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DIOGUARDI, Francesco, Saverio, Via Manara, 15, I-20122 Milano, Italien(74) Fuldmægtig i Danmark: **Zacco Denmark A/S, Arne Jacobsens Allé 15, 2300 København S, Danmark**(54) Benævnelse: **Sammensætninger baseret på aminosyrer med forgrenet kæde til forbedring af den myocardiale ventrikulære funktion hos patienter, der lider af diabetes**

(56) Fremdragne publikationer:

EP-A- 0 483 614**WO-A-02/02092****WO-A-02/02103****FR-A- 2 710 243****O'KEEFE J H ET AL: "IMPROVING THE ADVERSE CARDIOVASCULAR PROGNOSIS OF TYPE 2 DIABETES"**
MAYO CLINIC PROCEEDINGS, MAYO MEDICAL VENTURES, ROCHESTER, MN, US, vol. 74, no. 2, February 1999 (1999-02), pages 171-180, XP000856581 ISSN: 0025-6196**SCHWALB H ET AL: "THE PROTECTIVE EFFECT OF ENRICHED BRANCHED CHAIN AMINO ACID FORMULATION IN THE ISCHEMIC HEART A PHOSPHORUS-31 NMR STUDY" JOURNAL OF MOLECULAR AND CELLULAR CARDIOLOGY, vol. 19, no. 10, 1987, pages 991-998, XP009007218 ISSN: 0022-2828****POGATSA G: "NEW CONCEPTS IN THE THERAPY OF DIABETIC CARDIOPATHIES" MEDLINE, XP002158323**
LI AI-LING ET AL: "Protection of branched-chain amino acids against ischemic myocardial injury in rats." BIOMEDICAL AND ENVIRONMENTAL SCIENCES, vol. 12, no. 1, March 1999 (1999-03), pages 62-65, XP009007240 ISSN: 0895-3988

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Description

[0001] The present invention refers to the use of compositions based on amino acids for improving the myocardial ventricular function in patients suffering from diabetes, in particular type II diabetes.

[0002] Experimental studies carried out on patients of the indicated type have shown, at myocardium level, a depression of the energetic metabolism, a reduction of the synthesis velocity and an increase of the proteins degradation. A general degradation of the mechanical function of the cardiac muscle derives from the above, whose main pathogenic mechanisms are the reduced availability of energetic material and the presence of contractile proteins having low ATPase activity.

[0003] WO0202103 and WO0202092 discloses the use of amino acids for treating chronic heart failure. These documents fall under Art. 54(3) EPC.

[0004] The article "Protection of branched-chain amino acid against ischemic myocardial injury in rats", by Li-Ai Ling et al., Biomedical and Environmental Sciences, 12(1) 1999, p. 62-65 reports that BCAA have a protective effect against the reduction of ATP during the ischemic phase.

[0005] The article "The protective effect of enriched branched chain amino acid formulation in the ischemic heart: a phosphorous-31 nuclear magnetic resonance study", by H. Schwalb et al., J. of Mol. And Cell. Cardiol. 19(10) 1987, p. 991-998, reports that BCAA have beneficial biochemical and hemodynamic effects on the ischemic heart.

[0006] EP-A-0 483 614 discloses the use of amino acids for the preparation of a composition for improving cardiac function that can be used as a cardiac therapy in which pre-existing ischemia is being treated or in which ischemia may result from a therapeutic intervention.

[0007] At present, no therapeutic approaches are known aimed at producing a noticeable improvement of the ventricular myocardial function in patients suffering from diabetes, in order to favorably influence the natural story of said patients, by retarding or preventing the appearance of cardiac insufficiency, which represents the main cause of morbidity and mortality within said population of patients.

[0008] The present invention has the aim of indicating an absolutely innovative therapeutic approach to the above mentioned problem.

[0009] Within this frame, a first aim of the invention is that of indicating compositions capable of determining a noticeable improvement of the myocardial ventricular function in patients suffering from diabetes, particularly but not exclusively type II diabetes.

[0010] A further aim of the invention is that of indicating compositions capable of determining, in patients of the above type, a noticeable increase of the ventricular ejection fraction, at rest and at peak of the isometric exercise.

[0011] A further aim of the invention is that of indicating compositions capable of eliminating the reduction of the ventricular ejection fraction which, in patients suffering from diabetes, occurs during isometric strain.

[0012] The inventors arrived to the use of compositions based on amino acids, as per the enclosed claims which are an integral part of the present description, which prove to be particularly effective for the proposed purposes.

[0013] Said compositions, being provided either for oral and parenteral use, are characterized by comprising, as main active ingredients, the branched chain amino acids leucine, isoleucine and valine, up to 75% of all the amino acids or active ingredients being present, by expressing the value in molecular weights.

[0014] The compositions according to the invention also comprise, as further active ingredients, threonine and lysine, where in particular threonine plus lysine are present up to 50% of all the amino acids or active ingredients being present, by expressing the value in molecular weights.

[0015] The compositions provide for, as further active ingredients, other essential amino acids, in particular methionine and phenylalanine and histidine and tryptophan, and non essential amino acids, in particular tyrosine and cyst(e)ine (i.e. cystine and cysteine).

[0016] The sum of the amounts expressed in molecular weights of threonine and lysine is greater than the sum of the single amounts of the other essential amino acids being provided, but in any case lower than the sum of the single amounts of the branched chain amino acids being provided. In addition, the amounts expressed in molecular weight of threonine and of lysine can be each greater than the single amounts of the other essential amino acids being provided, but in any case lower than the single quantities of the branched chain amino acids being provided.

[0017] The use of compositions according to the invention can also comprise one or more further amino acids, with respect to those as previously indicated, the sum of which, expressed in molecular weight, is preferably lower than 20% with respect to the active ingredients, and less than 10% for each single further amino acid.

[0018] It should be noticed that, in general terms, a mixture of amino acids particularly suitable for nutritional use in humans should satisfy different requirements:

- the pH of the solution of the mixture should be substantially neutral, in order to prevent urinary calcium losses;
- the mixture should be safe, in respect to calcium balance (i.e.: with no urinary losses) and homocyst(e)ine production (i.e., preferably related to the amount of all amino acids, a strictly correct ratio of sulphur containing amino acids, with a ratio cyst(e)ine / methionine of at least 2:1 on a stoichiometric basis).

[0019] In addition, the content of essential amino acids in the mixture should be preferably in an adequate ratio to fulfill real human nutritional needs (and this can be optimized by the co-operative adjunction of adequate and small ratios of some non essential amino acids).

[0020] Within this frame, a preferred use of the compositions according to the invention, comprising essential amino acids (leucine, isoleucine, valine, threonine, lysine, methionine, phenylalanine, histidine, tryptophan) and some non essential amino acids (tyrosine and cyst(e)ine), in different but fixed and co-operative molar ratios among them, is the following one:

- branched chain amino acids leucine (40-60% in molecular weight), isoleucine (20-40% in molecular weight) and valine (20-40% in molecular weight), preferentially in a stoichiometric ratio 2:1:1 among them, covering from 30 to 60% of the weight of the whole mixture;
- threonine plus lysine, preferably in a molar ratio with the said branched chain amino acids between 20 and 50%, preferably in a threonine to lysine ratio in which lysine is from 10 to 50% more represented than threonine;
- the above said branched chain amino acids plus threonine and lysine, whose sum of the molecular weight is in a stoichiometric ratio of 50 to 70% of a mixture also comprising histidine and other amino acids, were histidine is present in molar fraction up to 50% of the following amino acids:
 - cyst(e)ine (i.e., cystine and cysteine) and methionine, up to 50% of histidine (the ratio between cyst(e)ine and methionine should be preferably of 50 to 200% greater for cyst(e)ine in molar ratio),
 - phenylalanine and tyrosine, in molar ratio up to 50% of histidine (in which tyrosine is preferably represented up to 50% of the molar weight of phenylalanine),
 - tryptophan, up to 10% of the weight of all the other amino acids, on a molar weight basis.

[0021] It has to be noticed that any other amino acid can be added to the above formulation, without altering the expected effects, provided that the sum of the additional amino acids is in a percentage lower than 20% with respect to the other active ingredients (less than 10% for each single amino acid).

[0022] It should also be noticed that a significant characteristic of the above said formulation is that of having a pH in water solution comprised between 6.5 and 8.5, and therefore suitable for a safe oral or parenteral use, in humans or animals, according to needs. This feature prevents the excessive calcium urinary losses induced by protein sources of amino acids.

[0023] The effects of an amino acids mixture according to the above suggested formulation were the subject of a comparative study.

[0024] To this purpose, 18 patients suffering from II type diabetes (mellitus) were recruited

(M/F 16/2, age 62 ± 6 years, body mass index (BMI) $27.4 \pm 3.0 \text{ kg/m}^2$). The average duration of the disease was 12 ± 8 years. Glycated haemoglobin was $8.2 \pm 0.8\%$. After the basal evaluation, patients were randomized at the treatment with a composition of amino acids according to the above said preferred formulation of the invention (12 g/die) or with placebo, for a period of 3 weeks. The treatment was subsequently exchanged and maintained for a further period of 3 weeks.

[0025] The analyzed main metabolic parameters were: glycaemia, insulinemia, C-peptide, free fatty acids (FFA), total and fractioned cholesterol, triglycerids and fibrinogen.

[0026] The left ventricular function was evaluated by means of 2D echocardiography, using a Hewlett-Packard Sonos 5500 system, with dedicated program for the execution of echo-stress methodologies and the quantification of ventricular images. The echocardiographic studies were encoded and blind analyzed, by two independent observers, without knowing the identity of the patient and the experimental condition. The echocardiographic analysis was carried out using a digital *cine-loop* method (Prevue System, Nova Microsonics Inc.). The ventricular volumes were calculated, in the various experimental conditions, with a biplane area-length method, from which the ejection fraction (EF) was derived as index of ventricular pump function: $EF = VTD-VTS / VTD$, wherein VTD and VTS represent the telediastolic and telesistolic volume of the left ventricle, respectively.

[0027] The parietal contractile function was evaluated by analyzing the myocardium systolic thickening, in each segment obtained by subdividing the left ventricle into 16 segments (according to the *American Society of Echocardiography* standards) and by using a semiquantitative score system (1=normal, 2=hypocinesia, 3=akinesia, 4=dyscinesia).

[0028] The general and regional ventricular function was studied at rest conditions and during isometric strain through *hand-grip test*.

[0029] After having determined the maximal voluntary contraction by means of a dynamometer, an isometric strain at 40% was carried out for 3 minutes. During strain, ventricular function was monitored by 2D echocardiography and the arterial pressure was monitored through a continuous oscillometric method (Nippon Colin Co. Ltd).

[0030] The results of the study are expressed as mean \pm SD. The multiple comparisons were carried out by means of the *two-way* variance analysis for repeated measures, followed by the Fisher's test. A *two-tailed* value ≤ 0.05 as been considered as a significant one.

[0031] None of the metabolic parameters was significantly modified, neither by the treatment with the amino acids mixture according to the invention, nor by the administration of placebo.

[0032] As it is apparent from Table 1 which follows, the treatment with the mixture according to the invention did not brought to significant variations, concerning glycaemia at

fast, insulinemia and sensitivity to insulin considered through the *insulin tolerance test* technique.

[0033] Also concerning lipidic metabolism, no variations were observed in relation to total cholesterol, HDL, triglycerids, free fatty acids and Lp(a). In addition, no substantial modifications occurred concerning both pressure values and body mass index.

Table 1 - Clinical characteristics of patients

	Placebo	Invention	P
Demography			
Age (years)	62 ± 6		
Clinical characteristics			
Disease duration (years)	10 ± 7		
Body mass index (SD) (kg/m ²)	27 ± 3	27 ± 3	n.s.
Anti-hypertension treatment (%)	77%		
Sistolic pressure (SD) (mm Hg)	151 ± 12	154 ± 17	n.s.
Diastolic pressure (SD) (mmHg)	84 ± 5	84 ± 6	n.s.
HbA1c (SD) (%)	8.7 ± 1.7	8.6 ± 1.6	n.s.
Insulinemia (SD) (μm/ml)	14 ± 8	20 ± 15	n.s.
Cholesterolemia (SD) (mg/dl)	225 ± 27	214 ± 24	n.s.
HDL cholesterol (SD) (mg/dl)	47 ± 13	46 ± 15	n.s.
Triglyceridemia (SD) (mg/dl)	165 ± 91	115 ± 66	n.s.
Free fatty acids (SD) (μmol/l)	699 ± 395	656 ± 370	n.s.
Lp(a) (SD) (mg/dl)	12 ± 10	13 ± 10	n.s.
Proteinuria (mg/die)	40 ± 35	45 ± 29	n.s.
Anti-diabetes therapy (D/ADO/ADO+I/I)	2/9/3/4		

[0034] By analyzing the general ventricular function of the patients being the subject of the study, it was possible to draw the following conclusions:

- 1) the treatment with the amino acids mixture according to the invention does not modify, in a significant manner, the ventricular dimensions (considered as telediastolic volume), neither at rest (77 ± 24 vs. 78 ± 24 ml/m², p=ns), nor at the peak of the isometric strain (86 ± 26 vs. 88 ± 25 ml/m², p=ns). In addition, the same increment induced by the isometric strain is maintained;
- 2) during treatment with the amino acids mixture according to the invention, the ejection fraction increases in a significant manner, both at rest (58 ± 8 vs. 52 ± 12%, p=0.009) and at the peak of the isometric strain (58 ± 10 vs. 43 ± 13, p=0.0001); it is then particular interesting that

3) the reduction of the ejection fraction during isometric effort is abolished, with respect to the basal condition ($p=0.188$), which is instead maintained during placebo ($p<0.0001$).

[0035] Upon analyzing the regional myocardial contractile function of the patients, the following remarks are possible:

- the administration of the amino acids mixture according to the invention determines a reduction of the extension of the regional contractile dysfunction at rest (considered as *wall motion score index*, WMSI) (1.32 ± 0.42 vs. 1.26 ± 0.41 , $p=0.005$);
- the isometric strain causes an extension of the regional contractile dysfunction independently of the type of treatment, but the extension of the contractile dysfunction at the strain peak is smaller during the treatment with the amino acids mixture according to the invention (1.49 ± 0.45 vs. 1.29 ± 0.41 , $p<0.05$).

[0036] It results clear from the above that the oral administration of the amino acids mixture according to the invention determines remarkable variations of the myocardial and ventricular function in diabetes patients, in particular type II diabetes.

[0037] Said administration has in fact positively influenced the left ventricular myocardial function, both at rest and during isometric strain. The fact is particularly interesting that said administration prevents the depression of the function caused by isometric strain which characterizes diabetes patients.

[0038] The above data also highlight a positive action on myocardial inotropism and contractile recruitment during conditions of increased load (such as during hand grip characterized by an acute increase of the postload). This effect can be the result of the combined action of an improvement of the energetic metabolism and an inversion of the *shift* of the synthesis of contractile proteins to the production of fast ATPase activity elements.

[0039] The results deriving from the study of the regional contractile function also highlight a reduction of the extension of the reversible chronic contractile dysfunction, which is a sign of a favorable influence on hibernating myocardium.

[0040] Finally, the administration of the amino acids mixture according to the invention reduces the further extension of the contractile dysfunction induced by acute ischemia during isometric strain.

[0041] From the given description the features of the present invention are clear, as well as its advantages. In particular, the oral administration of the described mixture of amino acids positively influences the myocardial ventricular function of patients suffering from diabetes, in particular type II diabetes. The positive influence is evident either at rest and

during the acute overload imposed by an isometric strain during *hand-grip*, and also on the parietal contractile function, by means of a reduction of the extension of myocardial hibernation phenomena and of the extension of the contractile dysfunction induced by acute ischemia during isometric strain.

[0042] It results therefore clear that the proposed use of amino acids allows for favorably influencing the natural story of diabetic patients, by retarding or preventing the appearance of cardiac insufficiency, which represents the main cause of morbidity and mortality within said population of patients.

Patentkrav

1. Anvendelse af aminosyrerne leucin, isoleucin og valin med forgrenet kæde i kombination med threonin, lysin methionin, phenylalanin, histidin, tryptophan, tyrosin, cystein og cystin som aktiv bestanddel til fremstilling af sammensætninger til forsinkelse eller forebyggelse af hjerteinsufficiens hos patienter, der lider af diabetes, især men ikke udelukkende type II-diabetes, hvor summen af mængden i molekylvægte af threonin og lysin er større end summen af mængderne af methionin, phenylalanin, histidin, tryptophan, der tilvejebringes, men lavere end summen af mængderne af aminosyrerne med forgrenet kæde.

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2. Anvendelse ifølge krav 1, hvor aminosyrerne med forgrenet kæde forekommer i en mængde på op til 75% af aminosyrerne eller de aktive bestanddele, der tilvejebringes, hvor værdien udtrykkes i molekylvægte.

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3. Anvendelse ifølge krav 1, hvor threonin plus lysin forekommer i en mængde på op til 50% af aminosyrerne eller de aktive bestanddele, der er til stede, hvor værdien udtrykkes i molekylvægte.

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4. Anvendelse ifølge mindst et af de foregående krav, hvor mængden i molekylvægt af threonin og af lysin i hvert tilfælde er større end de enkelte mængder af methionin, phenylalanin, histidin, tryptophan, der tilvejebringes, men mindre end de enkelte mængder af aminosyrerne med forgrenet kæde.

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5. Fremgangsmåde til fremstilling af en sammensætning til forsinkelse eller forebyggelse af hjerteinsufficiens hos diabetespatienter, **kendetegnet ved** anvendelse af aminosyrerne leucin, isoleucin og valin med forgrenet kæde i kombination med threonin, lysin methionin, phenylalanin, histidin, tryptophan, tyrosin, cystein og cystin som aktiv bestanddel, hvor summen af mængden i molekylvægte af threonin og lysin er større end summen af mængderne af methionin, phenylalanin, histidin, tryptophan, der tilvejebringes, men lavere end summen af mængderne af aminosyrerne med forgrenet kæde.

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6. Fremgangsmåde ifølge krav 5, hvor aminosyrerne leucin, isoleucin og valin med forgrenet kæde forekommer som følger:

- fra 40 til 60% leucin i molekylvægt,
- fra 20 til 40% isoleucin i molekylvægt,
- fra 20 til 40% valin i molekylvægt,
og hvor summen af mængderne af aminosyrerne leucin, isoleucin og valin
5 med forgrenet kæde er mellem 30 og 60% af summen i molekylvægt af alle
de aktive bestanddele.

10 **7. Fremgangsmåde ifølge krav 6, kendetegnet ved tilvejebringelse af threonin plus lysin i et molforhold (Mw/Mw) med aminosyrerne med forgrenet kæde mellem 20 og 50%, især med et threonin til lysin-forhold, hvor lysin er fra 10 til 50% mere repræsenteret end threonin.**

15 **8. Fremgangsmåde ifølge krav 5, hvor histidin forekommer i molforhold (Mw/Mw) på op til 50% af de følgende aminosyrer:**
- cystin og cystein og methionin,
- phenylalanin og tyrosin,
- tryptophan.

20 **9. Fremgangsmåde ifølge krav 8, hvor cystein og cystin repræsenteres på en basis fra 50 til 200% i molforhold (Mw/Mw) af mængden af methionin.**

10. Fremgangsmåde ifølge krav 8, hvor tyrosin er repræsenteret i op til 50% af molvægten af phenylalanin.