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(54) Title: ANTI-INFLAMMATORY AND PRO-ANABOLIC EFFECTS OF THE COMBINATION OF OMEGA-3 POLYUNSATURATED FATTY ACIDS AND GLUTAMINE

(57) Abstract: The present invention provides uses of a composition comprising fish oil and glutamine for improving skeletal muscle function and metabolism in a subject. In one embodiment, the subject has heart failure or chronic inflammatory disease.



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**ANTI-INFLAMMATORY AND PRO-ANABOLIC EFFECTS OF THE
COMBINATION OF OMEGA-3 POLYUNSATURATED FATTY ACIDS AND
GLUTAMINE**

[0001] This application claims the priority of U.S. Serial No. 61/416,695, filed November 23, 2010. The entire contents and disclosures of the preceding applications are incorporated by reference into this application.

FIELD OF THE INVENTION

[0002] This invention relates to improving skeletal muscle function and metabolism using glutamine and fish oil.

BACKGROUND OF THE INVENTION

[0003] In normal metabolism of the heart, 95% ATP comes from oxidative phosphorylation; 60-90% Acetyl-CoA comes from β -oxidation of fatty acid and 10-40% originates in glycolysis. In normal metabolism of skeletal muscle (SkM) tissue, substrate preference is glucose over fatty acid. During abnormal metabolism, the heart switches to glucose, glycogen, and lactate as fuel source. This in turns leads to regression to the fetal gene program (decreased CPT, MCAD, citrate synthase), mitochondrial dysfunction and decreased oxidative metabolism. In abnormal skeletal muscle metabolism, Type II glycolytic fibers increases, citrate synthase activity decreases, which leads to reduced COXIII activity and decreased mitochondrial density.

[0004] In 1956, Dr. Otto Heinrich Warburg hypothesized that cancer, malignant growth, and tumor growth are caused by the fact that tumor cells mainly generate energy (adenosine triphosphate / ATP, etc.) by non-oxidative breakdown of glucose (glycolysis). This is in contrast to "healthy" cells which mainly generate energy from oxidative breakdown of pyruvate. Thus a fundamental difference between normal and cancerous cells was found to be the ratio of glycolysis to respiration. This is now known as the Warburg effect - tumor cell proliferation had high glycolytic flux under normoxic conditions. This normally would lead to inefficient production of ATP, but if the flux is great enough, it can exceed oxidative phosphorylation.

[0005] In U.S., heart failure affects about 2% (~5 million) of the population. Although there have been considerable medical progress, annual mortality due to heart failure is still ~10%. To combat the health issues caused by heart failure, there is a need for novel and improved therapy, and nutritional supplementation is a promising approach.

SUMMARY OF THE INVENTION

[0006] The prevalence of heart failure is increasing throughout the world with high morbidity and mortality. Heart failure is associated with exercise intolerance and functional impairment, the degree of which identifies risk and heralds increased mortality. Patients with heart failure develop profound metabolic abnormalities including insulin resistance, cytokine activation, and an abnormal oxidative metabolism in skeletal muscle and myocardium. Abnormalities in skeletal muscle include local inflammation, depletion of substrates for oxidative metabolism and impaired mitochondrial biogenesis. This progressive catabolic state leads to skeletal muscle and myocardial atrophy with prognostic significance for patients with heart failure.

[0007] The present invention discloses a method of improving skeletal muscle function and metabolism in heart failure patients using glutamine and fish oil. A randomized, double-blind, placebo controlled study is designed to compare the combined supplementation of fish oil and glutamine with placebo in patients with stable heart failure. Thirty eight patients with heart failure will be randomized to either receiving 6.5 g fish oil/d and 8 g glutamine/d or placebo for 30 days. The primary outcome in this study is the change in muscle functional capacity measured as changes in maximum muscle strength and fatigability, peak VO₂ and exercise time after supplementation. A secondary outcome is the measurement of systemic and local markers of inflammation.

[0008] The present study aims to bridge the gap between basic cardiovascular research and clinical practice. Further, data obtained from the proposed study will be used to design larger clinical trials on micronutrient supplementation in chronic and acute disease states.

[0009] It is expected that the combined treatment with glutamine and fish oil will be pro-anabolic and anti-inflammatory and improve skeletal muscle function and metabolism.

DETAILED DESCRIPTION OF THE INVENTION

[0010] The syndrome of heart failure is associated with high morbidity and mortality that have been linked to progressive development of metabolic abnormalities, inflammation, and atrophy in the myocardium and skeletal muscle in these patients. Therapeutic management addressed at improving peripheral skeletal muscle function is lacking. Therefore, nutritional approaches with dietary supplementation in addition to current therapies are particularly appealing as they are novel and mechanistically different.

[0011] In patients with advanced heart failure, there is a metabolic switch from fatty acids to glucose as the principal energy substrate for ATP generation in the myocardium. It is hypothesized that the switch is the result of regression to the fetal gene program, with decreased expression of carnitine palmitoyl transferase (CPT), medium chain acyl-CoA dehydrogenase (MCAD) and citrate synthase [1]. This may then account for the mitochondrial dysfunction and ultimately, a preferential flux through the glycolytic pathway. Interestingly, this metabolic switch is also seen in the skeletal muscle as there is an increase in the number of type II muscle fibers, which are dependent on glycogen stores and glucose metabolism and a decrease in the number of type I fibers, which are dependent on oxidative metabolism [2]. This intrinsic metabolic defect in skeletal muscle is thought to contribute to exercise intolerance.

[0012] Glutamine is of particular interest because it is proposed to replete intermediates in the tricarboxylic acid (TCA) cycle and perhaps activates the suppressed oxidative metabolism in heart failure. Previous studies found that replenishing TCA cycle intermediates, termed anaplerosis, with pyruvate or glutamine acutely increased flux through the TCA cycle and suggests improved oxidative metabolism. It can also be stated that anaplerotic flux may be more important than TCA content or the total concentration of any of the TCA cycle intermediates in determining the overall TCA flux [3].

[0013] The predominant anaplerotic reaction in exercising human muscle is regulated by alanine aminotransferase (ALAT), an enzyme that catalyzes the conversion between

pyruvate and glutamate and alanine and -ketoglutarate. Most studies with glutamine supplementation have been completed in acute settings where it is infused and testing is done within minutes or after a bout of exercise. The data for these experiments is not clear. Some authors believe that early metabolic support can improve the recovery in response to ischemia. When myocardial infarction was induced in rats, those reperfused with glutamine, versus aspartate and glutamate, showed a significant recovery of cardiac output and no decrease in the ATP/ADP ratio [4]. Others argue that ingestion of glutamine before exercise augments the pool of TCA cycle intermediates but does not increase TCA cycle flux nor extend endurance capacity [5]. The contribution to anaplerosis in skeletal muscle has not been studied.

[0014] Amino acid intake in elderly sedentary patients results in increased ambulatory capacity (increased distance in a 6 minute walk test) and maximal isometric muscle strength [6]. Amino acid supplementation (4 grams twice daily) for 30 days in elderly patients with heart failure resulted in improved exercise capacity, reduced circulatory dysfunction, and increased peripheral oxygen availability [7]. There was an increase in peak VO₂; the recovery time was quicker and the time at peak VO₂ was less in the amino acid group. In a group of subjects with Type 2 diabetes (at risk for heart failure) and no history of coronary artery disease, 12 g amino acids/day were administered for 12 weeks in a randomized single-blind crossover study and results showed that exercise LVEF was higher during amino acid intake [8].

[0015] Cardiac cachexia is also a common phenotype in heart failure and is associated with impairment of exercise tolerance. Cachexia is weight loss with a preferential loss of lean muscle mass, a pattern that is similar across chronic diseases, such as cancer and AIDS. It is associated with an increase in systemic inflammation and oxidative stress which then uncouple TCA cycle intermediates.

[0016] Glutamine has been shown to attenuate inflammation, especially in the critically ill. Glutamine decreases inflammation and oxidative stress by promoting heat shock protein (HSP) activity and serves as a substrate for glutathione, a scavenger of reactive oxygen species (ROS). In a systematic review of parenterally fed critically ill patients, glutamine led to significant improvement in mortality, hospital stay, and infections [9]. Bongers et al. also suggested that glutamine may be a major fuel source for immune cells,

and that an adequate supply of glutamine is necessary for phagocytic and secretory activity [10].

[0017] Fish oil has been shown to reduce mortality in large studies of patients with heart failure and is a potent anti-inflammatory agent. The pathways in which fish oil acts are still uncertain but it is believed that the double bonds of EPA and DHA may act as scavengers of ROS. Anti-inflammation may be attributed to the metabolites of EPA and DHA, such as eicosanoids [11].

[0018] Subjects with heart failure will be treated with both glutamine and fish oil to see if there is an improvement in metabolism and a synergistic reduction in inflammation to ultimately improve muscle function as measured by exercise tolerance testing. The primary hypothesis to be tested is that the combined treatment with glutamine and fishoil will be pro-anabolic and anti-inflammatory and improve skeletal muscle function and metabolism.

[0019] Future studies will analyze the effects of glutamine and fish oil potentially in combination with other micronutrients in patients with heart failure to better discern the actions of these nutritional supplements and their therapeutic utility in patients with heart failure. A search for the metabolic mechanisms will provide stronger evidence for our initial hypothesis. The working hypothesis that anti-inflammatory and pro-anabolic interventions will positively affect the clinical status of patients with chronic inflammatory disease states is applicable to other conditions such as rheumatoid arthritis, chronic kidney disease, or cancer.

[0020] The present invention provides a method for improving skeletal muscle function and metabolism in a subject, comprising the step of administering to the subject a composition comprising fish oil and glutamine. In one embodiment, the subject has heart failure. In another embodiment, the subject has chronic inflammatory disease. Examples of chronic inflammatory diseases include, but are not limited to, rheumatoid arthritis, chronic kidney disease, and cancer. In one embodiment, the composition may comprise fish oil and other amino acids such as alanine or aspartate.

[0021] In general, the composition can be administered orally or intravenously. For example, the composition can be administered orally twice daily (e.g. in the morning and at night). One of ordinary skill in the art would readily formulate the composition into various

forms for oral or intravenous administration. In one embodiment, the composition can be formulated as emulsion drink. The oral formulations can be flavored or flavor masked to avoid the fish oil smell. In another embodiment, the composition can be formulated as gels or gelcaps as an alternative to liquid formulation. In yet another embodiment, the composition can be microencapsulated or formulated as enteric-coated capsules.

[0022] In one embodiment, the glutamine can be pure glutamine or L-alanyl glutamine. In another embodiment, the glutamine may be a modified glutamine (e.g. modified by addition of another amino acid or other side molecules) that nevertheless retains the same functional effects. One of ordinary skill in the art would readily devise a modified glutamine that would exhibit the functional effects described herein. Hence, the glutamine in the present invention encompasses glutamine in naturally occurring forms as well as chemically modified forms that exhibit the functional effects described herein. Examples of suitable glutamine include, but are not limited to, glutamine malate, glutamine alpha ketoglutarate, L glutamine, N acetyl L glutamine, L alanyl L glutamine, and glutamic acid.

[0023] The present invention also provides uses of a composition comprising fish oil and glutamine to improve skeletal muscle function and metabolism in a subject. In one embodiment, the subject has heart failure. In another embodiment, the subject has chronic inflammatory disease. Examples of chronic inflammatory diseases include, but are not limited to, rheumatoid arthritis, chronic kidney disease, and cancer. In one embodiment, the glutamine in the composition is L-alanyl glutamine.

[0024] The present invention also provides uses of a composition for the preparation of a medication for improving skeletal muscle function and metabolism in a subject, said composition comprises fish oil and glutamine. In one embodiment, the glutamine in the composition is L-alanyl glutamine.

[0025] The invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative, and are not meant to limit the invention as described herein, which is defined by the claims which follow thereafter.

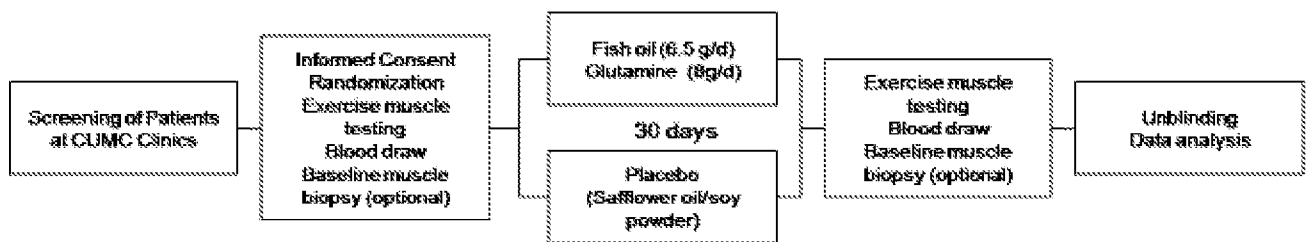
[0026] Throughout this application, various references or publications are cited. Disclosures of these references or publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. It is to be noted that the transitional term “comprising”, which is synonymous with “including”, “containing” or “characterized by”, is inclusive or open-ended and does not exclude additional, un-recited elements or method steps.

EXAMPLE 1

Materials and Methods

Study Design

[0027] A prospective, randomized double-blind, placebo-controlled study was designed. 38 subjects will be randomized to two groups (19 patients in each group), one taking active fish oil and glutamine supplementation and the other taking placebo for 30 days. Three study visits will be required for patients declining muscle biopsy. For those that agree to the muscle biopsy, 2 additional visits will be required.



Study Nutrients

[0028] Subjects randomized to receive active treatment will receive 3.285 g of EPA and 3.285 g of DHA provided by Barlean’s Organic Oils & Company in 30 ml packets, and glutamine (8g/d) provided by Natural Organic’s Laboratories, Inc. in 500 mg capsules. This will result in an additional 405 calories per day and an additional intake of 90 mg/day of cholesterol. An additional 36 g of carbohydrate/day will be consumed, half of which will come from xylitol. The fish oil mixture also includes other omega 3s, omega 6s and omega 9s. Subjects will add pure glutamine powder into the emulsion of fish oil, or swallow capsules separately.

[0029] *Placebo:* The emulsifiers in the fish oil are benign, and will not affect results. The placebo for fish oil will be safflower oil. For glutamine, soy powder or lactose free milk will serve as the placebo.

[0030] The active fish oil and glutamine supplements and the placebo fish oil and glutamine will be packaged together and randomized by the manufacturing company and will be blinded to all study investigators.

Study Subjects

[0031] Inclusion criteria are age greater than 18 years, left ventricular ejection fraction less than or equal to 35%, stable on standard heart failure medications, and optimal medical therapy. Exclusion criteria are major cardiovascular events, procedures in the last 6 months, dementia, presence of cardiovascular diseases that may lead to harm if the patient took part in the study (congenital heart disease, long QT syndrome, hypertrophic cardiomyopathy, active myocarditis).

Recruitment

[0032] Subjects will be recruited from the CUMC/NYPH Heart Failure Clinic. There are greater than 1000 heart failure patients seen at the clinic, including men and women of diverse racial backgrounds. Initial patient contact will be made via telephone after obtaining permission from the subject's personal physician. An IRB approved telephone script will be followed when speaking to the patient and informed consent will be obtained I person. Patients will be made aware of the study and it will be made clear that participating in the study is completely voluntary. An ongoing total of the number of participants contacted will be tracked with weekly progress reports and patients will be discussed at weekly team meetings.

Study Procedures

[0033] Using equipment partially available in the Department of Medicine, we will perform the assessment of muscle function and metabolism. Isokinetic testing under EMG control will be used to measure maximal muscle tension throughout a full range of motion in knee flexion and knee extension, with slow and constant speed using a BIODEX unit (located on PH4-465). Isometric strength will be taken as the average of 5 maximal voluntary contractions lasting 3 seconds with a minimum recovery period of 15 seconds between each. The subject's peak VO₂ (oxygen consumption) will be measured by cardiopulmonary exercise testing using the Modified Naughton Protocol. During the exercise, the subject will keep a lightweight pneumotach device in his or her mouth. Fatigability will be measured by having the subject repeat movements of flexion and extension at 80% of the maximal tension until the subject reaches exhaustion. The duration of exercise time before exhaustion will be

used as a measure of fatigability. Fatigue in a “real-life” setting will be measured by a six-minute walk test. A 25-meter course will be marked in an open corridor and chairs will be placed at each end. Subjects will be asked to walk as fast as possible over a period of six minutes. The test will be scored by the rounded meters walked in six minutes.

[0034] Blood will be collected before and after the trial period. Serum will be aliquoted into separate cryovials and frozen at -80C. The assessment of serum markers will be performed with the assistance of the Biomarker Core Unit of the Irving Institute for Clinical and Translational Research at Columbia University Medical Center. TNF-a, IL-1b, IL-6, CRP, insulin, glucose, IGF-1, IGF-1BP, aspartate, glutamate, and pyruvate will be measured in all serum samples. In collaboration with Dr. William Blaner, Division of Preventive Medicine and Nutrition, a detailed analysis of lipid content and composition in skeletal muscle biopsies and erythrocyte membrane fractions using LC/MS was conducted. This will allow the analysis of the impact of the fishoil and glutamine treatment on lipid metabolism and further confirm individual patient compliance and pharmacokinetics of the treatment. The analysis of blood lipid subfractions will also be performed at baseline and after 2 and 4 weeks using a rapid analysis kit (Ideal Omega, Inc).

[0035] Percutaneous muscle biopsies of the vastus lateralis muscle of the non-dominant leg will be taken with a Bergstrom needle through a small skin area at mid thigh (15 cm above the patella) before and after the trial period. Biopsy specimens will be frozen in liquid nitrogen and stored at -80°C for further processing.

Study Instruments

[0036] Baseline characteristics to be obtained through interview and chart review include age, gender, etiology of heart failure (dilated cardiomyopathy, coronary ischemic, valvular, and other), NYHA functional class, heart failure duration, past medical history, body mass index (BMI), current medications, smoking history, and alcohol and drug history. Medical history will be electronically coded via Microsoft Access. The study nurse or coordinator will be prompted these questions electronically and the subject will answer them in an interview format at the subject's first visit. The demographics form will be completed by the subject during the initial encounter. A medication form will be completed prior to the visit by the study coordinator or nurse from patient records and then confirmed during the interview. Notes during a subject's sign-in at the start of the study, progress notes during the trial, and notes at the end of the study will be recorded electronically or on paper. All paper

information will be entered into a Microsoft Excel database for storage and/or stored in a locked cabinet. All electronic files will be in secure form, password-protected format accessible only by the study nurse, coordinator, and principle investigators.

Statistical Procedures

[0037] The assessments for group size and study design as well as power calculations and statistics have been performed with the assistance of the Statistical Core Unit of the Irving Institute for Clinical and Translational Research at Columbia University Medical Center. Patient characteristics will be compared between patient groups using the chi-square test for categorical variables. Continuous variables with normal distribution will be compared using Student's t-test and the Mann-Whitney-U test for non-parametric distribution. The paired Student's t-test will be used for the analysis of changes between groups with normal distribution of values and the Wilcoxon test will be used in case of non-parametric distribution of values.

[0038] *Maximum Oxygen Uptake (peak VO₂)* In the Heart failure-ACTION trial, baseline peak VO₂ for chronic heart failure patients was 17.5 ml/kg/min [12]. Amino acid intake in elderly sedentary patients results in increased ambulatory capacity and isometric muscle strength [7]. Amino acid supplementation (4 g bid) for 30 days in elderly with heart failure improved VO₂ by 12.7±13% [7]. The effect of fish oil on peak VO₂ is not known. A clinically significant change in peak VO₂ would be approximately 10%.

[0039] *Maximum Muscle Strength and Fatigability* In a cohort of patients with heart failure, baseline quadriceps isometric strength was 249.8±49.9 N [13]. To compare muscle strength at day 3 across study groups using an unpaired t-test and a sample size of 15, a SD of 50 N, power of 80% and significance level of 0.05, we can detect a minimum difference of 53 N (21%) of muscle strength between treatment groups. Changes are not expected in muscle function or metabolism in the placebo group over the short period of 30 days given the clinically stable status of the patients on standard heart failure management and medication.

[0040] A paired t-test will be used to compare the change in VO₂ within groups. The active supplementation group to have an increase of approximately 10% in VO₂, which would be approximately 2 ml/kg/min±0.5 ml/kg/min using 17.5 as a baseline approximation. The sample size needed at a power of 0.80 and a significance level of 0.05 is fewer than six

subjects. If 10 patients in each group were included, with a SD of 0.5 ml/kg/min, a difference of 0.5 ml/kg/min within the group and 0.66 ml/kg/min across groups would be detected

[0041] *Six Minute Walk Test (6MWT)* In the HF-ACTION trial, the baseline 6MWT for patients with systolic heart failure was 365 ± 105 m [13]. Using a sample size of 10 and a SD of 105 m, a power of 80% and significance level of 0.05, a minimum difference of 139 m between the placebo and treatment group would be detected.

[0042] Based on the combined statistical assessment of all three exercise tests (VO₂max during exercise, maximal muscle strength and 6MWT), 15 patients will be sufficient to detect a clinically significant difference within and across both study groups. In order to account for a drop-out rate of 20%, 3 people will be added to the target enrollment, bringing the total to 19 for each arm of the study.

Potential Risks

[0043] A data monitoring committee will review the data over time to make sure that there is no adverse event rate. The risks associated with exercise testing are small with an average rate of emergencies being 3.4 out of 10,000 tests. Highly experienced personnel will perform all tests. If the subject experiences symptoms of angina, dyspnea, dizziness, syncope, cyanosis or pallor at any point during the exercise testing, the testing will be terminated, the subject will be treated appropriately and events will be recorded by the study coordinator. The testing will also be stopped if the subject requests to stop. Emergency equipment and medical personnel will be in the vicinity.

[0044] Although the risk of hemorrhage as a result fish oil supplementation is often stated as a concern, clinical evidence has shown otherwise. *In vitro* studies have shown that fish oil competitively binds to cyclooxygenase and decreases the synthesis of thromboxane A₂, resulting in a decrease in platelet aggregation, raising a theoretical possibility of hemorrhage. However, clinical data do not support an increase in risk, even for patients taking aspirin and warfarin [12]. In a small study in healthy individuals comparing the effects of prescription omega-3 fatty acids and aspirin, alone and in combination, on platelet aggregation, it was concluded that omega-3 fatty acids did not inhibit platelet aggregation [13]. Six weeks of supplementation with 8 g/d fish oil did not significantly increase post-operative bleeding in patients undergoing cardiac surgery [15]. There should be no complications associated with hemorrhaging and fish oil supplementation. However, in the

rare case of an adverse effect or significant complication, treatment will be discontinued. All significant adverse events will be reported to the CUMC Institutional Review Board within 24 hours as per institutional guidelines.

Potential Benefits

[0045] If the results of the study are promising, the present protocol may be applied to other diseases with a background of metabolic derangements and systemic inflammation. Patients might benefit from the intense observation during the study period and at follow-up visits. Novel data obtained from the present study will be used to help secure funding from various funding agencies for larger, more interdisciplinary studies.

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What is claimed is:

1. A method for improving skeletal muscle function and metabolism in a subject, comprising the step of administering to the subject a composition comprising fish oil and glutamine.
2. The method of claim 1, wherein the subject has heart failure.
3. The method of claim 1, wherein the subject has chronic inflammatory disease.
4. The method of claim 3, wherein the chronic inflammatory disease is selected from the group consisting of rheumatoid arthritis, chronic kidney disease, and cancer.
5. The method of any one of claims 1-4, wherein the composition is administered orally or intravenously.
6. The method of any one of claims 1-5, wherein the glutamine is L-alanyl glutamine.
7. A composition for improving skeletal muscle function and metabolism in a subject, wherein the composition comprises fish oil and glutamine.
8. The composition of claim 7, wherein the subject has heart failure.
9. The composition of claim 7, wherein the subject has chronic inflammatory disease.
10. The composition of claim 9, wherein the chronic inflammatory disease is selected from the group consisting of rheumatoid arthritis, chronic kidney disease, and cancer.
11. The composition of any one of claims 7-10, wherein the glutamine is L-alanyl glutamine.

12. Use of a composition for the preparation of a medication for improving skeletal muscle function and metabolism in a subject, said composition comprises fish oil and glutamine.
13. The use of claim 12, wherein the glutamine is L-alanyl glutamine.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/30688

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 25/00 (2011.01)

USPC - 514/784

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC: 514/784

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

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

PubWEST (PGPB, USPT, EPAB, JPAB)

skeletal, muscle, function and metabolism, subject, patient, human, mammal, animal, fish oil, glutamine, heart failure, cardiovascular disease, chronic inflammatory disease, rheumatoid arthritis, chronic kidney disease, and cancer, orally intravenously, oral, intravenous,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HAMMARQVIST et.al.; Alanyl-glutamine Counteracts the Depletion of Free Glutamine and the Postoperative Decline in Protein Synthesis in Skeletal Muscle; November 1990 (11.1990); Annals of Surgery Vol. 212 No. 5; abstract; page 638, col1, para 1-2, col 2, para 4; page 640, col 1, para1-2; page 641, col 1, para 2; page 642, col 1, para 1; page 643, col 2, para 2	1-5, 7-13
Y	VON HAEHLING et.al.; Nutrition, metabolism, and the complex pathophysiology of cachexia in chronic heart failure; 1 September 2006 (01.09.2006) Cardiovascular Research 73; abstract; page 298, col 1, para 1, col 2, para 1; page 303, col 2, para 3; page 305, col 2, para 2	1-5, 7-13

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


* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

9 May 2011 (09.05.2011)

Date of mailing of the international search report

03 JUN 2011

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/30688

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 6
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.