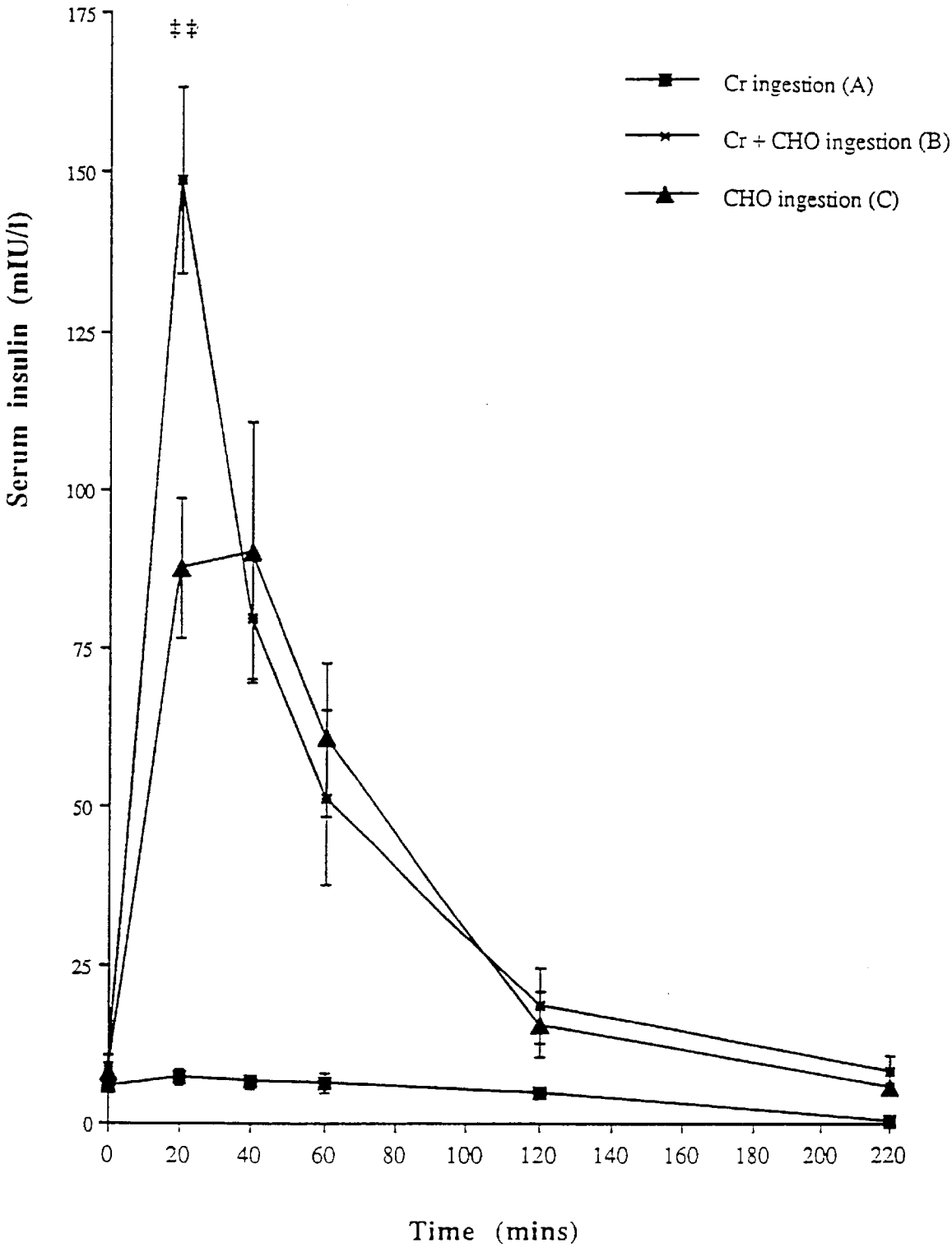
175
150
125
100
75
50
25
0
‡‡
Serum insulin (mIU/l)
Cr ingestion (A)
Cr + CHO ingestion (B)
CHO ingestion (C)
0
20
40
60
80
100
120
140
160
180
200
220
Time (mins)

FIGURE 3

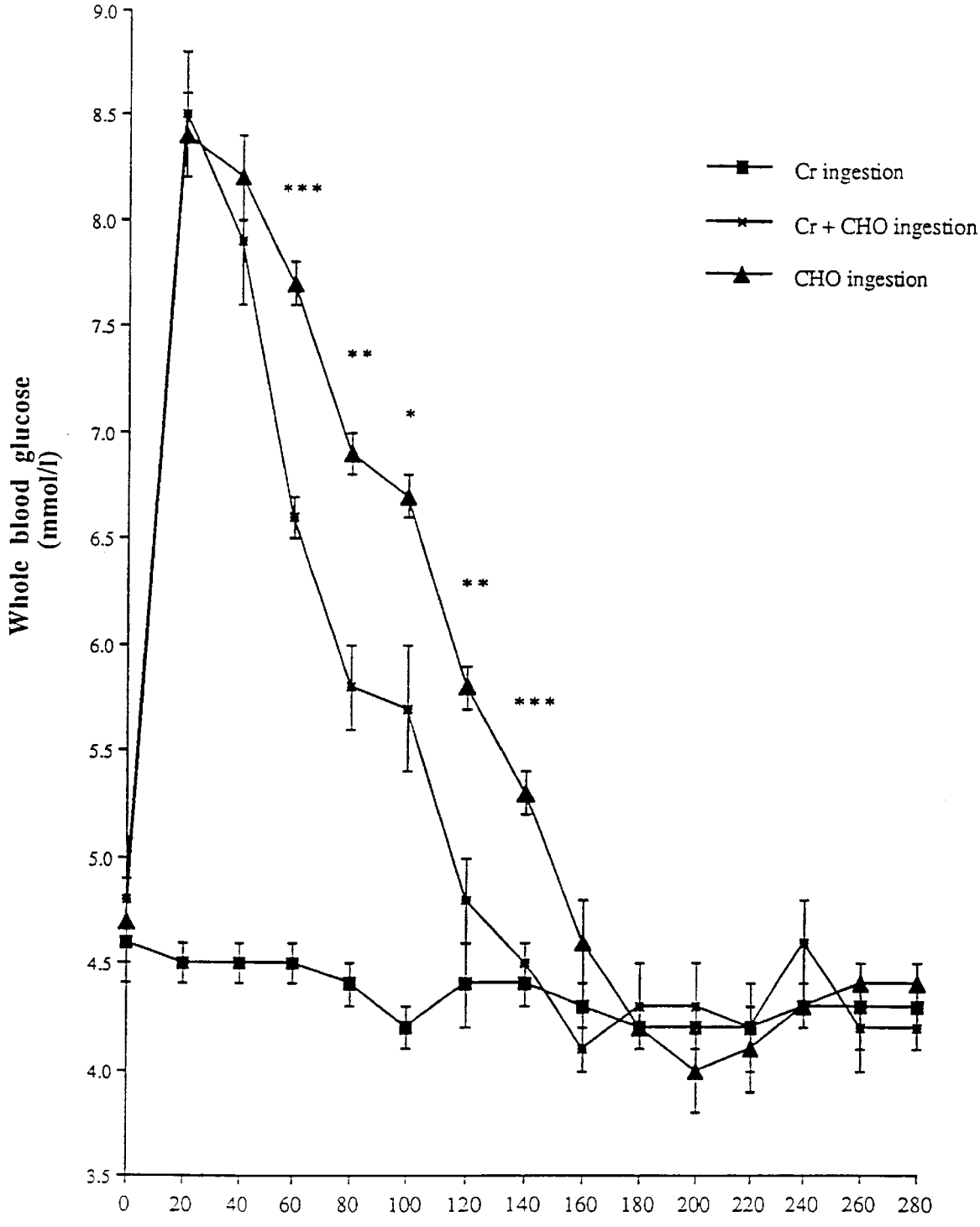
Whole blood glucose (mmol/l)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
Time (mins)
Cr ingestion
Cr + CHO ingestion
CHO ingestion
***
**
*
**
***

FIGURE 4

INCREASING CREATINE AND GLYCOGEN CONCENTRATION IN MUSCLE

This is a 371 of PCT/6B95/02933, filed Dec. 15, 1995.

The present invention concerns the retention of creatine within the body, and relates in particular but not exclusively to a method and composition for increasing creatine uptake in humans. The invention also concerns a method and composition for simultaneously increasing glycogen concentration in muscle.

Creatine (methylglycocyamine, $H_2NC{=}NH{\cdot}N(CH_3)CH_2CO_2H$) is known to be present in the muscles of vertebrates. It is present in a phosphorylated and a non-phosphorylated form and has been shown to be involved in muscular contraction and the development of fatigue. Creatine is produced naturally by the body, but is also obtained from animal foods.

Most bodily creatine is present in muscle, and it is believed that increasing the amount of creatine within muscle favorably affects muscular performance and the amount of work which can be done by the muscle. Accordingly, it is held desirable to be able to influence creatine retention in the body.

Glycogen, $(C_6H_{10}O)x$, is a carbohydrate found in animal cells and is convertible from and to glucose. Athletes endeavour to increase muscle glycogen content before competing in order to enhance muscle performance.

In this specification the term "active derivative" means anything derived from or a precursor of the relevant substance that acts in the same or similar way in the body to the substance, or which is processed into the substance when placed into the body. The terms serum and plasma can be interchanged.

According to the invention there is provided a method of increasing creatine retention in the human or animal body by causing an increase in blood plasma creatine concentration and causing a substantially simultaneous increase in blood plasma insulin concentration.

The plasma creatine concentration may be increased by ingestion and/or in fusion of creatine or an active derivative thereof.

The plasma insulin concentration may be increased by infusion of insulin or an active derivative thereof and/or by the ingestion of an agent operable to cause an increase in the blood plasma insulin concentration.

The agent may be a carbohydrate or an active derivative thereof, preferably a simple carbohydrate. Preferably the carbohydrate is glucose.

Preferably the method comprises the simultaneous ingestion of creatine and an agent operable to cause an increase in the blood plasma insulin concentration substantially simultaneously with the arrival in the plasma of the creatine.

The creatine and/or the agent is preferably orally ingested.

The invention further provides a method of increasing glycogen storage, and particularly glycogen concentration in muscle of the human or animal body by causing an increase in blood plasma carbohydrate concentration and insulin concentration and causing a substantially simultaneous increase in blood plasma creatine concentration.

The plasma creatine concentration may be increased by ingestion and/or infusion of creatine or an active derivative thereof. The plasma carbohydrate, which is desirably glucose and insulin concentrations may be increased by ingestion of carbohydrate or an active derivative thereof, but desirably glucose and/or any other simple carbohydrate and/or by infusion of a carbohydrate or an active derivative thereof, such as glucose or any other simple carbohydrate.

Preferably creatine or an active derivative thereof and glucose and/or another simple carbohydrate are orally ingested.

According to the invention there is further provided a composition for increasing creatine retention in the human or animal body, the composition comprising creatine or an active derivative thereof together with a carbohydrate or an active derivative thereof.

Preferably the composition is in the nature of a dietary supplement.

Preferably the carbohydrate is glucose and/or another simple carbohydrate.

The composition preferably comprises 2 to 8% by weight creatine and 92 to 98% by weight glucose and/or another simple carbohydrate.

According to the invention there is also provided a method of increasing creatine retention in the human or animal body by ingestion and/or injection of a composition as hereinbefore described. Preferably the composition is ingested in an amount of 100 g to 700 g per day. Which may be taken in four equal parts throughout the day.

Further according to the present invention there is provided a composition for increasing creatine retention in the human or animal body, the composition comprising creatine or an active derivative thereof together with insulin or an active derivative thereof.

Further according to the present invention there is provided a composition for increasing glycogen storage in the human or animal body and particularly glycogen concentration in muscle, the composition comprising creatine or an active derivative thereof together with insulin or an active derivative thereof.

The composition may be in a form to be ingested and/or injected into the body.

According to the invention there is also provided a method of increasing creatine retention in the human or animal body by ingestion and/or injection of a composition as described above.

According to a further aspect of the invention there is provided a method increasing glycogen storage in the human or animal body and particularly glycogen concentration in muscle by ingestion and/or injection of a composition as described above.

Preferably a carbohydrate, or an active derivative thereof, is also ingested and/or injected desirably such that an increase in blood plasma carbohydrate concentration and insulin concentration occurs substantially simultaneously with an increase in blood plasma creatine concentration.

According to the invention there is also provided a composition for increasing glycogen storage in the animal or human body and particularly glycogen concentration in muscle of the human or animal body, the composition comprising creatine or an active derivative thereof together with a carbohydrate or an active derivative thereof.

Preferably the composition is in the nature of a dietary supplement.

Preferably the carbohydrate is glucose and/or another simple carbohydrate.

The composition preferably comprises 2 to 8% by weight creatine and 92 to 98% by weight glucose and/or another simple carbohydrate.

According to the invention there is also provided a method of increasing glycogen storage in the human or animal body and particularly glycogen concentration in muscle by ingestion and/or injection of a composition as hereinbefore described.

Preferably the composition is ingested in an amount of 100 g to 700 g per day, which may be taken in four equal parts throughout the day.

According to the invention there is further provided a composition comprising creatine or an active derivative thereof and a carbohydrate or an active derivative thereof for use as an active pharmaceutical composition.

The invention also provides a composition comprising creatine or an active derivative thereof and insulin or an active derivative thereof for use as an active pharmaceutical preparation. The composition may also comprise a carbohydrate or an active derivative thereof.

The invention further provides creatine or an active derivative thereof and a carbohydrate or an active derivative thereof for use in the manufacture of a substance for increasing creatine retention in the human or animal body.

The invention also provides a composition comprising creatine or an active derivative thereof, and insulin or an active derivative thereof, for use in the manufacture of a substance for increasing creatine retention and/or glycogen storage in the human or animal body, such as muscle. Carbohydrate or an active derivative thereof may also be provided.

The invention further provides a composition comprising creatine or an active derivative thereof and a carbohydrate or an active derivative thereof for use in the manufacture of a substance for increasing glycogen concentration in muscle of the human or animal

Preferably the carbohydrate is glucose and/or another simple carbohydrate.

The composition preferably comprises 2 to 8% by weight creatine and 92 to 98% by weight glucose and/or another simple carbohydrate.

The methods and compositions of the invention may be used to increase bodily creatine retention in humans. This is desired, for example, by sportsmen and athletes to avoid or delay the onset of muscular fatigue. The ability to increase creatine retention may also be desired in individuals having relatively low general creatine levels, for example vegetarians who do not take animal protein, and sufferers of disease which affects muscle. The present invention enables creatine retention to be increased to a greater extent than is achieved by making creatine available to the body alone.

The invention also permits the increase of muscle glycogen concentration. This is desired by athletes to enhance performance. Also, increasing the glycogen concentration in muscle is of interest where insulin sensitivity of the body is impaired by, for example, obesity, diabetes, heart failure or post-surgical trauma.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be further described for the purposes of illustration only with reference to the following examples and to the drawings, in which:

FIG. **1** is a graph showing increase in total creatine concentration against change in glycogen concentration in subjects of group A of Example 2;

FIG. **2** is a similar graph for subjects of group B of Example 2;

FIG. **3** is a graph showing serum insulin concentration against time for all groups in Example 4; and

FIG. **4** is a graph showing blood plasma glucose concentration against time, for all groups in Example 4.

EXAMPLE 1

Experimental

16 men were randomly divided into groups 1 (6 members), 2 (6 members) and 3 (4 members). On day one, fasted subjects gave a blood sample and then consumed the following preparations:

Group 1–5 g creatine in 250 ml low calorie hot orange

Group 2–5 g creatine in 250 ml low calorie hot orange plus 500 ml of a glucose drink (LUCOZADE(™) manufactured by Smith Kline Beecham), containing 90–100 g simple sugars.

Group 3–250 ml of low calorie hot orange

Arterialized-venous blood samples were then obtained at 20 minute intervals for the next 4½ hours, while subjects remained in a supine position. For the remainder of the day, and throughout day two, subjects ingested the mentioned preparations at 4 hourly intervals, representing a total daily creatine dose of 20 g. On the morning of day three the subjects reported back to the laboratory and underwent the same procedures as on the first day. All subjects weighed and recorded their dietary intake throughout the study, subjects in group 2 consuming a prescribed high carbohydrate diet, and undertook 24 hour urine collections on day one and day three. Plasma and urine creatine were measured using high performance liquid chromatography and serum insulin was measured using a radioimmunoassay technique.

Results

The results are shown in Table 1, in which CR=creatine. Plasma creatine concentration (u mol/l) was plotted against time for each group, and the area under each curve was determined. Urinary creatine (g) and peak serum insulin (mIU/l) were also determined.

Plasma creatine concentrations peaked within 90 minutes of creatine ingestion and declined towards resting values during the remaining 180 minutes of the 4½ hour period. The area under the plasma creatine curve was lower in group 2 than in group 1, as was urinary creatine content. Following carbohydrate ingestion, serum insulin levels peaked within 30 minutes in group 2 and returned to the pre-ingestion concentration over the remaining 240 minutes. Plasma insulin concentration did not change in group 1 or group 3 over the course of the experiment.

TABLE 1

	Group 1		Group 2	
	Mean	SE	Mean	SE
		Day 1		
Area under plasma CR (umol/l/min)	2834.1	298.1	883.9^{++}	109.9
Urinary CR (g)	9.5	1.2	5.0*	0.8
Peak serum insulin (mIU/l)	7.8	1.3	72.0^{++}	11.2
		Day 3		
Area under plasma CR (umol/l/min)	2637.5	228.6	948.3*	454.5
Urinary CR (g)	11.9	1.1	5.7^{+}	1.2
Peak serum insulin (mIU/l)	9.5	2.0	84.2^{++}	11.5

*$P < 0.05$; $^{+}P < 0.01$; $^{++}P < 0.001$ - significantly different from corresponding value.

The reduced area under the plasma creatine curve and the lower urinary creatine content of those subjects which had ingested creatine and carbohydrate compared with those

which had ingested only creatine shows that bodily uptake of creatine is greater in the second group. This increase in creatine uptake is believed to be insulin mediated, the plasma insulin concentration being increased by the ingested carbohydrate.

EXAMPLE 2

Experimental

A muscle biopsy sample was taken from the vastus lateralis muscle of each of 21 healthy males and was frozen in liquid nitrogen for subsequent biochemical analysis. Beginning the following day, 12 subjects (group A) each ingested 5 g of creatine dissolved in hot sugar-free orange juice, four times a day for 5 days. The remaining 9 subjects (group B) proceeded as group A, but in addition consumed 500 ml of LUCOZADE, 30 minutes after each creatine preparation had been ingested. Subjects returned the day after the 5th day of supplementation and further muscle biopsy samples were taken. 24 hour urinary collections were made prior to the first biopsy sample (control) and on the first day of creatine supplementation (day 2). Urinary creatine content (in grams) was then measured using high performance liquid chromatography.

Results

Table 2 shows the muscle concentration (mmol/kg dry mass, mean +S.E.M.) of phosphorylated creatine (PCr) non-phosphorylated creatine (Cr) and total creatine (TCr) before and after creatine supplementation. Significant differences between the groups are indicated by an asterisk $p<0.05$.

TABLE 2

	Before Creatine Supplementation	After Creatine Supplementation
	PCr	
Group A	85.1 ± 2.5	92.4 ± 2.1
Group B	84.4 ± 3.8	99.4 ± 2.6*
	Cr	
Group A	36.4 ± 1.7	49.8 ± 1.5
Group B	39.0 ± 2.3	57.1 ± 3.4*
	TCr	
Group A	121.5 ± 3.1	142.2 ± 2.6
Group B	123.4 ± 4.3	156.4 ± 5.4*

The increase in total creatine concentration after supplementation in group B was approximately 60% greater than that in group A. This increase comprises increases in both phosphorylated and non-phosphorylated creatine. Urinary creatine content was greater in group A than in group B on day 2 but there was no difference between the groups on the control day.

These results indicate that carbohydrate ingestion increases uptake of creatine in muscle in man, and to a far greater extent than to that seen when creatine alone is ingested.

EXAMPLE 3

The muscle samples obtained in the study of Example 2 were additionally analysed for muscle glycogen concentration. Muscle samples from a further group C containing 8 subjects were also analysed. This group has followed a similar regime to groups. A and B but ingested a preparation of carbohydrate but no creatine, in the form of 500 ml LUCOZADE, at same times as subjects of Groups A and B.

Table 3 shows the muscle concentration (mmol/kg) of glycogen before and after supplementation, and also the difference in the concentration.

TABLE 3

	before supplementation	after supplementation	difference
Group A			
mean	364.8	366.1	1.2
sd	63.4	65.8	67.9
se	19.1	19.8	20.5
Group B			
mean	331.1	488.7	157.6
sd	32.5	125.4	126.8
se	10.8	41.8	42.3
Group C			
mean	337.5	413.3	75.8
sd	37.3	55.9	33.2
se	13.2	19.8	11.7

sd = standard deviation, se = standard error

Table 3 shows that the mean glycogen difference after supplementation in Group A, who took creatine only, was very small.

The subjects of Group C, who took glucose only, showed an increase in muscle glycogen concentration after supplementation. However, a more marked increase in muscle glycogen concentration was shown by Group B, who took creatine and glucose together. The results of individual subjects in Group B varied greatly. However, referring to FIG. **2** it is shown that there was a linear relationship between the increase in creatine concentration and the increase in glycogen concentration in subjects of this group, showing a synergistic effect. No such relationship was observed in the subjects in Group A, who ingested only creatine (FIG. **1**).

EXAMPLE 4

Experimental

Twenty nine fasted subjects were divided randomly into three groups, group A (12 subjects), group B (9 subjects) and group C (8 subjects). Each member of group A ingested 5 g of creatine dissolved in hot sugar-free orange juice. Each member of group B ingested 5 g of creatine dissolved in hot sugar-free orange juice along with 500 ml of LUCOZADE, 30 minutes after the creatine preparation had been ingested. Group C ingested 500ml of LUCOZADE alone.

Arterialised-venous blood samples were obtained from each member of each group before ingestion and at 20 minute intervals immediately following ingestion for the following 220 minutes, while subjects remained in a supine position. Blood serum insulin concentration was measured in each sample, and the results are shown in Table 4 below. Serum insulin concentration (mIU/l) was plotted against time (mins) for each group and is shown in FIG. **3**.

The whole blood glucose concentration was also measured before ingestion and at 20 minute intervals for the following 280 minutes and the results obtained are shown in Table 5 below. Whole blood glucose (mmol/l) was plotted against time (mins) for each group and is shown in FIG. **4**.

TABLE 4

Gp	Time (min)	Plasma Insulin (mIU/l, mean ± SEM) 0	20	40	60	120	220
A	Creatine	5.8 ± 0.8	7.3 ± 1.2	6.5 ± 1.0	6.3 ± 1.6	4.7 ± 0.3	4.7 ± 0.4
B	Creatine + carbohydrate	8.8 ± 2.0	148.7 ± 14.7	79.7 ± 9.3	51.5 ± 13.7	18.6 ± 6.0	8.2 ± 2.4
C	Carbohydrate	8.0 ± 1.5	87.6 ± 11.1	90.2 ± 20.4	60.8 ± 12.1	15.6 ± 5.2	5.8 ± 0.5

TABLE 5

Gp	Time (min)	Plasma Glucose (mmol/l, mean ± SEM) 0	20	40	60	80	100
A	Creatine	4.6 ± 0.2	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.2 ± 0.1
B	Creatine + carbohydrate	4.8 ± 0.2	8.5 ± 0.3	7.9 ± 0.2	6.6 ± 0.1	5.8 ± 0.5	5.7 ± 0.3
C	Carbohydrate	4.7 ± 0.2	8.4 ± 0.2	8.2 ± 0.2	7.7 ± 0.1	6.9 ± 0.0	6.7 ± 0.1

Gp	Time (min)	120	140	160	180	200	220	240	280
A	Creatine	4.4 ± 0.2	4.4 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.2	4.3 ± 0.1	4.3 ± 0.1
B	Creatine + carbohydrate	4.8 ± 0.2	4.5 ± 0.1	4.1 ± 0.1	4.3 ± 0.2	4.3 ± 0.2	4.2 ± 0.2	4.6 ± 0.2	4.2 ± 0.1
C	Carbohydrate	5.8 ± 0.1	5.3 ± 0.3	4.6 ± 0.2	4.2 ± 0.1	4.0 ± 0.2	4.1 ± 0.2	4.3 ± 0.1	4.4 ± 0.1

The results shown in Table 4 and FIG. **3** clearly show that when creatine is ingested along with carbohydrate (group B), the serum insulin concentration is considerably greater than that found when creatine (group A) and carbohydrate (group C) are ingested alone.

Further, the results shown in Table 5 and FIG. **4**, clearly show that when creatine and carbohydrate (group B) are ingested together, there is a considerably more rapid decline in blood plasma glucose concentration, than when carbohydrate is ingested alone. This is a direct result of the augmented release of insulin into the blood caused by the ingested creatine and glucose composition.

This rapid decline in blood plasma glucose concentration is indicative of an increased uptake of glucose into muscle for glycogen synthesis (as seen in Example 3). In other words, the ingestion, or infusion, of creatine in conjunction with carbohydrate increases muscle glycogen storage.

Modifications may be made within the scope of the invention. In particular the carbohydrate may be varied. for example by the use of another simple carbohydrate such as a di-or trisaccharide, although glucose is preferred because of the rapidity with which it enters the bloodstream after ingestion, causing substantially simultaneous peaks in blood insulin and creatine concentrations, and to maximise plasma insulin increase. The creatine, glucose and/or insulin or active derivatives of any of these may be infused into the blood in any suitable manner, for example by injection.

Further, the carbohydrate may be substituted or accompanied by insulin or an active derivative thereof. Ingestion or injection of compositions comprising creatine (or an active derivative thereof) and insulin (or an active derivative thereof) may be complimented by ingestion of carbohydrate, such as glucose, for example in the form of a drink. The timing of ingestion or injection (infusion) of the composition and carbohydrate is such that the increase in blood plasma carbohydrate concentration and insulin concentration and plasma creatine concentration peak substantially simultaneously.

Whilst endeavouring in the foregoing Specification to draw attention to those features of the invention believed to be of particular importance it should be understood that the Applicant claims protection in respect of any patentable feature or combination of features hereinbefore referred to and/or shown in the drawings whether or not particular emphasis has been placed thereon.

What is claimed is:

1. A method of increasing creatine retention in a human or animal body comprising causing an increase in blood plasma creatine concentration and causing a substantially simultaneous increase in blood plasma insulin concentration.

2. The method according to claim **1** comprising increasing the plasma creatine concentration by ingestion of creatine or an active derivative thereof.

3. The method according to claim **1** comprising increasing the plasma creatine concentration by infusion of creatine or an active derivative thereof.

4. The method according to claim **1** comprising increasing the plasma insulin concentration by infusion of insulin or an active derivative thereof.

5. The method according to claim **1** comprising increasing the plasma insulin concentration by ingestion of an agent operable to cause an increase in the blood plasma insulin concentration.

6. The method according to claim **5** wherein the agent is a carbohydrate or an active derivative thereof.

7. The method according to claim **5** wherein the agent is a simple carbohydrate.

8. The method according to claim **7** wherein the simple carbohydrate is glucose.

9. The method according to claim **5** wherein at least one of the creatine and the agent is orally ingested.

10. The method according to claim **1** comprising increasing the blood plasma creatine concentration by administering creatine or an active derivative thereof and increasing the blood plasma insulin concentration by administering a carbohydrate or an active derivative thereof, wherein the composition comprises the carbohydrate or its derivative in an amount by weight which is greater than an amount of the creatine or its derivative.

11. The method according to claim **10** wherein the composition comprises, in % by weight based upon a total weight of the composition: the creatine or its derivative present in an amount ranging from 2 to 8% and the carbohydrate or its derivative present in an amount ranging from 92 to 98%.

12. The method according to claim 1 comprising ingesting creatine and an agent operable to cause an increase in the blood plasma insulin concentration substantially simultaneously with the arrival in the plasma of the creatine.

13. A method of increasing glycogen storage in a human or animal body comprising causing an increase in blood plasma carbohydrate concentration and insulin concentration and causing a substantially simultaneous increase in blood plasma creatine concentration.

14. The method according to claim 13 comprising increasing the plasma creatine concentration by administering creatine or an active derivative thereof by at least one of ingestion and infusion.

15. The method according to claim 13 comprising increasing the plasma carbohydrate and insulin concentrations by administering a carbohydrate or an active derivative thereof by at least one of ingestion and infusion.

16. The method according to claim 13 comprising increasing the plasma glucose and insulin concentrations by infusion of a carbohydrate or an active derivative thereof, the carbohydrate being selected from the group consisting of glucose and other simple carbohydrates.

17. The method according to claim 13 comprising orally ingesting creatine or an active derivative thereof and a carbohydrate or an active derivative thereof, the carbohydrate being selected from the group consisting of glucose and other simple carbohydrates.

18. The method according to claim 13 comprising increasing the blood plasma creatine concentration by administering creatine or an active derivative thereof and increasing the blood plasma insulin concentration by administering a carbohydrate or an active derivative thereof, wherein the composition comprises the carbohydrate or its derivative in an amount by weight which is greater than an amount of the creatine or its derivative.

19. The method according to claim 18 wherein the composition comprises, in % by weight based upon a total weight of the composition: the creatine or its derivative present in an amount ranging from 2 to 8% and the carbohydrate or its derivative present in an amount ranging from 92 to 98%.

20. A composition for use in a human or animal body, the composition comprising creatine or an active derivative thereof together with a carbohydrate or an active derivative thereof, wherein the composition comprises the carbohydrate or its derivative in an amount by weight which is greater than an amount of the creatine or its derivative, and the amount of the creatine or its derivative and the amount of the carbohydrate or its derivative are effective to increase creatine retention in the body.

21. The composition according to claim 20 wherein the composition is in the nature of a dietary supplement.

22. The composition according to claim 20 wherein the carbohydrate is selected from the group consisting of glucose and other simple carbohydrates.

23. The composition according to claim 20 wherein the composition comprises in % by weight based upon a total weight of the composition: the creatine or its derivative present in an amount ranging from 2 to 8% and the carbohydrate or its derivative present in an amount ranging from 92 to 98%.

24. The composition according to claim 20 wherein the amount of the creatine or its derivative and the amount of the carbohydrate or its derivative are effective to increase glycogen storage in the body.

25. A method of increasing creatine retention in a human or animal body comprising administering a composition comprising creatine or an active derivative thereof together with a carbohydrate or an active derivative thereof by at least one of ingestion and injection, wherein the composition comprises the carbohydrate or its derivative in an amount by weight which is greater than an amount of the creatine or its derivative.

26. The method according to claim 25 wherein the composition is ingested in an amount of 100 g to 700 g per day.

27. The method according to claim 25 wherein the composition is administered in four equal parts throughout the day.

28. The method according to claim 25 wherein the composition comprises, in % by weight based upon a total weight of the composition: the creatine or its derivative present in an amount ranging from 2 to 8% and the carbohydrate or its derivative present in an amount ranging from 92 to 98%.

29. The method of claim 25 wherein the carbohydrate is selected from the group consisting of glucose and other simple carbohydrates.

30. A composition for use in a human or animal body comprising creatine or an active derivative thereof together with insulin or an active derivative thereof.

31. The composition according to claim 30 wherein the creatine or its derivative and the insulin or its derivative are present in amounts effective to increase glycogen storage in the body.

32. The composition according to claim 30 wherein the creatine or its derivative and the insulin or its derivative are present in amounts effective to increase creatine retention in the body.

33. The composition according to claim 30 wherein the composition is in a form that can be administered by at least one of ingestion and injection.

34. 8 The composition according to claim 30 further comprising a carbohydrate or an active derivative thereof.

35. A method of increasing creatine retention in a human or animal body comprising administering a composition comprising creatine or an active derivative thereof together with insulin or an active derivative thereof by at least one of ingestion and injection.

36. A method according to claim 35 further comprising administering a carbohydrate or an active derivative thereof such that an increase in blood plasma carbohydrate concentration and insulin concentration occurs substantially simultaneously with an increase in blood plasma creatine concentration.

37. A method of increasing glycogen storage in a human or animal body comprising administering a composition comprising creatine or an active derivative thereof together with insulin or an active derivative thereof by at least one of ingestion and injection.

38. A method according to claim 37 further comprising administering a carbohydrate or an active derivative thereof such that an increase in blood plasma carbohydrate concentration and insulin concentration occurs substantially simultaneously with an increase in blood plasma creatine concentrations.

39. A method of increasing glycogen storage in the human or animal body comprising administering a composition comprising creatine or an active derivative thereof together with a carbohydrate or an active derivative thereof by at least one of ingestion and infusion, wherein the composition comprises the carbohydrate or its derivative in an amount by weight which is greater than an amount of the creatine or its derivative.

40. A method according to claim 39 wherein the composition is ingested in an amount of 100 g to 700 g per day.

41. A method according to claim 39 wherein the composition is administered in four equal parts throughout the day.

42. The method according to claim 39 wherein the composition comprises, in % by weight based upon a total weight of the composition: the creatine or its derivative present in an amount ranging from 2 to 8% and the carbohydrate or its derivative present in an amount ranging from 92 to 98%.

43. The method according to claim 39 wherein the carbohydrate is selected from the group consisting of glucose and other simple carbohydrates.

44. A pharmaceutical having a composition comprising creatine or an active derivative thereof together with a carbohydrate or an active derivative thereof, wherein the composition comprises the carbohydrate or its derivative in the amount by weight which is greater than an amount of the creatine or its derivative, and the amount of said creatine or its derivative and the amount of said carbohydrate or its derivative are effective to increase creatine retention in the body.

45. A pharmaceutical having a composition comprising creatine or an active derivative thereof together with insulin or an active derivative thereof.

46. The pharmaceutical according to claim 45 wherein the composition further comprises a carbohydrate or an active derivative thereof in an amount by weight which is greater than an amount of the creatine or its derivative.

47. A composition for use in a human or animal body, the composition comprising creatine or an active derivative thereof together with a carbohydrate an active derivative thereof, wherein the composition comprises the carbohydrate or its derivative in an amount by weight which is greater than an amount of the creatine or its derivative, and the amount of said creatine or its derivative and the amount of said carbohydrate or its derivative are effective to increase glycogen storage in the body.

48. The composition according to claim 47 wherein the composition is in the nature of a dietary supplement.

49. The composition according to claim 47 wherein the carbohydrate is selected from the group consisting of glucose and other simple carbohydrates.

50. The composition according to claim 47, wherein the composition comprises, in % by weight based upon a total weight of the composition: the creatine or its derivative present in an amount ranging from 2 to 8% and the carbohydrate or its derivative in an amount ranging from 92 to 98%.

51. The composition according to claim 47 wherein the amount of said creatine or its derivative and the amount of said carbohydrate or its derivative are effective to increase creatine retention in the body.

52. A pharmaceutical having a composition comprising creatine or an active derivative thereof together with a carbohydrate or an active derivative thereof, wherein the composition comprises the carbohydrate or its derivative in an amount by weight which is greater than an amount of the creatine or its derivative, and the amount of the creatine or its derivative and the amount of the carbohydrate or its derivative are effective to increase glycogen storage in the body.

53. A composition for use in a human or animal body comprises, in % by weight based upon a total weight of the composition: creatine or its derivative present in an amount ranging from 2 to 8% and a carbohydrate or its derivative in an amount ranging from 92 to 98%.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.: 5,968,900

DATED: October 19, 1999

INVENTOR(S): Paul Leonard Greenhaff

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page, item

[30] **Foreign Application Priority Data**

Aug. 17, 1994 [GB] United Kingdom.....9425514.8

Signed and Sealed this

Twelfth Day of September, 2000

Attest:

Q. TODD DICKINSON

Attesting Officer

Director of Patents and Trademarks

Exhibit C

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(12) **United States Patent**
Dente

(10) **Patent No.: US 6,277,396 B1**
(45) **Date of Patent: Aug. 21, 2001**

(54) **DIETARY SUPPLEMENT CONTAINING A THERMOGENIC SUBSTANCE AND AN ADRENAL SUPPORT SUBSTANCE**

(75) Inventor: **Gerard Dente**, Cedar Grove, NJ (US)

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(58) **Field of Search** 424/436, 439, 424/725

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(57) **ABSTRACT**

A dietary supplement system having a daytime component and a nighttime component is provided, wherein the daytime component comprises at least one thermogenic substance and the nighttime component comprises at least one adrenal support substance.

6 Claims, No Drawings

DIETARY SUPPLEMENT CONTAINING A THERMOGENIC SUBSTANCE AND AN ADRENAL SUPPORT SUBSTANCE

FIELD OF THE INVENTION

The present invention relates to a dietary supplement comprising at least one thermogenic substance, at least one adrenal support substance and/or at least one anxiolytic substance. More particularly, the presently claimed invention relates to a dietary supplement system having daytime and nighttime conmponents wherein the daytime component contains a thermogenic substance and the nighttime component contains an adrenal support substance.

BACKGROUND OF THE INVENTION

Dietary and nutritional supplements have become a significant element of the human diet. Most dietary supplements contain stimulants as their active ingredient. Generally, stimulants can have undesirable side effects. The most common side effect is a general "jittery" feeling, but other side effects include stress on adrenal glands, restlessness, nervousness, gastro intestinal disturbances, muscle twitching, and in some extreme cases, cardiac arrhythmia. In view of the above, dietary supplements containing stimulants are not designed for nighttime usage. Because of the stimulants, dietary supplements are formulated for daytime consumption and not recommended for nighttime usage. The present invention provides a 24-hour dietary supplement system that can be consumed for daytime and nighttime usage.

SUMMARY OF THE INVENTION

The present invention provides a dietary supplement comprising at least one thermogenic substance and at least one adrenal support substance. In one embodiment, the thermogenic substance is selected from a group consisting of caffeine, catechin, MaHuang, ephedrine, synephrine (*Citrus aurantium*), norephedrine, psuedoephedrine, and White Willow (*salicin*) and extracts thereof and mixtures thereof. For purposes of this invention, the term "thermogenic" is defined as any natural or synthetic substance, nutrient, vitamin, mineral, herb or compound used to increase metabolism and accelerate calorie expenditures. In one embodiment, the term thermogenic means heat producing or fat burning. In another embodiment, the adrenal support substance is selected from a group consisting of Cordyceps (*Cordyceps sinensis*), Ashwagandha (*Withania somniferum*), Astragalus (*Asgragalus mebranaceus*), ginseng (*Panax ginseng*), Schisandra (*Schizendra chinensis*), Siberian ginseng (*Eleutherococcus senticosus*), licorice (*Glycerrhiza glabra*), Asian ginseng, Codonopsis ("Dangshen"), Vitamin B complex, Vitamin C, adrenal glandular extract, embryo extract, chromium, Vitamin B5 (pantothenic acid) and extracts thereof and mixtures thereof. The term "adrenal support" substance is defined as any natural or synthetic substance, nutrient, vitamin, mineral, herb, or compound used to support, maintain and/or improve adrenal functions and to reduce stress.

In still another embodiment, the supplement further comprises at least one thyrogenic substance. In yet another embodiment, the thyrogenic substance is selected from a group consisting of Guggul (*Commiphora mukul*) or guggulsterones, iodine, copper, selenium, thyroid glandular extract, tyrosine, phosphates and extracts thereof and mixtures thereof. In one embodiment, the iodine sources include, but are not limited to, seaweed, kelp, seafood, shellfish, and bladderwrack (*Focus vesiculosus*). For purposes of this invention, a "thyrogenic" substance is any natural or synthetic substance, nutrient, vitamin, mineral, herb or compound used to support, maintain, and/or improve thyroid functions.

In still yet another embodiment, the supplement further comprises at least one blood sugar regulation substance. In a further embodiment, the blood sugar regulation substance is selected from a group consisting of Bitter Melon (*Momordica charantia*), vanadium, allano lactone, Fenugreek (*Trigonella foenumgraecum*), garcinia (*Garcinia cambogia*), gymnema (*Gymnema sylvestra*), marshmallow (*Althaea officinalis*), chromium, chromium GTF, chromium picolinate, chromium polynicotinate, alpha lipoic acid, inula racemusa, zinc, magnesium, cyclo-hispor, Agaricus campestris (mushroom), *Medicago sativa* (Lucerna), pinitol (*Bougainvillea spectabilis*) and extracts thereof and mixtures thereof. A "blood sugar regulation" substance is defined as any natural or synthetic substance, nutrient, vitamin, mineral, herb, or compound used to regulate or manipulate blood sugar levels and/or glucose metabolism. The current US diet consist of high amounts of carbohydrates and refined sugars. This can result in elevated blood sugar levels. High levels of blood sugar can increase the production of insulin, which accelerates the storage of body fat. The blood sugar regulation substance of the present invention, functions to help stabilize normal blood sugar levels and increase the body's ability to lose stored body fat.

In still a further embodiment, the supplement further comprises at least one anxiolytic substance. An "anxiolytic" substance is defined as any natural or synthetic substance, nutrient, vitamin, mineral, herb or compound used as a calming agent, to reduce stress and anxiety, or improve sleep. In yet a further embodiment, the anxiolytic substance is selected from a group consisting of valerian (*Valeriana officinalis*), damiana, chamomile (*Matricaria chamomila*), kava kava (*Piper methysticum*), passionflower (Passilflora spp.), hops (*Humulus lupulus*), skullcap, St. John's wort (*Hypericum perforatum*), hawthorn (*Crataegus oxyacantha*), lavender (*Lavendula officinalis*), melatonin, 5-Hydroxytrytophan and extracts thereof and mixtures thereof.

In one embodiment, the supplement further comprises at least one diuretic or water balancing substance. Normal diets contain high amounts of sodium, which can lead to excessive amounts of water retention. The water balancing substance of the present invention will help regulate and relieve excessive water retention. In another embodiment, the water balancing substance is selected from a group consisting of cranberry (*Vaccinum magrocapon*), dandelion, elder (*Sambucus nigra*, Sambucus Canadensis), horsetail (*Equisetum arvense*), uva ursi (*Arctostaphylos uva-ursi*), parsley (*Petroselinum crispum*), B-6 and extracts thereof and mixtures thereof.

In still yet a further embodiment, the present invention relates to dietary supplement system comprising a daytime component and a nighttime component, said daytime component comprising at least one thermogenic substance and said nighttime component comprising at least one adrenal support substance. In another embodiment, the system is a 24-hour system. In still another embodiment, the thermogenic substance is selected from a group consisting of caffeine, catechins (epigalocatechin-EGCG), MaHuang (8% Ephedra alkaloids) ephedrine HCI, synephrine, norephedrine, psuedoephedrine, and White Willow and extracts thereof and mixtures thereof. In one embodiment, the caffeine may be green tea (*Camilla sinensis*) and guarana

(methylxanthines). In yet another embodiment, the adrenal support substance is selected from a group consisting of Cordyceps, Ashwagandha, Astragalus, ginseng, Schisandra, Siberian ginseng, licorice, Asian ginseng, Codonopsis, Vitamin B complex, pantothenic acid, Vitamin C, adrenal glandular extract, chromium and extracts thereof and mixtures thereof.

In still yet another embodiment, the daytime component further comprises at least one thyrogenic substance; the thyrogenic substance is selected from a group consisting of Guggul (guggulsterones), iodine, copper, selenium, thyroid glandular extract, tyrosine, phosphates and extracts thereof and mixtures thereof In a further embodiment, the daytime component further comprises at least one blood sugar support substance; the blood sugar support substance being selected from a group consisting of Bitter Melon, vanadium, allano lactone, Fenugreek, garcinia, gymnema, marshmallow, chromium, chromium GTF, chromium picolinate, chromium polynicotinate, alpha lipoic acid, inula racemusa, zinc, magnesium, cyclo-hispor, *Agaricus campestris, Medicago sativa*, pinitol and extracts thereof and mixtures thereof.

In still a further embodiment, the nighttime component further comprises at least one anxiolytic substance; the anxiolytic substance is selected from a group consisting of valerian, damiana, chamomile, kava kava, passionflower, hops, skullcap, St. John's wort, hawthorn, lavender, melatonin, 5-Hydroxytrytophan and extracts thereof and mixtures thereof In still yet a further embodiment, nighttime component further comprises at least one thyrogenic substance; the thyrogenic substance being selected from a group consisting of Guggul (guggulsterones), iodine, copper, selenium, thyroid glandular extract, tyrosine and extracts thereof and mixtures thereof. In a further embodiment, the nighttime component of the system of the present invention further comprises at least one water balancing substance; the water balancing substance being selected from a group consisting of cranberry, dandelion, elder, urva ursi, parsley, B-6 and extracts thereof and mixtures thereof In yet another embodiment, the supplement comprises a water balance blend. In still another embodiment, the water balancing blend comprises the water balancing substance and other ingredients. In still yet another embodiment, the water balance blend comprises Buchu leaf, cornsilk stylus, couchgrass rhizome, hydrangea root, juniper berry, uva ursi leaf, cranberry fruit, dandelion root, artichoke leaf, and extracts thereof and mixtures thereof. In a further embodiment, the supplement further comprises calcium sulfate, gelatin, magnesium stearate, and silica.

In a further embodiment, the system further comprises at least one of following: inert diluents, granulating and disintegrating agents, binding agents, lubricating agents, plasticizers, humectants, electrolytes, buffers, colorants, aromatic agents, flavoring agents, emulsifying agents, compounding agents, formulation agents, permeation enhancers and bulking agents.

In another embodiment, the present invention relates to a dietary supplement comprising at least one thermogenic substance, at least one thyrogenic substance and at least one blood sugar support substance. In still another embodiment, the thermogenic substance being selected from a group consisting of caffeine, catechins, MaHuang, ephedrine, synephrine, norephedrine, psuedoephedrine, and White Willow and extracts thereof and mixtures thereof, the thyrogenic substance being selected from a group consisting of Guggul (guggulsterones), iodine, copper, selenium, thyroid glandular extract, tyrosine and extracts thereof and mixtures thereof; and the blood sugar support substance being selected from a group consisting of Bitter Melon, vanadium, allano lactone, Fenugreek, garcinia, gymnema, marshmallow, chromium, chromium GTF, chromium picolinate, chromium polynicotinate, alpha lipoic acid, inula racemusa, zinc, magnesium, cyclo-hispor, *Agaricus campestris, Medicago sativa*, pinitol and extracts thereof and mixtures thereof.

In yet another embodiment, the supplement further comprising at least one adrenal support substance, the adrenal support substance is selected from a group consisting of Cordyceps, Ashwagandha, Astragalus, ginseng, Schisandra, Siberian ginseng, licorice, Asian ginseng, Codonopsis, Vitamin B complex, pantothenic acid, Vitamin C, adrenal glandular extract, chromium and extracts thereof and mixtures thereof.

In still yet another embodiment, the supplement further comprises at least one anxiolytic substance, the anxiolytic substance is selected from a group consisting of valerian, damiana, chamomile, kava kava, passionflower, hops, skullcap, St. John's wort, hawthorn, lavender, melatonin, 5-Hydroxytrytophan and extracts thereof and mixtures thereof.

In a further embodiment, the present invention relates to a method of manufacturing a dietary supplement with daytime and nighttime components, said method comprising: formulating a daytime component comprising at least one thermogenic substance and a nighttime component comprising at least one adrenal support substance; the thermogenic substance is selected from a group consisting of caffeine, catechins, MaHuang, ephedrine, synephrine, norephedrine, psuedoephedrine, and White Willow and extracts thereof and mixtures thereof; the adrenal support substance is selected from a group consisting of Cordyceps, Ashwagandha, Astragalus, ginseng, Schisandra, Siberian ginseng, licorice, Asian ginseng, Codonopsis, Vitamin B complex, pantothenic acid, Vitamin C, adrenal glandular extract, chromium and extracts thereof and mixtures thereof. In still a further embodiment, the method further comprises utilizing the supplement as a fat burning composition.

In yet a further embodiment, the presently claimed invention relates to a fat burning dietary supplement having daytime and nighttime components, said supplement comprising a daytime component comprising at least one thermogenic substance and a nighttime component comprising at least one anxiolytic substance. In still yet a further embodiment, the thermogenic substance is selected from a group consisting of caffeine, catechins, MaHuang, ephedrine, synephrine, norephedrine, psuedoephedrine, and White Willow and extracts thereof and mixtures thereof. In another embodiment, the anxiolytic substance is selected from a group consisting of valerian, damiana, chamomile, kava kava, passionflower, hops, skullcap, St. John's wort, hawthorn, lavender, melatonin, 5-Hydroxytrytophan and extracts thereof and mixtures thereof. In another embodiment, the daytime component further comprises at least one adrenal support substance. In a further embodiment, the nighttime component comprises at least one adrenal support substance. In still a further embodiment, the adrenal support substance is selected from a group consisting of Cordyceps, Ashwagandha, Astragalus, ginseng, Schisandra, Siberian ginseng, licorice, Asian ginseng, Codonopsis, Vitamin B complex, pantothenic acid, Vitamin C, adrenal glandular extract, chromium and extracts thereof and mixtures thereof.

DETAILED DESCRIPTION OF THE INVENTION

As required, detailed embodiments of the present invention are disclosed herein; however, it is to be understood that

the disclosed embodiments are merely exemplary of the invention that may be embodied in various forms. Therefore, specific structural and functional details disclosed herein are not to be interpreted as limiting, but merely as a basis for the claims and as a representative basis for teaching one skilled in the art to variously employ the present invention.

The present invention relates to a 24-hour dietary supplement system having a daytime component and a nighttime component and related methods of manufacturing the same. In one embodiment, the daytime component comprises a thermogenic substance and the nighttime component comprises an adrenal support substance. The thermogenic substance of the daytime component of the system of the present invention functions as a fat burner, metabolism booster and/or weight loss aid. The fat burning effects of the thermogenic decreases over time and places stress upon the adrenal glands. The adrenal support substance of the nighttime component functions to support the adrenal glands and maintain effective thermogenic fat burning. In one embodiment, the nighttime components may be consumed after about 4 to about 12 hour intervals of consuming the daytime component.

In another embodiment, the thermogenic substance of the daytime component comprises a guarana seed extract (contains naturally occurring caffeine), Ma haung herb extract, and White Willow bark extract. In one embodiment, White Willow bark functions as a catalyst that enhances the effect of the stimulants or thermogenic substance. In another embodiment, the thermogenic substance of the present invention is tea extract, specifically, green tea extract. Tea is derived from *Camellia sinensis*, a plant native to China. Green tea is the most common beverage in many Asian countries. Green tea has been shown to reduce body fat by promoting fat oxidation, exhibit thermogenic properties, and provide other health benefits, including helping control body composition. The active ingredients in the green tea are caffeine and catechin polyphenols. Studies have shown that the active thermogenic ingredient in the green tea extract is the catechins (epigallocatechins-EGCG).

A thermogenic substance is a substance that increases caloric expenditure. The most commonly used thermogenics are caffeine and Ma Huang. These thermogenic compounds may have stimulatory effects. In one embodiment, the thermogenic substance of the present invention may act as stimulants and the stimulants employed in the supplement of the present invention, is a methylxanthine, or mixtures of methylxanthines. The most widespread stimulant is caffeine, which is primarily ingested by drinking tea or coffee. Caffeine affects the central nervous system, mainly the cerebrum. Caffeine is found in coffee beans, tea, cola nuts, guarana, cacao seeds, and mate. Mate is made from a South American evergreen tree (*llex paraguariensis*) whose leaves contain caffeine. Mate is customarily consumed as a tea-like beverage. Guarana is a vine that climbs trees in South America, and grows as a shrub when cultivated in the open. The botanical name is *Paullinia cupana* H.B.K., variety sorbilis. Seeds cultivated from the plant yield guaranine, which has the same chemical composition as caffeine. A syrup extract is obtained from the seeds and used in soft drinks, or the seeds can be roasted and ground into powder. Caffeine may also be manufactured synthetically. The chemical name for caffeine is 1,3,7-trimethylxanthine. Other common methylxanthine stimulants include 1,3-trimethylxanthine (found in tea and commonly called theophylline), and 3,7-dimethylxanthine (found in cacao seeds and tea, and commonly called theobromine). Products containing caffeine are ubiquitous. Ma Huang is an herb that contains ephedra-alkaloids (ephedrine). Ephedra has a thermogenic fat burning effect and increases caloric expenditure. Research has shown that caffeine and ephedra work synergistically to further increase thermogenesis. Thermogenesis is the process by which the increase in body temperature increases caloric expenditure.

In still another embodiment, the adrenal support substance of the nighttime component comprises chromium as chromium picolinate, licorice root extract, Siberian ginseng root extract, Asian ginseng root and astragalus root. Ginseng includes active ingredients such as saponins termed ginsenosides, essential oils, phytosterol, carbohydrates, amino acids, peptides, vitamins, minerals and other ingredients. During stressful situations, the adrenal glands release corticosteroids and adrenaline. When these hormones are depleted, the organism reaches an exhaustive phase. Adrenal support substances, such as ginseng, delay the exhaustive phase and allow a more economical and efficient release of these hormones. Adrenal support substances also reduce stress. In another embodiment, the adrenal support substance includes adaptogens, such as (chick) embryo extract. Adaptogens are substances that help the body respond and adapt to stress by normalizing bodily functions that have been disrupted by various types of stress. Other than the brain, the most important target organ for adaptogens is the adrenal glands. The adrenal glands are a target organ because the glands produce various hormones (adrenaline, noradrenaline, androgens,estrogens, glucocorticoids and mineralcorticoids) and because of their overriding influence on metabolism and other aspects of the physical and mental functions. As stated above, the use of thermogenics have negative effects on the adrenal glands. If the adrenal functions are depleted, the resulting mental irritation, loss of muscle and regression of strength may take a long time to recover and recoup. One of the functions of the adrenal support substance of the nighttime component of the system of the present invention is to normalize adrenal functions and to revitalize the adrenal glands from the stressful effects placed upon the glands from the consumption of the thermogenic substance in the daytime component. If you can normalize adrenal hormone output, virtually all physiological functions improve-from sex and sleep to immune response. For bodybuilding purposes, normalizing adrenal output can increase the potential for fat loss and muscle growth.

In yet another embodiment, the daytime component further comprises a thyrogenic substance and a blood sugar support substance. In still yet another embodiment, the thyrogenic substance comprises a blend of guggulsterones extract of Commiphora mukul resin, bladderwrack kelp, Atlantic kelp, and sargassi seaweed. Guggul is a resin from a tree native to India. This resin has been used in Ayurvedic medicine, which combined it with other plant products to cleanse and rejuvenate the body, especially the blood vessels and joints. It was also used for sore throats and digestive complaints. In Chinese medicine, guggul is known as mo yao and is used to activate blood flow, relieve pain and speed recovery. Guggul is also known to lower cholesterol and increase thyroid functions and the production of thyroid hormones. The active ingredients in guggul include essential oils, myrcene, Z and E guggulsterones, alpha-camphorene, various other guggulsterones, and makulol. The Z and E guggulsterones, extracted with ethyl acetate, are the constituents that appear to be responsible for lowering blood lipids. In another embodiment, the thyrogenic substance may also include selenium as selenomethionine and copper as copper gluconate. The thyrogenic substance also provides

weight loss control benefits. Dieting and calorie restrictions slow down human metabolism and the thyroid gland functions to regulate metabolism. The thyrogenic substance supports the thyroid functions and increases the metabolism, thereby increasing the burning of body fat.

In a further embodiment, the blood sugar support substance comprises garcinia cambogia fruit extract, gymnema sylvestre leaf and chromium as chromium picolate.

In still a further embodiment, the nighttime component comprises an anxiolytic substance, in particular, kava kava root. An anxiolytic substance is a relaxant, and the most widespread used relaxant is kava. The anxiolytic substance of the present invention, functions to help improve sleep which is often compromised when dieting or taking supplements to increase metabolism. Kava, which is also known as kava-kava, yaquona, ava, ava-ava, awa, or kawa, is a member of the pepper family Piperceae. Kava is obtained from the rhizome and roots of Piper methysticum Forst. Kava is the most relaxing botanical herb with the exception of the opium poppy. Kava is known to induce general relaxation in humans when orally ingested, but it does not cause drowsiness or involuntary sleep. A liquid macerate of the kava root has been used on islands in the South Pacific in social gatherings and religious rituals for over three thousand years.

Recently, kava has been scientifically scrutinized and its psychoactive ingredients identified. These ingredients are referred to as kavalactones. A total of fifteen kavalactones have been identified to date, including kavain (a.k.a. kawain), dihydrokavain (a.k.a. dihydrokawain), methysticin, dihydromethysticin, yangonin, and demethoxyyangonin. A synthetic version of kava, known as D, L-kavain is also available. The specific kavalactones in kava root extract vary depending upon the origin of the kava plant. Kava roots, and their rhizomes, or distal root tips, are preferred, but other parts of the plant may be used. High quality extracts of kava are sold based upon the total kavalactone content, rather than upon analysis of the individual lactones contained therein.

Studies indicate that kavalactones can relieve nervous anxiety, tension, restlessness, as well as promote muscle relaxation. Studies have also shown that consumption of kavalactones does not irnpair neurophysiological activity, as evidenced by measurements of recognition rates, and driving ability. Further, kavalactones are nonaddictive and do not induce involuntary sleep or symptoms of drunkenness.

Traditionally, kava root is prepared for human consumption by pulverizing the root and/or rhizome and mixing it with water to obtain a liquid which can be consumed orally. Presently, kava root extracts are manufactured using ethanol, as a solvent, as the kavalactones are readily soluble in ethanol. The extracted material is a yellowish brown paste or powder, which is tested to determine the weight percentage of kavalactones. Synthetic versions of kava are also available.

In still a further embodiment, the nighttime component may include one or more of the following: a thyrogenic substance, a cleansing blend and/or a water balancing blend. In another embodiment, the thyrogenic substance of the nighttime component comprises a thyrogenic blend of guggulsterone extracts, Atlantic kelp, bladderwrack kelp, and sargassi seaweed. In still yet a further embodiment, the cleansing blend comprises Senna leaf, rhubarb root, cascara sagrada bark, apple fruit, cassia powder, St. John's bread, tamarind fruit, date fruit and fig fruit. In a further embodiment, the water balancing blend comprises Buchu leaf, cornsilk stylus, couchgrass rhizome, hydrangea root, juniper berry, uva ursi leaf, cranberry fruit extract, dandelion root extract, and artichoke leaf extract.

The supplement of the present invention may be formulated for administration to any suitable human by any conventional route such as oral, rectal, topical or nasal. Any carriers known in the art for oral application may be used. For solid form preparation, such as, for example, powders, tablets, disbursable granules and capsules, a solid carrier may be one or more substances such as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, tablets disintegrating agents, encapsulating materials and the like. Suitable carrier materials may include, for example, magnesium carbonate, calcium carbonate, sodium bicarbonate, magnesium stearate, calcium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, cellulose derivatives, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter, alginates, gelatin, polyvinyl pyrrolidone, polyethyl glycols, quaternary ammonium compounds and the like.

Liquid form preparations include solutions, suspensions and emulsions. Suitable carriers may include, for example, water, coloring, flavoring agents, stabilizers and thickening agents. Viscous materials, such as natural synthetic gums, resins, methyl cellulose, sodium carboxymethyl cellulose and other agents known to the pharmaceutical art may also be used.

The composition to be administered may be prepared in accordance with any dose preparation method known in the art, for example mixing, encapsulation, etc., and is not limited. The components of the composition may be added in any order without limitation.

For rectal applications, suitable formulations for compositions according to the present invention include suppositories (emulsion or suspension type), and rectal gelatin capsules (solution or suspensions). In a typical suppository formulation, the active ingredients are combined with an appropriate pharmaceutically acceptable suppository base such as cocoa butter, esterified acids, glycerinated gelatin, and various water soluble or dispersable bases like polyethylene glycols and polyoxyethylene glycols and polyoxyethylene sorbitan fatty acid esters.

Numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the attendant claims attached hereto, this invention may be practiced otherwise than as specifically disclosed herein.

What is claimed is:

1. A dietary supplement comprising at least one thermogenic substance, at least one adrenal support substance and at least one thyrogenic substance, wherein said thermogenic substance is selected from a group consisting of caffeine, catechins, MaHuang, ephedrine, synephrine, norephedrine, psuedoephedrine, and White Willow and extracts thereof and mixtures thereof, wherein said adrenal support substance is selected from a group consisting of Cordyceps, Ashwagandha, Astragalus, ginseng, Schisandra, Siberian ginseng, licorice, Asian ginseng, Codonopsis, adrenal glandular extract, embryo extract, and extracts thereof and mixtures thereof, and said thyrogenic substance is selected from a group consisting of guggelsterones, thyroid glandular extract, tyrosine and extracts thereof and mixtures thereof.

2. A dietary supplement comprising at least one thermogenic substance, at least one adrenal support substance and at least one blood sugar regulation substance, wherein

said thermogenic substance is selected from a group consisting of caffeine, catechins, MaHuang, ephedrine, synephrine, norephedrine, psuedoephedrine, and White Willow and extracts thereof and mixtures thereof, wherein said adrenal support substance is selected from a group consisting of Cordyceps, Ashwagandha, Astragalus, ginseng, Schisandra, Siberian ginseng, licorice, Asian ginseng, Codonopsis, adrenal glandular extract, embryo extract, and extracts thereof and mixtures thereof, and wherein said blood sugar regulation substance is selected from a group consisting of Bitter Melon, vanadium, allano lactone, Fenugreek, garcinia, gymnema, marshmallow, alpha lipoic acid, inula racemusa, cyclo-hispor, *Agaricus campestris, Medicago sativa*, pinitol and extracts thereof and mixtures thereof.

3. A dietary supplement system comprising a daytime component and a nighttime component, said daytime component comprising at least one thermogenic substance and at least one thyrogenic substance, and said nighttime component comprising at least one adrenal support substance, said thermogenic substance of said daytime component is selected from a group consisting of caffeine, catechins, MaHuang, ephedrine, synephrine, norephedrine, psuedoephedrine, and White Willow and extracts thereof and mixtures thereof, said thyrogenic substance of said daytime component is selected from a group consisting of guggulsterones, thyroid glandular extract, tyrosine and extracts thereof and mixtures thereof, and said adrenal support substance of said nighttime component is selected from a group consisting of Cordyceps, Ashwagandha, Astragalus, ginseng, Schisandra, Siberian ginseng, licorice, Asian ginseng, Codonopsis, adrenal glandular extract, embryo extract and extracts thereof and mixtures thereof.

4. A dietary supplement system comprising a daytime component and a nighttime component, wherein said daytime component comprising at least one thermogenic substance and at least one blood sugar support substance, and said nighttime component comprising at least one adrenal support substance, said thermogenic substance of said daytime component is selected from a group consisting of caffeine, catechins, MaHuang, ephedrine, synephrine, norephedrine, psuedoephedrine, and White Willow and extracts thereof and mixtures thereof, said blood sugar support substance of said daytime component being selected from a group consisting of Bitter Melon, vanadium, allano lactone, Fenugreek, garcinia, gymnema, marshmallow, alpha lipoic acid, inula racemusa, cyclo-hispor, *Agaricus campestris, Medicago sativa*, pinitol and extracts thereof and mixtures thereof, and said adrenal support substance of said nighttime component is selected from a group consisting of Cordyceps, Ashwagandha, Astragalus, ginseng, Schisandra, Siberian ginseng, licorice, Asian ginseng, Codonopsis, adrenal glandular extract, embryo extract and extracts thereof and mixtures thereof.

5. A dietary supplement system comprising a daytime component and a nighttime component, said daytime component comprising at least one thermogenic substance, and said nighttime component comprising at least one adrenal support substance and at least one thyrogenic substance, said thermogenic substance of said daytime component is selected from a group consisting of caffeine, catechins, MaHuang, ephedrine, synephrine, norephedrine, psuedoephedrine, and White Willow and extracts thereof and mixtures thereof, said adrenal support substance of said nighttime component is selected from a group consisting of Cordyceps, Ashwagandha, Astragalus, ginseng, Schisandra, Siberian ginseng, licorice, Asian ginseng, Codonopsis, adrenal glandular extract, embryo extract and extracts thereof and mixtures thereof, and said thyrogenic substance of said nighttime component is selected from a group consisting of guggulsterones, thyroid glandular extract, tyrosine and extracts thereof and mixtures thereof.

6. A dietary supplement comprising at least one thermogenic substance, at least one thyrogenic substance and at least one blood sugar support substance, wherein said thermogenic substance being selected from a group consisting of caffeine, catechins, MaHuang, ephedrine, synephrine, norephedrine, psuedoephedrine, and White Willow and extracts thereof and mixtures thereof, said thyrogenic substance being selected from a group consisting of guggulsterones, thyroid glandular extract, tyrosine and extracts thereof and mixtures thereof, and said blood sugar support substance being selected from a group consisting of Bitter Melon, vanadium, allano lactone, Fenugreek, garcinia, gymnema, marshmallow, alpha lipoic acid, inula racemusa, cyclo-hispor, *Agaricus campestris, Medicago sativa*, pinitol and extracts thereof and mixtures thereof.

* * * * *

Exhibit D

US005719119A

United States Patent [19]

Veech

[11] Patent Number: 5,719,119

[45] Date of Patent: Feb. 17, 1998

[54] **PARENTERAL NUTRITION THERAPY WITH AMINO ACIDS**

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[73] Assignee: **British Technology Group, Ltd.**, London, England

[21] Appl. No.: **53,291**

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Related U.S. Application Data

[63] Continuation of Ser. No. 782,751, Oct. 21, 1991, abandoned, which is a continuation of Ser. No. 479,237, Feb. 12, 1990, abandoned, which is a continuation of Ser. No. 940,332, Dec. 17, 1986, which is a continuation-in-part of Ser. No. 810,916, Dec. 18, 1985, abandoned.

[51] **Int. Cl.**[6] **A61K 38/00**

[52] **U.S. Cl.** **514/2**; 514/546; 514/557; 514/561; 514/578; 424/601; 424/633; 424/677; 424/678; 424/679; 424/680; 424/719

[58] **Field of Search** 424/601, 633, 424/677, 678, 679, 680, 719; 514/561, 557, 578, 546, 2

[56] **References Cited**

U.S. PATENT DOCUMENTS

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4,279,917	7/1981	Takami et al.	514/400
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4,649,050	3/1987	Veech	424/601
4,663,166	5/1987	Veech	424/663
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[57] **ABSTRACT**

Parenteral nutrition aqueous solutions are provided which preferably contain glutamine together with other organic nitrogen containing compounds. The respective concentrations of the compounds present in any given such solution are typically approximately multiples of the concentration of the same compounds as found in normal human plasma, and the respective mole ratios of various such compounds in any given such solution relative to one another are approximately the same mole ratio associated with the same compounds as found in normal human plasma. Processes for using such solutions are provided.

14 Claims, No Drawings

PARENTERAL NUTRITION THERAPY WITH AMINO ACIDS

RELATED APPLICATIONS

This is a continuation of application Ser. No. 782,751, filed Oct. 21, 1991; now abandoned, which is a continuation of application Ser. No. 479,237, filed Feb. 12, 1990 which is now abandoned, which is a continuation of application Ser. No. 940,332, filed Dec. 17, 1986, which is a continuation-in-part of my previous now U.S. patent application Ser. No. 810,916 filed Dec. 18, 1985, now abandoned.

My previously filed U.S. patent application Ser. No. 747,792 (now U.S. Pat. No. 4,663,289), application Ser. No. 747,858 (now U.S. Pat. No. 4,649,050), application Ser. No. 748,232 (now U.S. Pat. No. 4,663,166), and application Ser. No. 748,184 (now U.S. Pat. No. 4,668,400) all filed Jun. 24, 1985 may be considered to be related to my present case.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention lies in the field of parenteral nutrition therapy, and especially in the field of amino-acid containing solutions and methods for practicing such therapy.

2. Prior Art

The use of acetate in parenteral fluids developed in the late 40's and early 50's following reports that "acetate could serve as an alternative source of fixed base such as bicarbonate" (Mudge G H, Manning J A, Gilman A. Sodium acetate as a source of fixed base. *Proc Soc Exptl Biol Med* 71:136–138, 1949; Fox C L, Winfield J M, Slobody L B, Swindler C M, Lattimer J K. Electrolyte solution approximating plasma concentrations with increased potassium for routine fluid and electrolyte replacement. *J Am Med Asso* 148: 827–833, 1952), The routine use of acetate in parenteral fluids has grown over the past 40 years to the point where the present commercially available amino acid supplements now contain from 40 to 150 mM acetate (*Facts and Comparisons* March 1984, pp 36–37d. Lippincott, St. Louis) even though the normal level of blood acetate is generally controlled at below 0.2 mM in blood (Bergmeyer H U, Moellering H. Enzymatische besimmung von acetat. *Biochem Z* 344:167–189, 1966).

Acetate was used in parenteral fluids for four major reasons, the first being low cost, and the second, ignorance of its toxic effects. The third was an attempt to avoid hyperchloremic acidosis. Amino acid solutions which included amino acids as their chloride salts lead to hyperchloremic acidosis. The use of acetate as an anion avoided this problem and created a solution which had a pH between 5.5 and 6.5, thus avoiding the precipitation of the divalent cations calcium and magnesium, often included in parenteral fluids. It has generally been assumed that the inorganic pyrophosphate which is formed during the metabolism of acetate is instantly hydrolyzed to 2 inorganic phosphates (Kornberg A. In: *DNA Replication* 1981, pp. 55–56, W H Freeman, San Francisco) by the ubiquitous and highly active inorganic pyrophosphatase (Shatton J B, Shah H, Williams A, "Pyrophosphatase in normal and hepatic tumors of the rat," *Cancer Res.* 41:1866–1872, 1981). This is now known not to be the case (Veech R L, Gitomer W L, King M T, Balaban R S, Costa J L, Eanes E D. "The effects of short chain fatty acid administration on hepatic glucose, phosphate, magnesium and calcium metabolism," In Brautbar N, ed., *Myocardial Bioenergentics and Compartmentation,* New York, Raven Press, 1986, pp. 617–646). Administration of unphysiological levels of acetate causes a 200 fold increase in hepatic inorganic pyrophosphate, a five fold increase in total liver calcium, and a doubling of total metabolizable liver phosphate within 5 minutes of administration. This massive increase in liver calcium, phosphate and pyrophosphate means that, in the rat, the entire blood content of calcium must be removed 4 times to provide this increase. This store can only come from bone destruction. This is the cause of the chronic bone pain and metabolic bone disease seen in patients on current amino acid parenteral nutrient supplements. (See *Facts and Comparisons* pp. 35f, J. B. Lippincott, St. Louis, 1984.)

The fourth reason acetate was included in the amino acid mixtures, particularly those containing sulfur, was to avoid a metabolic acidosis. By adding acetate to the mixture, an alkalizing agent was administered, which, at the time, was thought to be harmless. This has now been proven not to have been the case. The improvement here consists in substituting for acetate other anionic metabolites which may accomplish this function in a non-harmful manner.

Another adverse consequence of the use of acetate in parenteral nutrition fluids is the lowering of the phosphorylation potential which occurs in tissues exposed to high levels of acetate (Veech, R L et al, 1986). This inevitably results in an increased O_2 demand for any organ to do a comparable amount of work. In short, this decreases the metabolic efficiency of the organism because less energy is released from each ATP utilized.

Another well known and undesirable consequence of the use of acetate is the release of adenosine from tissues. (Laing C-S, Lowenstein J M, Metabolic control of the circulation, effects of acetate and pyruvate. *J Clin Invest* 62:1029–1038, 1978). Adenosine is a potent vasodilator. Given the well known occurrence of hypotension (Graefe U et al, Less dialysis induced morbidity and vascular instability with bicarbonate in dialysate. *Ann Intern Med* 88: 332–336, 1978) during dialysis where 35 to 45 mM acetate is used, the not uncommon occurrence of hypotension and nausea sometimes seen during the administration of parenteral nutrition solutions may be related to adenosine release which can be avoided by the choice of a different anion than acetate in the formulation.

Finally, the profound lethargy and weakness experienced by patients for up to 2 days following acetate hemodialysis is not dissimilar clinically from the weakness complained of by patients receiving parenteral nutrition. It is not unreasonable to expect that elevated muscle Ca^{2+} plays a role in the functional myopathy seen in both of these clinical situations.

Many current parenteral fluids marketed in the U.S. at present use, in their formulations, a racemic mixture of d,l-lactate. Because of the recognized dangers inherent in giving large volumes of normal saline to patients whose renal excretory capacity is often compromised, Ringer's lactate is the most common complex electrolyte fluid parenterally given in this country today. It was originally formulated because of the hyperchloremic acidosis which resulted from treating children with infantile diarrhea with normal saline (Hartmann A F, The theory and practice of parenteral fluid administration. *J Am Med Asso* 103:1349–1354, 1934). It is now recognized that much lower levels than the 14 mM d-lactate present in Ringer's lactate solution can have obvious untoward effects (Oh M S et al D-Lactic acidosis in a man with short bowel syndrome. *N Eng J Med* 301:249–251, 1979). What is not recognized widely is that d-lactate, while being metabolized only slowly by the body, nevertheless is readily transported into cells by

the monocarboxylate carrier present on the plasma membranes of most cells (Olendorf W H, Blood brain barrier permeability to lactate. *Eur J Neurol* 6:49–55, 1971). The result is that not only d-lactate, but also an equivalent amount of K^+ are transported into tissues, thereby increasing their osmotic burden. In some cases, this increase can significantly contribute to the so called "idiogenic osmoles" which can be fatal during the treatment of diabetic ketoacidosis with alkalinizing agents such as d,l-lactate. There seems no adequate rationale for the inclusion of d,l-lactate in parenteral fluids any longer. The alternative use of l-lactate, while preferable to the presently used d,l-lactate, is likewise not an optimum alternative to the use of acetate.

In addition to the inappropriate use of acetate, the prior art amino acid formulations suffer because they are commonly derived from the amino acid composition of casein, a common milk protein, and bear little resemblance to the normal human plasma concentrations of free amino acids. Certain classes of amino acids are even missing, particularly the major plasma amino acid, glutamine, which is essential to the function of many organs, such as kidney and gut. It is further known that the plasma concentrations of amino acids are carefully regulated. It is, therefore, desirable that the prior art compositions be replaced by mixtures wherein the respective amino acid concentrations, relative one to another, resemble the plasma to which such are being added.

Finally, the hormonal balance in many patients receiving such treatments favors the breakdown of protein with concurrent loss of muscle and tissue mass and the synthesis of glucose and urea. The action of hormones cab be effected by control of the redox state (Sistare F D, Haynes R D, *J Biol Chem* 260:12748–12753, 1985).

Present parenteral nutritional supplements in malnourished patients generally fail either to restore positive nitrogen balance, or to achieve a demonstrable increase in functional capacity when administered, for example, over a one week period.

The common conventional parenteral amino acid supplements currently in use are listed in Table 1 (below). It is clear from the Table that the current parenteral formulations in no way resemble the amino acid compositions seen in normal plasma.

Attempts have been made to develop amino acid formulations based on the normal amino acid compositions present in postprandial samples from blood (see U.S. Pat. No. 4,491,589, Dill R B, Waters R W, Hurd W C. Amino acid solutions for parenteral nutrition and methods of formulation and use). However, these attempts fail to address the acidosis resulting from the metabolism of amino acids and neither do they make any attempt to define the non-amino acid anionic components in such mixtures so as to prevent the development of bone pain and metabolic disease which accompanies such therapy. Further, the ratio scheme of purported non-essential to essential amino acids may not meet the various clinical situations requiring such therapy.

The "essential" amino acids listed by Dell et al. U.S. Pat. No. 4,491,589 are 11 in number whereas the "essential" groups listed in *Fact and Comparisons* are only 8, Omitting l-histidine, l-cysteine, and l-tyrosine. The essential nature of l-histidine in man is not universally agreed to. Further, most authorities would agree that if l-phenylalanine is given, then its hydroxylation product l-tyrosine is not "essential".

TABLE 1

Composition of Commercial Parenteral Nutrition Fluids
Values are given in millimoles/Liters (mMol/l). The values are taken from Facts and Comparisons March 1984, pp 35d-37d, Lippincott, St. Louis.

Component	Normal Plasma	Aminosyn 3.5% Abbott	Travasol 5.5% Travenol	FreeAmine 6.9% McGaw	Hepamine 9% McGaw
Essential					
l-Ile	0.036	19.2	20.1	58.0	68.7
l-Leu	0.076	25.1	26.0	105.0	84.0
l-Lys^+	0.106	17.2	21.8	28.1	41.8
l-Met	0.03	9.4	21.3	16.8	6.7
l-Phe	0.029	9.3	20.6	19.4	6.1
l-Thr	0.082	15.3	19.3	16.8	37.8
l-Trp	0.054	2.75	4.85	4.4	3.2
l-Val	0.136	23.9	21.5	75.2	71.8
Non-Essential					
l-Cyst**	0.24	—	—	—	—
l-Ala	0.142	50.3	128.0	44.9	86.5
l-Arg^+	0.041	19.7	32.8	33.3	34.5
l-Asp^-	0.02	—	—	—	—
l-Asn	0.02				
l-Glu^-	0.031	—	—	—	—
l-Gln	0.300	—	—	—	—
Gly	0.124	59.7	152.0	44	120.0
l-His	0.051	6.77	15.5	10.3	15.5
l-Pro	0.105	26.1	20.0	54.8	69.6
l-Ser	0.081	14	—	31.4	47.6
l-Tyr	0.030	1.71	1.22	—	—
Other N Compounds					
l-Carnitine	0.047	—	—	—	—
l-Citrulline	0.019	—	—	—	—

TABLE 1-continued

Composition of Commercial Parenteral Nutrition Fluids
Values are given in millimoles/Liters (mMol/l). The values are taken from Facts and Comparisons March 1984, pp 35d-37d, Lippincott, St. Louis.

Component	Normal Plasma	Aminosyn 3.5% Abbott		Travasol 5.5% Travenol		FreeAmine 6.9% McGaw	Hepamine 9% McGaw
1-Ornithine	0.033						
Electrolytes mEq/L							
Na^+	163–145	7	47*	3	70*	10	10
K^+	3.5–5.0	—	13	—	60	—	—
Mg^{2+}	–.53	—	3	—	10	—	—
Cl^-	100–106	—	40	—	70	3	3
Acetate	0.02	46	58	48	102	57	62
1-lactate$^-$	0.6–6	—	—	—	—	—	—
pyruvate$^-$	0.1–1	—	—	—	—	—	—
Pi mM/L	1–1.45	—	3.5	—	30	—	10
$S_2O_4^{2-}$	0	0.5	0.5	—	—	—	—
HSO_3	0	0	0	3	3	3	3
Glucose	3.9–5.6						
mOsm/L	311	357	447	581	850	620	785

*Formulation with added electrolytes.
**Sulfur containing amino acids contents of human plasma from Felig P, Owen OE, Wahren J, Cahill GF, J Clin Invest 48:584-594, 1969.

BRIEF SUMMARY OF THE INVENTION

This invention provides a new class of formulations of nitrogen containing parenteral nutrition fluids which overcomes the prior art deficiencies above referenced, which contain the major plasma amino acids, which avoid the toxicity of acetate and other prior art components, and which optimally may achieve normalized redox balance within the organs of mammals to which such are administered (thereby to control and normalize the cellular phosphorylation state). The invention further provides methods for accomplishing nutrition therapy using such formulations.

More particularly, this invention is directed in one aspect to a new and improved class of non-hyperchloremic, alkalinizing, compositions which prevent both metabolic acidosis and metabolic bone disease. Such compositions comprise water having dissolved therein:

(A) from about 1 to 150 mMoles/Liter of each of at least one of the metabolizable nitrogen containing compounds appearing in Table 5 below with the total quantity of such compounds in any given such composition being not more than about 1000 mMoles/L (and preferably from about 50 to 800 mM);

(B) from about 0.1 to 150 mMoles/L of at least one carboxylic metabolite anion selected from the group consisting of l-lactate, pyruvate, d-beta-hydroxybutyrate, acetoacetate, alphaketoglutarate and bicarbonate;

(C) from about 0.1 to 150 mM/L of at least one cation selected from the group consisting of sodium, potassium, calcium, magnesium, and ammonium.

The notation "mMoles/L" as used herein has conventional reference to millimoles per liter (sometimes shown as mM/l or the like).

Optionally and additionally such a composition can contain at least one osmotically active nonionic water soluble nutrient, such as glucose, in a total quantity ranging from about 5 to 400 mMoles/L.

Also, optionally, the solution may contain added electrolytes as defined in (C) above as accomplished in the prior art amino acid solutions (see Table 1).

In another aspect this invention is directed to an improved in vivo process for accomplishing parenteral nutrition which comprises introducing intravenously into a human being a composition from the class above described preferably at a rate which is sufficient to be nutritionally effective. Such a rate with such a composition can also be generally effective in normalizing organ function particularly when near equilibrium couples are employed as taught hereinbelow.

These new parenteral organic nitrogen containing fluids avoid the toxic effects of the current commercially available 40 to 140 mM acetate containing parenteral nutrition solutions which lead to pathological accumulations of calcium, phosphate, and inorganic pyrophosphate within liver and other organs, and which result in the chronic bone pain and metabolic bone disease seen in patients with long term parenteral nutrition.

Optionally, a composition from the class above described may additionally contain dissolved therein glutamine. Preferably, the quantity of glutamine employed in any given such composition is as herein below described.

The glutamine containing compositions of the present invention are applicable for use in various particular parenteral fluid therapy applications. The concentrations and the relationship of the component concentrations to one another in such application can be varied. In use, a glutamine containing composition may result in an increase in organ protein content and/or an increase in organ functional capacity compared to compositions of the class above described.

Preferably, in the compositions of this invention, the anions above indicated are employed in the form of pairs which correspond to near-equilibrium couples in accord with the following table:

TABLE 2

Carboxylate Near Equilibrium Couples

Anion Couple	mEq ratio
1-lactate⁻/ pyruvate⁻	1:1 to 20:1
d-betahydroxy-butyrate⁻/ acetoacetate⁻	0.5:1 to 6:1
bicarbonate⁻/CO_2	1:1 to 100:1

Preferably, also, a composition of this invention contains at least 5 of such nitrogen containing compounds of Table 5, more preferably at least 10, still more preferably at least 15, and most preferable all of the compounds of Table 5. Preferably, when more than one such compound is present, the relative quantities of each in relation to the other(s) thereof present follows the hierarchical listing order shown in Table 5.

An object of the present invention is to provide parenteral nutritional compositions which do not contain toxic levels of acetate or d,l-lactate.

Another object is to provide parenteral nutritional compositions which contain at least one l-amino acid and at least one monocarboxylic anionic metabolite.

Another object is to provide therapeutic organic nitrogen containing fluid compositions which include near equilibrium couples which comprise either metabolite carboxylic anions or amino acids.

Another object is to provide a class of organic nitrogen containing parenteral nutrition fluids which, when administered, regulate and control the cellular phosphorylation state, thus normalizing and improving the efficiency of organ function in a living mammal.

Another object is to provide a class of mixtures of organic nitrogen containing compounds which mixtures are adopted to be employed, if desired, in multiples of the physiologic concentrations of such compounds found in normal human plasma and which mixtures are not simple compositions derived from the amino acid content of casein or other low cost hydrolyzable proteins.

Another object is to provide aqueous compositions containing amino acids and certain redox action carboxylic acid near equilibrium couples which are suitable for use in parenteral nutrition therapy to restore and maintain muscle and other cellular functions.

Other and further objects, aims, purposes, features, advantages, embodiments, applications and the like will be apparent to those skilled in the art from the teachings of the present specification taken with the claims.

DETAILED DESCRIPTION

Loss of organ protein and mass, with the conversion of the constituent amino acids to glucose, acetyl CoA, and urea, is a normal consequence of starvation or malnutrition. This process, called negative nitrogen balance, is accelerated by trauma, burns or wounds, infections and malignancy, and by surgery. It is recognized that the morbidity and mortality associated with surgery or cancer chemotherapy can be decreased if seriously ill patients can be returned toward a nutritionally normal state prior to surgery, or can be maintained in such a state while in the postoperative period or while undergoing a chemotherapy. Currently, therefore, parenteral nitrogen infusions are used to treat negative nitrogen balance when (1) the alimentary tract, by oral, gastrostomy or jujunostomy cannot be used; (2) gastrointestinal absorption of protein is impaired by obstruction, inflammatory disease or complications of antineoplastic therapy; (3) bowel rest is needed because of GI surgery or its complications, such as ileus, fistulae or anastomotic leaks; or (5) burns, trauma, infections, or other such so called hypermetabolic states exist.

It was originally supposed that, if the urinary losses of nitrogen accompanying these states of excessive catabolism could be replaced by the intravenous administration of nitrogen containing compounds in the form of amino acids in conjunction with carbohydrates such as glucose, or alternatively fat emulsions, then protein wasting could be reversed. While this is theoretically true, in practice this often proves not to be the case. It is now documented that, in spite of the provision of up to 1.5 g/kg of parenteral nitrogen/day for about one month pre-operatively, there is often no measurable increase in total body nitrogen. This naturally raises questions, not only about the hormonal status of such patients, but also about the adequacy of the specific amino acid formulations that are being administered, and whether the present formulations take advantage of newer information concerning the metabolic factors responsible for control of the rates of protein synthesis and degradation. Recent work now indicates that the current forms of parenteral nutrition fluids, to which high levels of acetate are routinely added as a method to insure the solubility of the constituents of the solution and to avoid the complication of hyperchloremic acidosis which were present in the early formulations which included amino acids as their chloride salts, themselves result in a number of toxic side effects, such as hyperglycemia, chronic bone pain, metabolic bone disease, and also a functional myopathy characterized as weakness of effort which results from the use of acetate. These undesired side effects may be ameliorated, or avoided altogether, when the acetate used in the prior art formulations is replaced by other more physiologically normal constituents as taught herein. There is further evidence to suggest that the specific concentrations and components of the nitrogenous compounds present in the current commercial formulations are not optimized so as to promote a positive nitrogen balance and a return to physiologically normal status. For these reasons, the present and new improved formulations of parenteral amino acid supplements have been created as taught herein.

These compositions provide less toxic and more efficacious forms of parenteral nitrogenous nutritional supplements to treat patients in negative nitrogen balance for the above listed reasons.

The proper alternative to the use of acetate and/or d,l-lactate in nutritional nitrogen containing parenteral fluids is the inclusion of a balanced ratio of redox active carboxylic acid near equilibrium anion couples, as taught herein. While control of pH is widely recognized as important in parenteral fluid therapy, the importance of the control of the redox states in such fluids has not generally been appreciated in clinical practice. While the serious and usually fatal nature of lactic acidosis is recognized, and measurement of the blood lactate/pyruvate ratio in various disease states has been accomplished for many years (Huckabee W E, Relationships of lactate and pyruvate during anaerobic metabolism, *J Clin Invest* 37:244–254, 1957), no previous clinical attempts to use the control of this fundamental cellular property of the redox states as a therapeutic tool are now known. The cellular levels of many metabolites, including many crucial amino acids, are functions of the intracellular redox states (Krebs H A, Veech R L. The energy level

and metabolic control in mitochondria, pp. 329–384, *Adriatica Editrica,* Bari, 1969). Thus, the concentration of the central amino acid transaminase pairs, namely alpha ketoglutarate x glutamate, and oxaloacetate x aspartate, or pyruvate x alanine, as well as the ketoacids of the branched chain amino acids, ketoisovaleric, and ketoisocaproic, are related to the redox state of one or another of the cellular redox states as the result of highly active transaminase systems which maintain a state of near equilibrium between the various cellular components. Administration of solutions of amino acids alone, without simultaneous administration of one or another substrate couple with which that amino acid is in a state of near equilibrium, results in a change of the general cellular redox state towards that state which is characteristic of the starved state. During starvation, characterized by a general reduction of all the cellular redox states, or $[NA(P)D^+]/[NAD(P)H]$ ratios, there is a breakdown of protein into amino acids and an increase in gluconeogenesis. This is precisely the situation which parenteral nutrition with amino acids is trying to reverse. The same situation of reduction of cellular redox states occurs under the influence of catabolic hormones, such as glucagon, sympathomimetic agents, and steroids. The present class of formulations, therefore, may be used to insure that the cellular redox state of the cells in a living mammal during parenteral nutrition achieves a level characteristic of the fed state when protein synthesis generally exceeds the rate of protein breakdown.

A second factor of major importance bearing upon the control of the redox state is that the cytoplasmic $[NAD^+]/[NADH]$ is directly related to the major cellular energy level, or to the cytoplasmic phosphorylation state of $[ATP]/[ADP]\times[Pi]$ ratios through the action of the glyceraldehyde 3 phosphate dehydrogenase reaction and the 3 phosphoglycerate kinase reactions (Veech R L, Lawson J W R, Cornell N W, Krebs H A. The cytoplasmic phosphorylation potential. *J Biol Chem* 254:6538–6547, 1979). Control of the cytoplasmic phosphorylation potential allows one to manipulate, within limits, the cellular energy level which determines the degree of efficiency at which any organ operates. It has recently been shown, for example, that the maximum rate at which isolated hepatocytes are able to convert lactate to glucose is at the physiological redox state represented when the [lactate]/[pyruvate] ratio in the bathing media is 10:1 (Sistare F D, Haynes R C, *J Biol Chem* 260:12748–12753, 1985). The same paper shows that, for any class of hormone tested, whether acting through cyclic AMP, through changes on intracellular Ca^{2+}, or directly on a receptor and then on nuclear synthesis such as steroids, there is a particular redox state which will allow, or not allow, as the case may be, the hormone class members to maximally express their action. By changing the ratio of redox active metabolite pairs during parenteral fluid therapy, the physician is offered the opportunity to directly alter the redox state of the tissues during such parenteral fluid therapy so as to best achieve the result desired in a particular situation. Thus, in most clinical conditions, such as following trauma, burns or surgery, the hormonal status of the patient favors the catabolism of protein and the making of glucose. While the prevention of the secretion of catabolic hormones in such a situation cannot be prevented, by alteration of the redox state of the parenteral fluids, the effects of these hormonal changes may be modified.

By far the bulk of the protein eaten in the diet is broken down in the gut endothelium to the constituent amino acids. These amino acids are transported in the portal vein to the liver, where they are mainly broken down into urea and glucose (Krebs H A. Some aspects of the energy transformation in living matter. *Brit Med Bull* 9:97, 1953). The amino acid composition of each of the blood, plasma, and extracellular fluid is tightly controlled by the liver, interacting with the muscles and the gut. Depending upon the tissue in question, gradients of from one to almost 100 fold in amino acid concentration between extracellular and intracellular amino acids can build up between blood and tissue in some, but not all, amino acids.

In Table 3 (below) are given the amino acid concentrations in rat and human serum, and in liver as a representative tissue. Also included as a point of reference is the amino acid composition of the ubiquitous intracellular protein, actin, which has a molecular weight of 42,056 with 337 amino acid and amide residues. The values are given as if 1 mole of actin were hydrolyzed completely in 1 liter of intracellular water, since 1 mM is about the concentration of this protein in muscle.

As will be apparent (see Table 1 above), not only do the relative amounts of the various amino acids included in current parenteral nitrogen supplements vary from the physiologically normal distribution, but also important omissions of whole classes of nitrogen containing compounds occur characteristically in the present fluids. Simply to give "nitrogen" in an unphysiological mixture as amino acids whose concentration ratios, one to another, bear no relationship to the normal levels of such amino acids in plasma, is simply to stimulate urea synthesis. Such does nothing to increase the rate of protein synthesis, inhibit the rate of protein breakdown, correct the observed physiological myopathy, or inhibit the action of catabolic hormones which are usually present in excess in situations of trauma, malignancy, or simply malnutrition itself. The provision of adequate glucose to maintain cerebral function at all costs is an evolutionary imperative. The contemporary (prior art) compositions of parenteral nitrogen containing nutritional fluids do nothing to address these fundamental organizational imperatives.

TABLE 3

Amino Acid and Amide Composition of Plasma, Perfused Liver, and a Representative Protein
Values are given in mmoles/liter water. The values for rat serum are taken from Lunn PG, Whitehead RG, Baker BA, Br J Nutr 36: 219–230, 1976; for normal human plasma from Guanda OM, Aoki T, Soldner H, Cahill FG. J Clin Invest 57: 1403–1411, 1976. Liver values from perfused rat liver containing normal amino acid levels in a perfusate composed of Krebs-Henseleit are taken from Poso AR, Mortimore GE, Proc Nat'l Acad Sci USA 81: 4270–4274, 1984.

Amino Acid	MW	Normal Human[4] Plasma	Normal Rat[4] Plasma	Perfused Rat Liver Content	Liver Perfusate Gradient	Actin 1 mMol/ 1L
Essential						
1 l-Isoleucine	131	0.036	0.114			30
2 l-Leucine	131	0.076	0.204	0.356	1.7	26
3 l-Lysine$^+$	146	0.106	0.408			19
4 l-Methionine	149	0.03	0.060	0.114	1.9	17
5 l-Phenylalanine	165	0.029	0.096	0.074	0.8	12
6 l-Threonine	119	0.082	0.329			27
7 l-Tryptophane[1]	204	0.054	ND			4
8 l-Valine	117	0.136	0.250			21
Non-Essential						
1 l-Alanine	89	0.142	0.475	2.19	4.6	29
2 l-Arginine$^+$	174	0.041	0.220	0.04[3]		18
3 l-Aspartate$^-$	133	0.02	0.053	4.97	93.8	22
4 l-Asparagine	132	0.02	ND			12
5 l-Cysteine	121	0.24	0.034			6
6 l-Glutamate$^-$	147	0.031	0.158	9.19	58.2	28
7 l-Glutamine	146	0.300	ND	9.18		11
8 Glycine	75	0.124	0.370	5.09	13.7	28
9 l-Histidine	155	0.051	0.092	0.836	9.1	9
10 l-Proline	115	0.105	0.437	0.161	0.37	19
11 l-Serine	105	0.081	0.657			23
12 l-Tyrosine	181	0.030	0.098	0.106	1.1	16
Other N Compounds						
NH_4^+	18	0.05	0.02	0.71	35	
l-^{4}Aminobutyrate	103	0.016				
l-Carnitine[2]	161	0.047		2–4	50	
l-Citrulline	175	0.019		0.050[3]		
l-Ornithine	132	0.033		0.150[3]	50	
l-Taurine	125	0.024				

[1]Denckla WD, Dewey HK. The determination of tryptophane in plasma, liver and urine. J Lab Clin Med 69:160–169, 1967.
[2]Rudman D, Ansley JD, Sewell CW, in Carnitine Biosynthesis, Metabolism, and Functions pp. 307–321. Academic Press, 1980.
[3]Raijman L, in The Urea Cycle pp. 243–254, John Wiley, 1976.
[4]The values in this column are based upon the best information now known to the inventor; further study and evaluation may lead to slightly different values.

Table 3 shows the amino acid composition of normal human plasma and the amino acid composition of a normal intracellular protein, actin. It can readily be seen that the composition of plasma is very different from the amino acid composition of a protein. Plasma levels of amino acids often bear little relation to tissue concentrations. Table 2 further illustrates that the amino acid composition of plasma is approximately the same as the amino acid composition of liver for certain amino acids, such as the branched chain amino acids, valine, leucine, and isoleucine, the aromatic amino acids, phenylalanine and tyrosine, and the sulfur containing amino acid, methionine. In contrast, the liver/plasma gradient of other amino acids, particularly those which take place in near-equilibrium, redox-related reactions, such as alanine, glutamate$^-$ and aspartate^{2-}, may show concentration gradients from 5 to 100 between perfusing fluid and liver. The same large concentration gradients occur in the case of glutamine. In general, the major traffic in nitrogen between the various organs is borne by alanine, glutamine, and the branched chain amino acids, leucine, isoleucine and valine.

In a 70 kg normal man, the major repository of the 6 kg of nitrogen is the tissue proteins, while the free tissue amino acid pools comprise less than 1% of the total amino acids. Tissue protein is in a dynamic state with half lives ranging from less than one hour to weeks, and with the overall turnover being about 300 g/day in a 70 kg man.

Protein synthesis obviously requires a supply of amino acids. In addition, it requires that the gradients of amino acids between extracellular fluid and cells remain normal. The latter function of the cell is the result of a variety of active, energy-requiring uptake systems, which, in turn, demand that the cellular energy state, or [ATP]/[ADP]×[Pi], and its related redox state, or [NAD^+]/[NADH], are normal. In fact, by far the major fate of ingested amino acids is not for the synthesis of protein, but rather to serve either as substrates for gluconeogenesis, or as precursors of acetyl CoA for combustion in the Krebs cycle. Most of the nitrogen of ingested proteins, and their hydrolyzed amino acids, therefor, end as urea. The carbon skeletons end as either

glucose, or as ketone bodies, being metabolized by the various transaminase reactions with glutamate, or forming one or another forms of CoA, with or without an intermediate form of an acyl carnitine.

In trauma (Kinney J M. The metabolic response of injury. in *Nutritional aspects of care in the critically ill,* Richards J R, Kinney J M, eds. pp 95–133, Churchill Livingston, 1977), in malnutrition, and in many malignant states, the degradative reactions of proteins are accelerated over synthesis, with excessive catabolism of the released amino acids to glucose, ketone bodies, and urea. The result is that the patient shows negative nitrogen balance and muscle wasting. Attempts have been made using so called parenteral nutrition solutions of amino acids to reverse this degradation of muscle and other organ mass. Unfortunately, using conventional forms of parenteral amino acid formulations, no significant gain in muscle nitrogen can be seen in the first weeks or months of therapy (Yeung C K et al. Effect of an elemental-diet on body composition. A comparison with intravenous nutrition. *Gastroenterology* 77:652–657, 1979).

A first possibility or mode for improvement is the development of alternative formulations of nitrogen compound containing parenteral nutrient formulation which avoid the use of 40 to 150 mM acetate in all current amino acid containing parenteral nutrition formulation, (*Facts and Comparisons* March, 1984, pp. 36a–37d, J B Lippincott, St. Louis) so as to avoid the chronic bone pain, the metabolic bone disease, and the profound disordering of calcium, phosphate and pyrophosphate homeostasis which accompany the current use of unphysiological levels of acetate in parenteral nutrition formulations. Instead of acetate, at least one carboxylic metabolite, or more preferably, at least one redox balanced mixture as shown in Table 2 containing the near equilibrium carboxylic acid couples l-lactate/pyruvate, d-betahydroxybutyrate/acetoacetate, and/or bicarbonate/CO_2 are used as substitutes for acetate (see, for example, my copending U.S. patent application Ser. No. 748,232).

Another alternative or mode for improvement is to employ nitrogen containing parenteral nutrient solutions as provided by the present invention wherein the redox state is controllable after administration using certain amino acids themselves, along with the appropriate redox and transaminase partner, when employed in the concentration ratios found in normal healthy animals, or in designed variations of those ratios. Specific effects may be achieved, such as the modification of hormone action as described previously. For example, the normal redox partner of l-lactate is pyruvate, but, in solution, pyruvate tends to form the inactive dimer parapyruvate. This means that it preferably should be added just prior to use, as is now preferably done with cysteine. Such alternatives achieve generally the same desired redox control, but have the practical advantage of a longer shelf life. The use of the other major permeant redox active couple, [d-hydroxybutyrate]/[acetoacetate] also is limited since, in solution and over long periods of time, acetoacetate tends to decarboxylate. This property of acetoacetate means that it, too, must be added just prior to use. It may not be possible to avoid this problem and stay within the constraints imposed by physiological constraints. Nevertheless, certain methods, as taught herein, of achieving redox control are possible, which have the practical advantage of long shelf life.

As aforementioned, the metabolite anions used in the compositions of this invention exert a desirable alkalinizing action which avoids metabolic acidosis and thereby provides alkalinizing action as desired.

Thus, normal plasma contains concentrations of [ammonium$^+$, also characterized herein as NH_4^+]× [alphaketoglutarate^{2-}]/[glutamate$^-$] the product of which is equivalent to the estimated mitochondrial free [NAD^+]/[NADH] ratio (Veech R L, unpublished data) as determined by the intracellular concentration of these metabolites (Williamson, D H, Lund P, Krebs H A, The redox state of free nicotinamide-adenine dinucleotide phosphate in the cytoplasm and mitochondria of rat liver. *Biochem J* 115:609–619, 1967). The stability of alphaketoglutarate is somewhat greater in solution than that of either pyruvate or acetoacetate, depending upon the conditions. Even though large gradients of each of these compounds exist across plasma membranes, and their concentrations in plasma are very low relative to their intracellular concentrations, controlled transport of all of these substrates does occur. If fluids not containing these substrates are given, then the cells alter their metabolism so as to make the infused material contain a predetermined concentration gradient. Addition, therefore, of alphaketoglutarate and NH_4^+ in an amino acid solution containing glutamate can control the redox state of the mitochondria.

Another example is the use of various ratios, around the physiologically normal, of [ketoglutarate]/[glutamine] which avoid the use of free ammonia, but which generate the ammonia and the production of intracellular glutamate.

Finally, the glutamate-pyruvate-transaminase reaction makes it possible to control redox state in an amino acid containing parenteral solution using the ratio of [l-alanine]/[l-lactate]. This appears to be useful in situations where the limited shelf life of ketoacids, such as pyruvate, in solution, may call for premixed redox balanced solutions with a long stability. Using a combination of [l-alanine]/[l-lactate] in conditions where this solution stability is needed, and the extra ammonia resulting from the formation of pyruvate from alanine and its subsequent removal as urea, does not present a problem, make this alternative practically useful in certain settings, such as the treatment of battle casualties or civilian catastrophes.

Suitable amino acid containing couples are shown in Table 4 below:

TABLE 4

Couple	Ratio Range
[1-Lactate$^-$]/[1-Alanine]	2:1 to 25:1
[1-glutamine]/[alpha ketoglutarate^{2-}]	2:1 to 50:1
[1-glutamate^{1-}] [NH_4^+] [alpha ketoglutarate^{2-}]	$1 \times 10^{+3} - 100 \times 10^{+3}$ Molar

The use of one or more of these various redox couples is optionally employed in a solution of this invention whether or not other nitrogen containing compounds are present (including glutamine) when control of the redox state is desired. Other nitrogen containing components present in normal plasma optionally may also be present in a solution of this invention. These new solutions, when compared to the presently available commercial formulations evaluated, for example, using rats with implanted venous cannulae, both before and after the induction of surgical trauma, demonstrate substantially improved capacity to control the redox state.

As those skilled in the art of amino acids appreciate, it has heretofore been the common practice to prepare such in the form of their hydrochloride salts. Evidently, such salts offer the advantages of reliable preparation of crystalline salts in high yields from aqueous solutions or slurries which are

themselves prepared variously by conventional total synthesis methods, by acid hydrolysis of inexpensive proteins from a protein source such as casein, degummed white silk, etc., by microbial conversion, by resolution of racemates to produce l-amino acid etc. Such hydrochloride salts when redissolved in water to prepare l-amino acid therapeutic fluids produce hyperchloremia when administered to a patient. To prevent such hyperchloremia, the prior art solutions remove chloride and substitute acetate with others, but different complications described earlier.

To avoid such complications, it is preferred to prepare l-amino acids for use in the present inventive compositions in the form of salts of at least one of the metabolite anions identified herein.

The parenteral aqueous solutions of the present invention preferably include, as solutes, more than one amino acid. In such preferred solutions this quantitative relationship of individual amino acids one to another is preferably such as to correspond to the quantitative relationship of these same respective amino acids in normal plasma. Each of the component amino acids present in such a mixture is preferably present in an amount which is in excess of the amount present in normal plasma. Thus, such mixtures are preferably multiples of normal plasma levels. Leaving aside the concentration of amino acids in the portal vein, the diurnal variations in the amino acid composition of plasma in the systemic circulation are relatively small (Wurtman R J et al., Daily rhythms in the concentrations of various amino acids in human plasma. *N Eng J Med* 279:171–175, 1969). It is, therefore, striking to note that the amino acid concentrations, relative to one another, which are used in the current commercial amino acid nutritional supplements in clinical use, bear little relationship to the amino acid composition of human plasma (see Table 1). Thus, as shown in Tables 1 and 3, the order of decreasing concentration in normal plasma is roughly: 1 glutamine, 2 cysteine, 3 alanine, 4 valine, 5 glycine, 6 lysine$^+$, 7 proline, 8 threonine, 9 serine, 10 leucine, 11 methionine, 12 tryptophane, 13 histidine, 14 arginine$^+$, 15 isoleucine, 16 glutamate$^-$, 17 tyrosine, 18 phenylalanine, 19 asparagine, 20 aspartate$^-$, as shown in the following Table 5:

TABLE 5

Decreasing Concentration of Organic Nitrogen Materials in Normal Human Plasma

I.D. No.	Material
1	1-glutamine
2	1-cysteine
3	1-alanine
4	1-valine
5	glycine
6	1-lysine
7	1-proline
8	1-threonine
9	1-serine
10	1-leucine
11	1-methionine
12	1-tryptophane
13	1-histidine
14	1-arginine
15	1-isoleucine
16	1-glutamate
17	1-tyrosine
18	1-phenylalanine
19	1-asparagine
20	1-aspartate

In what is believed to be the most commonly used present commercial formulations, the concentrations are disordered from normal in both order and in concentration range. In, for example, the "Travasol" formulation, the concentrations are, relative to the natural order: 4 glycine, 2 alanine, 13 arginine$^+$, 9 leucine 5 lysine, 3 valine, 10 methionine, 19 phenylalanine, 14 isoleucine, 6 proline, 7 threonine, 12 histidine, 13 tryptophane, 14 tyrosine, with the major amino acid in plasma, l-glutamine being omitted altogether, as are the important redox active amino acids, 15 glutamate$^-$ and 19 aspartate$^-$, and also, inexplicably, 8 serine, a precursor of pyruvate.

Through the transaminase reactions, the tissue concentrations of many amino acids are related to one another through the concentration of common ketoacids, particularly pyruvate and alpha ketoglutarate (see Veech R L and Krebs H A, in *The energy level and metabolic control in mitochondria,* pp. 329–382, Adriatica Editrice, Bari, 1969; Brosnan J T, in *The Urea Cycle,* pp. 443–457, John Wiley, New York, 1976). The intracellular amino acid levels are, therefore, importantly related both to the cytoplasmic [NAD$^+$]/[NADH] ratio, and to the mitochondrial [NAD$^+$]/[NADH] ratio, and also to the concentration of ammonia (Williamson D H, Lund P, Krebs H A, *Biochem J* 103:514–427, 1967). Giving one, but not another component, of a near-equilibrium couple must inevitably lead to a distortion of the intracellular concentrations of a number of metabolites. More importantly, distortion of the cellular redox state leads to changes in the cellular energy level or [ATP]/[ADP]×[Pi] ratio because:

$$K_{G+G} = \frac{3PG}{DHAP} \times \frac{ATP}{ADP \times P_i} \times \frac{NADH \quad H^+}{NAD^+}$$

(see Veech R L, Cornell N W, Lawson J W R, Krebs H A, *J Biol Chem* 254:6538–6547, 1979). It would, therefore, seem reasonable that, during the administration of parenteral nutrition, supplements aimed at restoring muscle function and increasing in protein mass, some consideration be given to control the natural order of metabolite levels, in addition to taking steps to control the cellular redox state and the phosphorylation potential. The mere presentation of nitrogen containing compounds to the body in disordered amounts derived from the composition of hydrolyzed casein leads only to their conversion to urea, or, as is a common occurrence during present parenteral nutrition therapy, hyperammonemia and hyperglycemia. Even more to the point, the prior art parenteral amino acid supplements do not lead to an increased functional capacity in muscle which is desired to decrease operative mortality and morbidity in a reasonable pre-operative period of supplementation. Unlike the feeding of livestock, which dictates so much of nutritional thinking, the increase in protein mass per se is not the goal to be sought in a human patient in need of parenteral nutrition therapy. Rather, an increased functional capacity is such a goal, and that will be judged using NMR and ergometry.

Part of the difficulty in present formulations may be due to the use of acetate in these formulations, which leads to a decrease in the phosphorylation potential, and to a severe decrease in the free [NADP$^+$]/[NADPH] ratio, in addition to the abnormalities in calcium and pyrophosphate metabolism discussed earlier. It has been suggested that the persistence of the muscle weakness and the failure of muscle mass to increase in patients receiving conventionally formulated parenteral amino acid supplements may, in fact, be a myopathy secondary to increased intracellular calcium content (see Russell D. et al. Nitrogen versus muscle calcium in the genesis of abnormal muscle function in malnutrition. *J Paren Ent Nutr* 9:415–421, 1985).

Omitted from presently available commercial mixtures, but which may optionally and advantageously be included in the new compositions of this invention, are such nitrogen containing compounds as l-carnitine, l-ornithine, l-citrulline, and the like. Carnitine, in addition to its well known role in the metabolism of fatty acids, is an important co-factor in the metabolism of the branched chain amino acids leucine, isoleucine and valine. The serum and tissue levels of l-carnitine are frequently found to be decreased in patients with malnutrition, alcoholic cirrhosis, certain congenital myopathies, and lipidemias, in patients on chronic hemodialysis, or in patients who are eating no red meat, as is the case for those receiving nutrition totally parenterally.

Hyperammonemia is a common complication of the administration of the present amino acid supplements. The urea cycle intermediates, ornithine and citrulline, may, therefore, be included with the amino acids used in an infusable fluid composition of this invention in an attempt to avoid distortion of the normal physiological levels of intermediates that exist under natural conditions. In this way, optimum function of the urea cycle is maintained during the inevitable loss of amino acids into urea which is a natural accompaniment of the ingestion of protein.

The provision of (a) a naturally balanced mixture of amino acids in concentration ratios as they exist in blood in nature, and (b) the substitution for acetate by a redox balanced mixture of anions of lactate and pyruvate and/or of anions of d-betahydroxybutyrate and acetoacetate, and/or bicarbonate and dissolved carbon dioxide in an infusion mixture, means that (1) the bone pain and abnormal calcium, phosphorus and pyrophosphate metabolism of current formulations can be avoided, (2) the absolute requirements for amino acids are decreased far below the 1 to 1.5 g nitrogen now recommended on the basis of dietary intake, (3) a more rapid increase in muscular and other organ function is achieved during parenteral fluid administration, and (4) prevention of hyperammonemia and abnormal elevations of one or another amino acids due to a functional metabolic block may be avoided.

Thus, in one aspect, the present invention provides a class of aqueous solutions adaptable for use in human parenteral nutrition therapy. A solution of this class tends (a) to normalize muscle and other organ function, (b) to maintain normal cellular phosphorylation potential, and (c) avoid acidosis and bone pain characteristic of present formulations.

As indicated above, compositions of this invention preferably contain glutamine. A glutamine-containing such composition (solution) preferably contains from about 0.03 to 120 millimoles per liter of glutamine plus at least one metabolizable nitrogen containing compound selected from among those shown in the Table 7 listing below. In Table 7 the plasma amino acids are arranged in groups, each group indicating a specified (preferred) concentration range for use in the practice of this invention. The preferred amount of each such compound in mMoles/Liter present in dissolved form in such a preferred solution of this invention is determined by a constant K which interrelates concentration ratios shown in Table 6 glutamine concentration with (other) amino acid concentration as shown by the following formula:

K=glutamine concentration/nitrogen containing compound concentration

The respective ranges for the values of K which apply for each of the four classes of amino acids shown in Table 7 appear in Table 6 below:

TABLE 6

Values for K	
Concentration Range Class	Range of K concentration ratios
I	1.2–1.8
II	2.1–2.9
III	3.5–6.5
IV	7–19

The single member of Class I (that is, l-cysteine) in Table 7 is preferably added to a solution just prior to administration. The respective metabolizable nitrogen-containing compounds in each such concentration range class are as follows:

TABLE 7

Concentration Classes of Nitrogen Containing Compounds		
Class No.	Metabolizable organic nitrogen Containing Compound	Compound Reference Number
I	1-Cysteine	1
II	1-Alanine	2
	1-Valine	3
	Glycine	4
	1-Lysine$^+$	5
	1-Proline	6
III	1-Threonine	7
	1-Serine	8
	1-Leucine	9
	1-Trytophane	10
	1-Histidine	11
	NH_4^+	12
	1-Carnitine	13
IV	1-Arginine$^+$	14
	1-Isoleucine	15
	1-Ornithine	16
	1-Glutamate$^-$	17
	1-Methionine	18
	1-Tyrosine	19
	1-Phenylalanine	20
	1-Taurine	21
	1-Aspartate-	22
	1-Asparagine	23
	1-Citrulline	24
	1-Aminobutyrate	25

In general, solutions of this invention contain nitrogen-containing positively or negatively charged metabolizable compounds which are in solution with correspondingly oppositely charged metabolites or electrolytes.

As can be seen, solutions of this invention are acetate anion free and are electrically neutral. A solution of this invention also contains at least one inorganic cation selected from the group consisting of sodium, potassium, calcium, magnesium and ammonium. The total quantity of such metabolic cation(s) present in a given solution ranges from about 0.1 to 150 mM/l. Each such dissolved metabolized organic nitrogen containing compound (including glutamine), when present in a solution of this invention, is preferably present in a concentration range extending from about 1 to 150 mM/l, although larger and smaller concentrations can be used.

EMBODIMENTS

The present invention is further illustrated by reference to the following examples. Those skilled in the art will appreciate that other and further embodiments are obvious and

within the spirit and scope of this invention from the teachings of these present examples taken with the accompanying specification.

EXAMPLE A

To an aqueous solution of the hydrochloride of l-alanine is added a mole excess of dissolved l-lactic acid and the mixture is hyopholized until a solid precipitates which is the l-hydrolactate of l-alanine. Ethanol is added to promote crystallization.

Similarly, other l-lactate salts are prepared so that the following aqueous solution of l-hydrolactates of l-amino acids is formulated wherein the l-amino acid concentrations are approximately 100 times their levels in normal plasma:

Amino Acid	mM/L
l-glutamine	30.0
l-cysteine	24.0
l-alanine	14.0
l-valine	14.0
glycine	12.0
l-lysine	11.0
l-proline	11.0
l-threonine	9.0
l-serine	8.0
l-leucine	8.0

To this solution is added other components as in Example 1.1 to provide a composition of this invention.

EXAMPLE 1.1 AND 1.2

Examples of aqueous solutions of this invention are prepared having the following compositions:

TABLE 8

New Metabolizable Nitrogen Containing Parenteral Nutrition Solutions
Concentrations are in mMoles/L

	Normal Plasma	Example 1.1 200 × Normal Plasma	Example 1.2 150 × Normal Plasma
l-glutamine	0.30	60	45
Group I			
l-cysteine	0.24	48	36
Group II			
l-alanine	0.14	28	21
l-valine	0.14	27	20
glycine	0.12	25	19
l-lysine$^+$	0.11	21	16
l-proline	0.11	20	16
Group III			
l-threonine	0.09	16	12
l-serine	0.08	16	12
l-leucine	0.08	15	11
l-tryptophine	0.05	11	8
l-histidine	0.05	10	8
NH_4^+	0.05	—	—
l-carnitine	0.05	9	7
Group IV			
l-arginine$^+$	0.04	8	6
l-isoleucine	0.04	7	5
l-ornithine	0.03	7	5
l-glutamate$^-$	0.03	6	5
l-methionine	0.03	6	4
l-tyrosine	0.03	6	4

TABLE 8-continued

New Metabolizable Nitrogen Containing Parenteral Nutrition Solutions
Concentrations are in mMoles/L

	Normal Plasma	Example 1.1 200 × Normal Plasma	Example 1.2 150 × Normal Plasma
l-phenylalanine	0.03	6	4
l-taurine	0.02	—	—
l-aspartate$^-$	0.02	4	3
l-asparagine	0.02	4	3
l-citrulline	0.02	—	3
l-aminobutyrate	0.02	—	—
K^+	5	10	8
l-lactate$^+$	1–6	26	20
pyruvate$^-$		3	2
mMole/L	311	403	303

EXAMPLES 1.3–1.5

Further examples of aqueous solutions of this invention are prepared having the following components:

TABLE 9

New Metabolizable Nitrogen Containing Parenteral Nutrition Solutions
Concentrations are in mMoles/L.

	Example 1.3 200 × Normal	Example 1.4 200 × Normal	Example 1.5 200 × Normal
l-glutamine	60	60	60
Group I			
l-cysteine	48	48	48
Group II			
l-alanine	28	28	28
l-valine	27	27	27
glycine	25	25	25
l-lysine$^+$	21	21	21
l-proline	20	20	20
Group III			
l-threonine	16	16	16
l-serine	16	16	16
l-leucine	15	15	15
l-tryptophine	11	11	11
l-histidine	10	10	10
NH_4^+	—	—	—
l-carnitine	9	9	9
Group IV			
l-arginine$^+$	8	8	8
l-isoleucine	7	7	7
l-amithine	7	7	7
l-glutamate$^-$	6	6	6
l-methionine	6	6	6
l-tyrosine	6	6	6
l-phenylalanine	6	6	6
l-taurine	—	—	—
l-aspartate$^-$	4	4	4
l-asparagine	4	4	4
l-citrulline	—	—	—
l-aminobutyrate	—	—	—
Na^+	10	12.5	12.5
l-lactate$^-$	54	53	54
alphaketoglutarate^{2-}		1.2	1.2
NH_4^+			1
mMole/L	425	427	428

EXAMPLE 2

The route of administration has an effect upon maximum dosage of nitrogen containing compounds and caloric supplementation.

Parenteral nutrition is now characteristically given, either through normal intravenous lines, using only slightly hyperosmolar solutions, or through grossly hypertonic solutions administered through indwelling catheters placed in a deep vein. Both are unphysiologic in that the bulk of the nutrients normally provided to the body enter through the portal vein, where very large concentrations of substrates normally occur in a postprandial state (Veech R L, unpublished data).

After recovery from the implantation of a cannula in a deep vein each of the formulations Examples 1.1 and 1.2 at the dose rate of 2 ml are administered to three starved Sprague Dawley male rats over one hour in combination with 3 ml of 5% dextrose in water. The effects on the plasma and tissue metabolite levels and rate of protein turnover are measured following established methods, such as those described by Poso and Mortemore, 1984. In chronic experiments, change in lean body and bone mass is measured. Exercise tolerance and ^{31}NMR of their muscles at rest, and during exercise, is measured, and the animals are sacrificed. The accumulation of pyrophosphate, phosphate, calcium, and other relevant electrolytes and metabolic intermediates is determined in blood, liver and skeletal muscle after freeze clamping of these organs during administration of the two different parenteral nutrition formulations. In addition, the total protein content of liver and skeletal muscle on the two types of formulations is determined as is the liver, muscle and blood content of amino acids, soluble CoA's, phosphorylation potential or [ATP]/[ADP][Pi] ratio, the redox state of the free pyridine nucleotide couples or [NAD(P)$^+$]/[NAD(P)H] ratios using the two formulations. The ^{31}p NMR determinations are performed upon the rat hind limb placed in a NMR tube and pulsed by electrical stimulation. It is found that the function of skeletal muscle with the new formulations is approximately normal.

Other and further aims, objects, purposes, advantages, uses, and the like for the present invention will be apparent to those skilled in the art from the present specification. The problems in acetate administration, including pyrophosphate and calcium build-up in liver, post-dialysis hyperphosphatemia and hyperparathyroidism are avoided. Likewise, the abnormal redox state with diminished [ATP]/[ADP][Pi] ratio seen with acetate alone may be eliminated.

I claim:

1. A non-hyperchloremic, alkalinizing aqueous solution for parenteral nutrition comprising water having dissolved therein:

(A) from about 1 to 150 mMoles/L of at least one of the following metabolizable nitrogen containing compounds:

l-glutamine
l-cysteine
l-alanine
l-valine
glycine
l-lysine$^+$
l-proline
l-threonine
l-serine
l-leucine
l-tryptophane
l-histidine
ammonium$^+$
l-carnitine
l-arginine$^+$
l-isoleucine
l-ornithine
l-glutamate$^-$
l-methionine
l-tyrosine
l-phenylalanine
l-aspartate$^-$
l-asparagine
l-citrulline

but always containing l-glutamine the total quantity of all such compound(s) in any given such solution being not more than about 1000 mMoles/Liter,

(B) from about 0.1 to 150 mMoles/Liter of at least one carboxylate anion selected from the group consisting of l-lactate with substantially no d-lactate, pyruvate, d-betahydroxybutyrate, acetoacetate, alpha Ketoglutarate l-glutamate, and bicarbonate, and

(C) from about 0.1 to 150 mMoles/Liter of at least one cation selected from the group consisting of sodium, potassium, calcium, magnesium, and ammonium.

2. The solution of claim **1** wherein there is dissolved at least one osmotically active nonionic water soluble nutrient in a total quantity ranging from about 5 to 400 mMoles per liter.

3. The solution of claim **1** wherein said anions are employed as pairs and such pairs are selected from the group consisting of from about 1 to 150 mMoles/Liter total of

(A) l-lactate anions and pyruvate anions in a milliequivalent ratio ranging from about 1:1 to 20:1,

(B) from about 1 to 150 mMoles/Liter total of d-betahydroxybutyrate anions and acetoacetate anions in a milliequivalent ratio ranging from about 0.5:1 to 6:1,

(C) from about 1 to 150 mMoles/Liter total of bicarbonate anions and dissolved carbon dioxide in a milliequivalent ratio ranging from about 1:1 to 100:1,

there being at least one such pair in said solution.

4. The solution of claim **1** wherein said nitrogen containing compounds include at least one material selected from the group consisting of alanine, glutamine, glutamate, wherein said carboxylate anions include at least one selected from the group consisting of l-lactate and alpha ketoglutarate, and wherein:

(A) from 1 to 150 mMoles/Liter total of l-lactate anions and alanine are present in a ratio in moles per liter of l-lactate anions to alanine ranges from about 0.5:1 to 20:1,

(B) from 1 to 150 mMoles/Liter total of glutamine and alpha ketoglutarate anions are present, the ratio in moles per liter of glutamine to alphaketoglutarate anions ranges from about 1:1 to 50:1, and

(C) from about 1 to 150 mMoles/Liter total of when ammonium and glutamate and alpha ketoglutarate anions are present, the ratio in moles/liter of [glutamate$^-$] to the product of moles/liter ammonium$^+$ times moles/liter of alpha ketoglutarate^{2-} ranges from about 1000 to 100,000 Moles/Liter.

5. An aqueous solution adaptable for use in human parenteral nutrition therapy and which solution tends (a) to normalize muscle and other organ function and (b) to maintain normal cellular phosphorylation potential, said solution comprising from about 0.03 to 120 millimoles per liter of glutamine plus at least five metabolizable nitrogen containing compounds selected from among the following compounds:

Class No.	Metabolizing Nitrogen Containing Compound
I	1-Cysteine
II	1-Alanine
	1-Valine
	Glycine
	1-Lysine$^+$
	1-Proline
III	1-Threonine
	1-Serine
	1-Leucine
	1-Tryptophane
	1-Histidine
	ammonium$^+$
	1-Carnitine
IV	1-Arginine
	1-Isoleucine
	1-Ornithine
	1-Glutamate$^-$
	1-Methionine
	1-Tyrosine
	1-Phenylalanine
	1-Taurine
	1-Aspartate
	1-Asparagine
	1-Citrulline
	1-Aminobutyrate

the concentration range of each such compound in millimoles per liter being determined by the following formula:

$$\text{nitrogen containing compound concentration} = \frac{\text{glutamine concentration}}{K}$$

where the glutamine concentration is in millimoles per liter and the value of K for each given such nitrogen containing compound is determined by its particular Class above indicated which is associated with such compound in accordance with the following table:

Class	Range of values for K
I	1.2–1.8
II	2.1–2.9
III	3.5–6.5
IV	7–19

6. A solution of claim **5** additionally containing at least one cation selected from the group consisting of sodium$^+$, potassium$^+$, magnesium^{2+}, calcium$^+$, ammonium$^+$ and at least one anion selected from the group consisting of l-lactate$^-$ with substantially no d-lactate, pyruvate$^-$, d-betahydroxybutyrate$^-$, acetoacetate$^-$, and alphaketoglutarate^{2-}, such ions being present in a total amount ranging from about 0.1 to 120 mM/l.

7. The solution of claim **5** wherein the respective amounts of said anions are such as to define near equilibrium couples of

(A) l-lactate with substantially no d-lactate and pyruvate in a molar concentration ratio ranging from about 20:1 to 1:1 l-lactate to pyruvate, and

(B) d-betahydroxybutyrate and acetoacetate in a molar concentration ratio ranging from about 6:1 to 0.5:1 d-betahydroxybutyrate to acetoacetate.

8. An in vivo process for accomplishing parenteral nutrition which comprises introducing intravenously a composition of claim **5** into a mammal at a rate which is at least sufficient to normalize organ function and phosphorylation potential.

9. The solution of claim **5** comprising at least ten of said metabolizable nitrogen containing compounds.

10. The solution of claim **5** comprising all of said metabolizable nitrogen containing compounds.

11. A method for controlling the redox state and the phosphorylation potential during parenteral fluid therapy comprising intravenously administering to a human patient a solution of claim **4**.

12. A compound comprising a salt of at least one metabolizable acid selected from the group consisting of l-lactic acid with substantially no d-lactic acid, pyruvic acid, d-betahydroxybutyric acid, acetoacetic acid and alphaketoglutaric acid with at least one metabolizable nitrogen containing compound selected from the group consisting of

l-glutamine
l-cysteine
l-alanine
l-valine
glycine
l-lysine$^+$
l-proline
l-threonine
l-serine
l-leucine
l-tryptophane
l-histidine
ammonium $^+$
l-carnitine
l-arginine$^+$
l-isoleucine
l-ornithine
l-glutamate$^-$
l-methionine
l-tyrosine
l-phenylalanine
l-aspartate$^-$
l-asparagine
l-citrulline.

13. A solution comprising water having dissolved therein:

(A) from about 1 to 150 mMoles/L of at least one of the following metabolizable nitrogen containing compounds:

l-glutamine
l-cysteine
l-alanine
l-valine
glycine
l-lysine$^+$
l-proline
l-threonine
l-serine
l-leucine
l-tryptophane
l-histidine
ammonium$^+$
l-carmotome
l-arginine$^+$
l-isoleucine
l-ornithine

l-glutamate
l-methionine
l-tyrosine
l-phenylalanine
l-aspartate$^-$
l-asparagine
l-citrulline

but always containing l-glutamine the total quantity of all such compound(s) in any given such solution being not more than about 1000 mMoles/Liter,

(B) from about 0.1 to 150 mMoles/Liter of at least one carboxylate anion selected from the group consisting of l-lactate with substantially no d-lactate, pyruvate: d-betahydroxybutyrate, acetoacetate, l-glutamate, and bicarbonate, and

(C) from about 0.1 to 150 mMoles/Liter of at least one cation selected from the group consisting of sodium, potassium, calcium, magnesium, and ammonium.

14. A solution comprising water having dissolved therein:

(A) from about 1 to 150 mMoles/L of at least one of the following metabolizable nitrogen containing compounds:

l-glutamine
l-cysteine
l-alanine
glycine
l-lysine$^+$
l-proline
l-threonine
l-serine
l-leucine
l-tryptophane
l-histidine
ammonium$^+$
l-carmotome
l-arginine+
l-isoleucine
l-ornithine
l-glutamate$^-$
l-methionine
l-tyrosine
l-phenylalanine
l-aspartate$^-$
l-asparagine
l-citrulline

but always containing L-glutamine the total quantity of all such compound(s) in any given such solution being not more than about 1000 mMoles/Liter,

(B) from about 0.1 to 150 mMoles/Liter of at least one carboxylate anion selected from the group consisting of l-lactate with substantially no d-lactate, pyruvate, d-betahydroxybutyrate, acetoacetate, l-glutamate, and bicarbonate, and

(C) from about 0.1 to 150 mMoles/Liter of at least one cation selected from the group consisting of sodium, potassium, calcium, magnesium, and ammonium, wherein said nitrogen containing compounds include at least one material selected from the group consisting of alanine, glutamine, glutamate, wherein said carboxylate anions include at least one selected from the group consisting of l-lactate and alpha ketoglutarate, and wherein:

(A) from 1 to 150 mMoles/Liter total of l-lactate anions and alanine are present in a ratio in moles per liter of l-lactate anions to alanine ranges from about 0.5:1 to 20:1,

(B) from 1 to 150 mMoles/Liter total of glutamine and alpha ketoglutarate anions are present, the ratio in moles per liter of glutamine to alphaketoglutarate anions ranges from about 1:1 to 50:1, and

(C) from about 1 to 150 mMoles/Liter total of when ammonium and glutamate and alpha ketoglutarate anions are present, the ratio in moles/liter of glutamate– to the product of moles/liter ammonium$^+$ times moles/liter of alpha ketoglutarate^{2-} ranges from about 1000 to 100,000 Moles/Liter.

* * * * *

Exhibit E

US006814986B1

(12) **United States Patent**
Rombi

(10) **Patent No.: US 6,814,986 B1**
(45) **Date of Patent: Nov. 9, 2004**

(54) **COMPOSITION FOR TREATING OBESITY AND ESTHETIC TREATMENT PROCESS**

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(57) **ABSTRACT**

The invention relates to a composition for the curative and prophylactic treatment of obesity, comprising a catechol-rich extract of green tea, in particular containing from 20% to 50% by mass of catechols expressed as epigallocatechol gallate (EGCG).

8 Claims, No Drawings

COMPOSITION FOR TREATING OBESITY AND ESTHETIC TREATMENT PROCESS

The present invention relates to the general field of treating obesity. The invention is directed in particular toward compositions for the curative and prophylactic treatment of obesity, but it also relates to the esthetic treatment of a human being to enhance his or her figure.

The therapeutic objective as regards obesity is well defined: it is a matter either of allowing the individual to lose a significant amount of weight, or of helping the individual to maintain a weight level which is as low as desired.

Several types of approach have been envisaged to date.

Nutritional approaches are directed toward reducing the supply of energy in the form of foods. This can be achieved either by drastically reducing the energy supplies or by replacing high-energy nutrients with others which are lower in energy: such as indigestible substitute fats, structured triglycerides which are difficult to assimilate or dietary fibers which cannot be assimilated.

The therapeutic approaches may have a variety of targets. Reducing the food intake may be the first objective. Attempts to reduce the food intake may be made by using anorexigenic substances, whose short-term effects are proven, but whose duration of use is limited on account of adverse side effects. In fact, very few of these products can truly be used and their long-term efficacy remains highly debated. New molecules are undergoing assessment or may do so in the near future, but their value still remains to be shown.

A second objective may be to increase the expenditure of energy by using thermogenic substances which act at the central or peripheral level. The use of these substances still remains limited.

A third objective is to reduce the assimilation of the dietary lipids, or optionally even that of the carbohydrates. This is a more recent approach, but is gaining in interest. A reduction in the assimilation of the dietary lipids may be obtained either by reducing the activity of the digestive enzymes concerned, or by modifying the properties of the interfaces transporting the lipid molecules, emulsions, vesicles or micelles.

The traditional use of tea is in the form of an infusion, for which the tip of the stems, comprising the last two leaves and the bud, are used. After harvesting, these leaves may be subjected to a fermentation, resulting in a transformation of the chemical substances they contain and in particular the catechols, which corresponds to black tea, or else may be dried immediately, thus giving green tea.

Besides catechols, tea contains caffeine, the diuretic effect of which is well known. This diuretic effect is the reason for the traditional use of green tea as a medicinal plant to promote the elimination of water by the kidneys, either in the case of urinary disorders or as a supplement to weight-reducing diets. The presence of caffeine is also the reason for the traditional use of tea in conditions of fatigue (asthenia).

Numerous epidemiological studies carried out on certain populations have clearly demonstrated the beneficial effects of the chronic ingestion of tea, and more particularly of green tea. Thus, the long-term consumption of green tea is thought to be anti-atherogenic on account of its hypocholesterolemiant effects (Muramatsu et al. 1986, Yang et al. 1997) and its ability to prevent the oxidation of LDLs in the circulation (Tijburg et al. 1997). Green tea is also known for its anti-mutagenic and anti-carcinogenic effects. Thus, it has been shown that green tea significantly reduces the risk of colorectal, skin and breast cancers (Blot et al. 1997, Conney et al. 1997, Dreosti et al. 1997, Jankun et al. 1997, Ji et al. 1997).

The traditional use of green tea as a diuretic is currently performed in the form of infusions, liquid extracts, extracts of plant powders or extracts in gel capsules or tablets. In these various forms, the green tea, often combined with another diuretic plant, is generally used at a dose corresponding to 1 to 3 g of plant per day.

In the context of screening pharmacological properties of various plants, it has been discovered that extracts of green tea have noteworthy properties which allow them to be used in the treatment of obesity.

The human body continually expends energy in order to function. The origins of this expenditure of energy are threefold: the metabolism, muscular work and thermogenesis, which corresponds to the energy expended by the body to maintain a constant temperature.

The expenditure of energy is compensated for by the energy supplied by the assimilation of foods. If the energy supplied from the dietary ration is strictly identical to the energy expended, the individual maintains a stable weight. If there is an excess supply of energy, the body stores this energy in the form of fats (increase in weight), and if there is a deficit in the supply of energy, the body draws the energy it lacks by burning off the fats stored (loss of weight). However, in this latter situation of an energy deficit encountered in the course of weight-reducing diets, the body reacts to save energy and reduce thermogenesis. This is the control mechanism which accounts for the failure of weigh-reducing diets. Specifically, after losing weight for a few weeks, the individual's weight stabilizes. If they wish to continue to slim, they must further reduce their food intake.

The full value of being able continually to increase thermogenesis, in particular in the course of a low-calorie diet during which it is lowered, may thus be appreciated. Various chemical substances stimulate thermogenesis, such as nicotine, ephedrine, aspirin, caffeine, etc., but none of them has made it possible to produce a medicinal product for treating obesity since the doses required to obtain an increase in thermogenesis entail considerable side effects, which are incompatible with a treatment which is necessarily long-lasting, generally extending over several months.

This objective has been achieved in accordance with the present invention by means of a composition for the curative and prophylactic treatment of obesity, comprising an extract of green tea, Camellia sinensis, which is rich in catechols.

The present invention is also directed toward the use of an extract or powder of green tea which has anti-lipase and/or thermogenic properties, for the manufacture of a medicinal product intended for the curative and prophylactic treatment of obesity.

Finally, the present invention relates to a process for the esthetic treatment of a human being in order to enhance his or her figure, characterized in that it involves the oral administration of a catechol-enriched extract of green tea in order to bring about a loss of weight or to maintain a weight level which is as low as desired.

In the context of the present invention, the extract of green tea contains from 20% to 50%, in particular from 20% to 30%, by mass of catechols expressed as epigallocatechol gallate (EGCG).

The content of catechols, expressed as epigallocatechol gallate (EGCG), is, for example, advantageously determined in the context of the present invention by using the analytical method described below.

The process is performed by liquid chromatography.

Solution to be examined: 80 ml of methanol R are added to 0.200 g of extract. The mixture is placed under magnetic stirring for 5 min and then in an ultrasound bath for 5 min.

The resulting mixture is filtered through paper and the volume is made up to 100 ml with the same solvent. This solution is diluted fivefold with methanol R.

Caffeine stock solution: 30 mg of caffeine are dissolved in methanol and made up to 100 ml with the same solvent.

Epigallocatechol gallate stock solution: 6 mg of epigallocatechol gallate (EGCG) are dissolved in methanol and made up to 10 ml with the same solvent.

Control solution: 1 ml of each stock solution is taken and made up to 10 ml with the same solvent.

The chromatography can be carried out using:

- a stainless steel column of length 250 mm and inside diameter 4.6 mm, filled with octadecylsilyl silica gel for chromatography R (5 μm) and thermostatically maintained at 200° C. (Nucleosil C18) and a precolumn having the same characteristics as the column,
- as mobile phase, at a flow rate of 1 ml/min, a mixture of aqueous 2% V/V glacial acetic acid solution (A) and of acetonitrile (B), the linear elution gradient of which is as follows:

Time (min)	A (%)	B (%)
0	95	5
5	95	5
10	90	10
17	85	15
30	82	18
35	82	18
40	95	5

as detector, a spectrophotometer set at 278 nm.

10 μl of each of the solutions are injected separately, at least twice. The sensitivity of the detector is adjusted so as to obtain peaks whose height represents at least 50% of the total scale of the recorder.

The percentage caffeine content is calculated using the following expression:

$$\frac{SCE}{SCT} \times \frac{MCT}{MCE} \times 0.05$$

SCE: area of the peak corresponding to caffeine in the chromatogram obtained with the solution to be examined
SCT: area of the peak corresponding to caffeine in the chromatogram obtained with the control solution
MCE: test sample of extract in the solution to be examined, expressed in grams
MCT: test sample of caffeine in the control solution, expressed in milligrams.

The percentage content of catechols expressed as epigallocatechol gallate (EGCG) is calculated using the following expression:

$$\frac{\Sigma SE}{ST} \times \frac{MT}{ME} \times 0.5$$

ΣSE: sum of the areas of the peaks (2-5-6-7-8) corresponding to catechols in the chromatogram obtained with the solution to be examined
ST: area of the peak corresponding to the EGCG in the chromatogram obtained with the control solution
ME: test sample of extract in the solution to be examined, expressed in grams
MT: test sample of EGCG in the control solution, expressed in milligrams.

It should be pointed out that the epigallocatechol gallate represents on average about 70% of all the catechols present in an extract of green tea, with a range of between 50% and 90%.

According to one particular characteristic of the present invention, the extract of green tea contains from 5% to 10% by mass of caffeine.

According to another characteristic of the invention, the extract of green tea has a ratio of the concentration of catechols to the concentration of caffeine of between 2 and 10.

According to one preferred characteristic of the invention, the extract of green tea is titrated so as to allow the administration of a daily dose of from 250 mg to 500 mg, preferably about 375 mg, of catechols per day, and from 50 mg to 200 mg, preferably about 150 mg, of caffeine per day.

The increase in thermogenesis in rats by an extract of green tea according to the invention was studied according to the following protocol:

The oxygen consumption of rats, maintained in a hermetic chamber for two hours and more, is measured after administering the test product. Since the expenditure of energy is proportional to the oxygen consumption, this technique makes it possible to measure the increase in thermogenesis, the basal metabolism and the muscular work being constant before and after treatment.

The test product was an extract of green tea containing 24.7% catechols and 8.35% caffeine.

The following results were obtained:

Controls: 0.06 w/kg$^{0.75}$

0.5 mg of extract/kg: 0.45 w/kg$^{0.75}$

1.0 mg of extract/kg: 0.81 w/kg$^{0.75}$

2.0 mg of extract/kg: 1.10 w/kg$^{0.75}$

The increase in thermogenesis in man by an extract of green tea according to the invention was also determined.

A similar study was carried out on 10 volunteers receiving at each meal either 500 mg of an extract of green tea (corresponding to 125 mg of catechols and 50 mg of caffeine), or 50 mg of caffeine, or a placebo.

The total expenditure of energy over 24 h showed a statistically significant increase ($p<0.01$) in favor of the extract: 9867 kJ compared with 9538 kJ for the placebo and 9599 kJ for caffeine.

These results demonstrate the ability of an extract of green tea according to the invention to significantly increase thermogenesis. This property is not associated with the caffeine content of the extract, since the administration of caffeine alone, at the same dose as that provided by the extract of green tea, does not increase thermogenesis.

Furthermore, the fact that the significant decrease in the Respiratory Quotient is not accompanied by an increase in the urinary excretion of nitrogen, makes it possible to conclude that there has been an increase in the oxidation of lipids, which is the desired aim in any treatment of obesity.

Finally, it has been possible to demonstrate that the extract of green tea according to the invention leads to an inhibition of the digestive lipases. An in vitro study made it possible to demonstrate that the extract of green tea, at a dose of 6 mg of extract per 100 mg of lipids, partially eliminates the emulsification of the lipids, both in the stomach and in the intestine. As it is known that the emulsification of lipids is the essential step in the action of lipases on food lipids, these results may account for the inhibitory ability of digestive lipases.

Another in vitro study, carried out under conditions reproducing the physiological conditions (successive action on triolein of gastric lipase and then of pancreatic lipase)

demonstrated that the extract of green tea, at a dose of 6 mg/100 mg of lipids, allows virtually total inhibition of the gastric lipase (89% inhibition) and partial inhibition of the pancreatic lipase (32% inhibition), i.e. a total inhibition of lipolysis of close to 40%.

The use of a powder of green tea which has an intrinsically lower dose of catechols and/or caffeine, but in a larger amount making it possible to manufacture a medicinal product which has anti-lipase and/or thermogenic properties, obviously also falls within the context of the present invention.

In the context of the present invention, in vitro studies were carried out to demonstrate the existence of synergy between epigallocatechol gallate and caffeine. These studies were carried out on an ex vivo pharmacological model of thermogenesis. The principle is to measure the oxygen consumption of a sample of rat brown adipose tissue; the oxygen consumption is proportional to the thermogenesis induced in the adipose tissue by the various test substances.

The results below indicate the increase in oxygen consumption as a function of the concentration of EGCG and/or of caffeine.

Caffeine	100 μM	0	0	100 μM	100 μM
EGCG	0	100 μM	200 μM	100 μM	200 μM
Increase in oxygen consumption	no effect	no effect	+40%	no effect	+140%

These results clearly demonstrate the existence of synergistic stimulation of thermogenesis for a concentration of 200 μM of EGCG and 100 μM of caffeine, i.e. an EGCG/caffeine ratio of 2.

Given the other pharmacological effects of caffeine (tachycardia, insomnia), it may thus be desirable, in order to increase thermogenesis, to limit the amount of caffeine and to increase the EGCG/caffeine ratio. For this reason, according to one advantageous variant of the invention, this ratio will preferably be between 2 and 10.

EXAMPLE OF OBTAINING AN EXTRACT OF GREEN TEA

Green tea contains on average 6% to 7% catechols and 2% to 3% caffeine.

In order to obtain the properties of increasing thermogenesis and of inhibiting digestive lipases which are described above, a sufficient supply of catechols is necessary. It is thus necessary to carry out an extraction of the green tea making it possible to obtain an extract which is sufficiently concentrated in catechols.

By way of example, the following extraction process can be used: 1 kg of the tips of ground stems, comprising the last two leaves and the bud, of green tea are extracted by percolation for 6 to 8 h with 10 kg of 80% ethanol (m/m). After filtration, the extract is concentrated under partial vacuum at a maximum temperature of 60° C. The concentrated extract is then spray-dried at a maximum temperature of 250° C. with or without maltodextrin, depending on the plotter specifications selected. This process gives an extract containing 20% to 30% catechols and 5% to 10% caffeine

This example is not limiting, and other extraction processes for obtaining an extract which is sufficiently rich in catechols can be used, in particular by varying the proportions of water and ethanol, or by using other solvents such as water, ethyl acetate, methanol, etc., alone or in combination. The choice of solvents selected will make it possible to vary the catechol and caffeine contents, the objective being a high catechol content, since the catechols are the main source of the pharmacological properties demonstrated above.

In this context, the use of green tea which has been partially decaffeinated beforehand by any extraction process which does not have a negative effect on the catechols (for example methylene chloride or supercritical carbon dioxide) may be entirely envisaged in order to obtain a tea extract containing only a small percentage of caffeine.

Without at all wishing to be limited to such an interpretation, it appears likely that the mechanism of activity of the extracts of green tea, which is the subject of the present invention, can be explained as follows. The catechols present in high concentration in the extracts of green tea according to the invention exert an inhibitory effect on catechol-O-methyltransferase (COMT), whereas the caffeine concentration of the extracts of green tea according to the invention acts by inhibiting phosphodiesterases, which leads to a reinforced activity of noradrenalin on thermogenesis.

What is claimed is:

1. A composition for the treatment of obesity, suitable for oral administration, comprising an extract of green tea having from 20% to 50% by mass of catechols expressed as epigallocatechol gallate (EGCG), and from 5% to 10% by mass of caffeine, the ratio of the concentration of catechols to the concentration of caffeine in said extract being between 2 and 10.

2. A composition according to claim **1**, wherein said green tea extract has from 20% to 30% by mass of catechols expressed as epigallocatechol gallate (EGCG).

3. The composition according to claim **1**, wherein said green tea extract has from 250 mg to 500 mg of catechols, and from 50 mg to 200 mg of caffeine, per daily dose.

4. The composition according to claim **3**, wherein said green tea extract has about 375 mg of catechols and about 150 mg of caffeine.

5. A composition for the treatment of obesity, suitable for oral administration, comprising an extract of green tea having from 20% to 50% by mass of catechols expressed as epigallocatechol gallate (EGCG), and from 5% to 10% by mass of caffeine, the ratio of the concentration of catechols to the concentration of caffeine in said extract being between 2 and 10 and wherein said green tea extract is obtained using an 80% ethanol extraction.

6. The composition according to claim **5**, wherein said green tea extract has from 20% to 30% by mass of catechols expressed as epigallocatechol gallate (EGCG).

7. The composition according to claim **5**, wherein said green tea extract has from 250 mg to 500 mg of catechols, and from 50 mg to 200 mg of caffeine, per daily dose.

8. The composition according to claim **7**, wherein said green tea extract has about 375 mg of catechols and about 150 mg of caffeine.

* * * * *

Exhibit F

US006830765B2

(12) **United States Patent**
Rombi

(10) **Patent No.: US 6,830,765 B2**
(45) **Date of Patent: Dec. 14, 2004**

(54) **GREEN TEA EXTRACT FOR TREATING OBESITY**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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Related U.S. Application Data

(62) Division of application No. 09/601,019, filed as application No. PCT/FR00/00065 on Jan. 14, 2000.

(30) **Foreign Application Priority Data**

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(51) **Int. Cl.**[7] **A61K 35/78**
(52) **U.S. Cl.** **424/729**; 424/725; 424/774
(58) **Field of Search** 424/725, 729, 424/774

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(57) **ABSTRACT**

The invention relates to a composition for the treatment of obesity, comprising a catechol-rich extract of green tea, in particular containing from 20% to 50% by mass of catechols expressed as epigallocatechol gallate (EGCG).

12 Claims, No Drawings

GREEN TEA EXTRACT FOR TREATING OBESITY

This application is a divisional of U.S. Application No. 09/601,019, filed on Oct. 17, 2000, which was a national stage filing under 35 U.S.C. §371 of International Application No. PCT/FR00/00065 filed on Jan. 14, 2000, which International Application was not published by the International Bureau in English. This application also claims priority to French Application 99/00328, filed Jan. 14, 1999. These documents are hereby incorporated by reference in their entirety and relied upon.

FIELD OF THE INVENTION

The present invention relates to the general field of treating obesity. The invention is directed in particular toward compositions for the curative and prophylactic treatment of obesity, but it also relates to the esthetic treatment of a human being to enhance his or her figure.

BACKGROUND OF THE INVENTION

The therapeutic objective as regards obesity is well defined: it is a matter either of allowing the individual to lose a significant amount of weight, or of helping the individual to maintain a weight level which is as low as desired.

Several types of approach have been envisaged to date.

Nutritional approaches are directed toward reducing the supply of energy in the form of foods. This can be achieved either by drastically reducing the energy supplies or by replacing high-energy nutrients with others which are lower in energy: such as indigestible substitute fats, structured triglycerides which are difficult to assimilate or dietary fibers which cannot be assimilated.

The therapeutic approaches may have a variety of targets.

Reducing the food intake may be the first objective. Attempts to reduce the food intake may be made by using anorexigenic substances, whose short-term effects are proven, but whose duration of use is limited on account of adverse side effects. In fact, very few of these products can truly be used and their long-term efficacy remains highly debated. New molecules are undergoing assessment or may do so in the near future, but their value still remains to be shown.

A second objective may be to increase the expenditure of energy by using thermogenic substances which act at the central or peripheral level. The use of these substances still remains limited.

A third objective is to reduce the assimilation of the dietary lipids, or optionally even that of the carbohydrates. This is a more recent approach, but is gaining in interest. A reduction in the assimilation of the dietary lipids may be obtained either by reducing the activity of the digestive enzymes concerned, or by modifying the properties of the interfaces transporting the lipid molecules, emulsions, vesicles or micelles.

The traditional use of tea is in the form of an infusion, for which the tip of the stems, comprising the last two leaves and the bud, are used. After harvesting, these leaves may be subjected to a fermentation, resulting in a transformation of the chemical substances they contain and in particular the catechols, which corresponds to black tea, or else may be dried immediately, thus giving green tea.

Besides catechols, tea contains caffeine, the diuretic effect of which is well known. This diuretic effect is the reason for the traditional use of green tea as a medicinal plant to promote the elimination of water by the kidneys, either in the case of urinary disorders or as a supplement to weight-reducing diets. The presence of caffeine is also the reason for the traditional use of tea in conditions of fatigue (asthenia).

Numerous epidemiological studies carried out on certain populations have clearly demonstrated the beneficial effects of the chronic ingestion of tea, and more particularly of green tea. Thus, the long-term consumption of green tea is thought to be anti-atherogenic on account of its hypocholesterolemiant effects (Muramatsu et al. 1986, Yang et al. 1997) and its ability to prevent the oxidation of LDLs in the circulation (Tijburg et al. 1997). Green tea is also known for its anti-mutagenic and anti-carcinogenic effects. Thus, it has been shown that green tea significantly reduces the risk of colorectal, skin and breast cancers (Blot et al. 1997, Conney et al. 1997, Dreosti et al. 1997, Jankun et al. 1997, Ji et al. 1997).

The traditional use of green tea as a diuretic is currently performed in the form of infusions, liquid extracts, extracts of plant powders or extracts in gel capsules or tablets. In these various forms, the green tea, often combined with another diuretic plant, is generally used at a dose corresponding to 1 to 3 g of plant per day.

In the context of screening pharmacological properties of various plants, it has been discovered that extracts of green tea have noteworthy properties which allow them to be used in the treatment of obesity.

The human body continually expends energy in order to function. The origins of this expenditure of energy are threefold: the metabolism, muscular work and thermogenesis, which corresponds to the energy expended by the body to maintain a constant temperature.

The expenditure of energy is compensated for by the energy supplied by the assimilation of foods. If the energy supplied from the dietary ration is strictly identical to the energy expended, the individual maintains a stable weight. If there is an excess supply of energy, the body stores this energy in the form of fats (increase in weight), and if there is a deficit in the supply of energy, the body draws the energy it lacks by burning off the fats stored (loss of weight). However, in this latter situation of an energy deficit encountered in the course of weight-reducing diets, the body reacts to save energy and reduce thermogenesis. This is the control mechanism which accounts for the failure of weigh-reducing diets. Specifically, after losing weight for a few weeks, the individual's weight stabilizes. If they wish to continue to slim, they must further reduce their food intake.

The full value of being able continually to increase thermogenesis, in particular in the course of a low-calorie diet during which it is lowered, may thus be appreciated. Various chemical substances stimulate thermogenesis, such as nicotine, ephedrine, aspirin, caffeine, etc., but none of them has made it possible to produce a medicinal product for treating obesity since the doses required to obtain an increase in thermogenesis entail considerable side effects, which are incompatible with a treatment which is necessarily long-lasting, generally extending over several months.

BRIEF SUMMARY OF THE INVENTION

This objective has been achieved in accordance with the present invention by means of a composition for the curative and prophylactic treatment of obesity, comprising an extract of green tea, Camellia sinensis, which is rich in catechols.

The present invention is also directed toward the use of an extract or powder of green tea which has anti-lipase and/or thermogenic properties, for the manufacture of a medicinal product intended for the curative and prophylactic treatment of obesity.

Finally, the present invention relates to a process for the esthetic treatment of a human being in order to enhance his or her figure, characterized in that it involves the oral administration of a catechol-enriched extract of green tea in order to bring about a loss of weight or to maintain a weight level which is as low as desired.

DETAILED DESCRIPTION OF THE INVENTION

In the context of the present invention, the extract of green tea contains from 20% to 50%, in particular from 20% to 30%, by mass of catechols expressed as epigallocatechol gallate (EGCG).

The content of catechols, expressed as epigallocatechol gallate (EGCG), is, for example, advantageously determined in the context of the present invention by using the analytical method described below.

The process is performed by liquid chromatography.

Solution to be examined: 80 ml of methanol R are added to 0.200 g of extract. The mixture is placed under magnetic stirring for 5 min and then in an ultrasound bath for 5 min. The resulting mixture is filtered through paper and the volume is made up to 100 ml with the same solvent. This solution is diluted fivefold with methanol R.

Caffeine stock solution: 30 mg of caffeine are dissolved in methanol and made up to 100 ml with the same solvent.

Epigallocatechol gallate stock solution: 6 mg of epigallocatechol gallate (EGCG) are dissolved in methanol and made up to 10 ml with the same solvent.

Control solution: 1 ml of each stock solution is taken and made up to 10 ml with the same solvent.

The chromatography can be carried out using:

- a stainless steel column of length 250 mm and inside diameter 4.6 mm, filled with octadecylsilyl silica gel for chromatography R (5 μm) and thermostatically maintained at 20° C. (Nucleosil C18) and a precolumn having the same characteristics as the column,
- as mobile phase, at a flow rate of 1 ml/min, a mixture of aqueous 2% V/V glacial acetic acid solution (A) and of acetonitrile (B), the linear elution gradient of which is as follows:

Time (min)	A (%)	B (%)
0	95	5
5	95	5
10	90	10
17	85	15
30	82	18
35	82	18
40	95	5

as detector, a spectrophotometer set at 278 nm.

10 μl of each of the solutions are injected separately, at least twice. The sensitivity of the detector is adjusted so as to obtain peaks whose height represents at least 50% of the total scale of the recorder.

The percentage caffeine content is calculated using the following expression:

$$\frac{SCE}{SCT} \times \frac{MCT}{MCE} \times 0.05$$

SCE: area of the peak corresponding to caffeine in the chromatogram obtained with the solution to be examined

SCT: area of the peak corresponding to caffeine in the chromatogram obtained with the control solution

MCE: test sample of extract in the solution to be examined, expressed in grams

MCT: test sample of caffeine in the control solution, expressed in milligrams.

The percentage content of catechols expressed as epigallocatechol gallate (EGCG) is calculated using the following expression:

$$\frac{\sum SE}{ST} \times \frac{MT}{ME} \times 0.5$$

ΣSE: sum of the areas of the peaks (2-5-6-7-8) corresponding to catechols in the chromatogram obtained with the solution to be examined

ST: area of the peak corresponding to the EGCG in the chromatogram obtained with the control solution

ME: test sample of extract in the solution to be examined, expressed in grams

MT: test sample of EGCG in the control solution, expressed in milligrams.

It should be pointed out that the epigallocatechol gallate represents on average about 70% of all the catechols present in an extract of green tea, with a range of between 50% and 90%.

According to one particular characteristic of the present invention, the extract of green tea contains from 5% to 10% by mass of caffeine.

According to another characteristic of the invention, the extract of green tea has a ratio of the concentration of catechols to the concentration of caffeine of between 2 and 10.

According to one preferred characteristic of the invention, the extract of green tea is titrated so as to allow the administration of a daily dose of from 250 mg to 500 mg, preferably about 375 mg, of catechols per day, and from 50 mg to 200 mg, preferably about 150 mg, of caffeine per day.

The increase in thermogenesis in rats by an extract of green tea according to the invention was studied according to the following protocol:

The oxygen consumption of rats, maintained in a hermetic chamber for two hours and more, is measured after administering the test product. Since the expenditure of energy is proportional to the oxygen consumption, this technique makes it possible to measure the increase in thermogenesis, the basal metabolism and the muscular work being constant before and after treatment.

The test product was an extract of green tea containing 24.7% catechols and 8.35% caffeine.

The following results were obtained:

Controls: 0.06 w/kg$^{0.75}$

0.5 mg of extract/kg: 0.45 w/kg$^{0.75}$

1.0 mg of extract/kg: 0.81 w/kg$^{0.75}$

2.0 mg of extract/kg: 1.10 w/kg$^{0.75}$

The increase in thermogenesis in man by an extract of green tea according to the invention was also determined.

A similar study was carried out on 10 volunteers receiving at each meal either 500 mg of an extract of green tea (corresponding to 125 mg of catechols and 50 mg of caffeine), or 50 mg of caffeine, or a placebo.

The total expenditure of energy over 24 h showed a statistically significant increase ($p<0.01$) in favor of the extract: 9867 kJ compared with 9538 kJ for the placebo and 9599 kJ for caffeine.

These results demonstrate the ability of an extract of green tea according to the invention to significantly increase

thermogenesis. This property is not associated with the caffeine content of the extract, since the administration of caffeine alone, at the same dose as that provided by the extract of green tea, does not increase thermogenesis.

Furthermore, the fact that the significant decrease in the Respiratory Quotient is not accompanied by an increase in the urinary excretion of nitrogen, makes it possible to conclude that there has been an increase in the oxidation of lipids, which is the desired aim in any treatment of obesity.

Finally, it has been possible to demonstrate that the extract of green tea according to the invention leads to an inhibition of the digestive lipases. An in vitro study made it possible to demonstrate that the extract of green tea, at a dose of 6 mg of extract per 100 mg of lipids, partially eliminates the emulsification of the lipids, both in the stomach and in the intestine. As it is known that the emulsification of lipids is the essential step in the action of lipases on food lipids, these results may account for the inhibitory ability of digestive lipases.

Another in vitro study, carried out under conditions reproducing the physiological conditions (successive action on triolein of gastric lipase and then of pancreatic lipase) demonstrated that the extract of green tea, at a dose of 6 mg/100 mg of lipids, allows virtually total inhibition of the gastric lipase (89% inhibition) and partial inhibition of the pancreatic lipase (32% inhibition), i.e. a total inhibition of lipolysis of close to 40%.

The use of a powder of green tea which has an intrinsically lower dose of catechols and/or caffeine, but in a larger amount making it possible to manufacture a medicinal product which has anti-lipase and/or thermogenic properties, obviously also falls within the context of the present invention.

In the context of the present invention, in vitro studies were carried out to demonstrate the existence of synergy between epigallocatechol gallate and caffeine. These studies were carried out on an ex vivo pharmacological model of thermogenesis. The principle is to measure the oxygen consumption of a sample of rat brown adipose tissue; the oxygen consumption is proportional to the thermogenesis induced in the adipose tissue by the various test substances.

The results below indicate the increase in oxygen consumption as a function of the concentration of EGCG and/or of caffeine.

Caffeine	100 μM	0	0	100 μM	100 μM
EGCG	0	100 μM	200 μM	100 μM	200 μM
Increase in oxygen consumption	no effect	no effect	+40%	no effect	+140%

These results clearly demonstrate the existence of synergistic stimulation of thermogenesis for a concentration of 200 μM of EGCG and 100 μM of caffeine, i.e. an EGCG/caffeine ratio of 2.

Given the other pharmacological effects of caffeine (tachycardia, insomnia), it may thus be desirable, in order to increase thermogenesis, to limit the amount of caffeine and to increase the EGCG/caffeine ratio. For this reason, according to one advantageous variant of the invention, this ratio will preferably be between 2 and 10.

EXAMPLE OF OBTAINING AN EXTRACT OF GREEN TEA

Green tea contains on average 6% to 7% catechols and 2% to 3% caffeine.

In order to obtain the properties of increasing thermogenesis and of inhibiting digestive lipases which are described above, a sufficient supply of catechols is necessary. It is thus necessary to carry out an extraction of the green tea making it possible to obtain an extract which is sufficiently concentrated in catechols.

By way of example, the following extraction process can be used: 1 kg of the tips of ground stems, comprising the last two leaves and the bud, of green tea are extracted by percolation for 6 to 8 h with 10 kg of 80% ethanol (m/m). After filtration, the extract is concentrated under partial vacuum at a maximum temperature of 60° C. The concentrated extract is then spray-dried at a maximum temperature of 250° C. with or without maltodextrin, depending on the plotter specifications selected. This process gives an extract containing 20% to 30% catechols and 5% to 10% caffeine.

This example is not limiting, and other extraction processes for obtaining an extract which is sufficiently rich in catechols can be used, in particular by varying the proportions of water and ethanol, or by using other solvents such as water, ethyl acetate, methanol, etc., alone or in combination. The choice of solvents selected will make it possible to vary the catechol and caffeine contents, the objective being a high catechol content, since the catechols are the main source of the pharmacological properties demonstrated above.

In this context, the use of green tea which has been partially decaffeinated beforehand by any extraction process which does not have a negative effect on the catechols (for example methylene chloride or supercritical carbon dioxide) may be entirely envisaged in order to obtain a tea extract containing only a small percentage of caffeine.

Without at all wishing to be limited to such an interpretation, it appears likely that the mechanism of activity of the extracts of green tea, which is the subject of the present invention, can be explained as follows. The catechols present in high concentration in the extracts of green tea according to the invention exert an inhibitory effect on catechol-O-methyltransferase (COMT), whereas the caffeine concentration of the extracts of green tea according to the invention acts by inhibiting phosphodiesterases, which leads to a reinforced activity of noradrenalin on thermogenesis.

What is claimed is:

1. A method for treating obesity in a patient in need of said treatment comprising orally administering to said patient an extract of green tea comprising from 20% to 50% by mass of catechols expressed as epigallocatechol gallate (EGCG), and from 5% to 10% by mass of caffeine, said catechols and caffeine being present in said extract in a thermogenically effective amount, the ratio of the concentration of catechols to the concentration of caffeine in the extract of green tea being between 2 and 10.

2. A method according to claim **1**, wherein the extract of green tea contains from 20% to 30% by mass of catechols expressed as epigallocatechol gallate (EGCG).

3. A method according to claim **1**, wherein the extract of green tea comprises from 250 mg to 500 mg of catechols, and from 50 mg of caffeine and wherein said extract is administered daily.

4. A method according to claim **3**, wherein the extract of green tea comprises about 375 mg of catechols and about 150 mg of caffeine and wherein said extract is administered daily.

5. A method for promoting weight loss in a human being in need thereof, comprising orally administering thereto an extract of green tea comprising from 20% to 50% by mass

of catechols expressed as epigallocatechol gallate (EGCG), and from 5% to 10% by mass of caffeine, said catechols and caffeine being present in said extract in a thermogenically effective amount, the ratio of the concentration of catechols to the concentration of caffeine in the extract of green tea being between 2 and 10, in order to bring about a loss of weight or to maintain a desired weight level.

6. A method according to claim **5**, wherein the extract of green tea contains from 20% to 30% by mass of catechols expressed as epigallocatechol gallate (EGCG).

7. A method according to claim **5**, wherein the extract of green tea comprises from 250 mg to 500 mg of catechols, and from 50 mg to 200 mg of caffeine and wherein said extract is administered daily.

8. A method according to claim **7**, wherein the extract of green tea comprises about 375 mg of catechols and about 150 mg of caffeine and wherein said extract is administered daily.

9. A method for inhibiting the onset of obesity in a subject in need thereof comprising orally administering to said subject an extract of green tea comprising from 20% to 50% by mass of catechols expressed as epigallocatechol gallate (EGCG), and from 5% to 10% by mass of caffeine, said catechols and caffeine being present in said extract in a thermogenically effective amount, the ratio of the concentration of catechols to the concentration of caffeine in the extract of green tea being between 2 and 10.

10. A method according to claim **9**, wherein the extract of green tea contains from 20% to 30% by mass of catechols expressed as epigallocatechol gallate (EGCG).

11. A method according to claim **9**, wherein the extract of green tea comprises from 250 mg to 500 mg of catechols, and from 50 mg to 200 mg of caffeine and wherein said extract is administered daily.

12. A method according to claim **11**, wherein the extract of green tea comprises about 375 mg of catechols and about 150 mg of caffeine and wherein said extract is administered daily.

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