(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 February 2017 (23.02.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/031131 A1

(51) International Patent Classification:
A61K 31/192 (2006.01) *A61P 1/16* (2006.01)
A61K 31/198 (2006.01)

(21) International Application Number:
PCT/US2016/047211

(22) International Filing Date:
16 August 2016 (16.08.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/206,466 18 August 2015 (18.08.2015) US

(71) Applicant: **OCERA THERAPEUTICS, INC.** [US/US]; 525 University Avenue, Suite 610, Palo Alto, CA 94301 (US).

(72) Inventors: **ROSE, Christopher, F.**; 525 University Avenue, Suite 610, Palo Alto, CA 94301 (US). **ROUSSEAU, Franck**; 525 University Avenue, Suite 610, Palo Alto, CA 94301 (US).

(74) Agent: **MALLON, Joseph, J.**; Knobbe, Martens, Olson & Bear, LLP, 2040 Main Street, Irvine, CA 92614 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

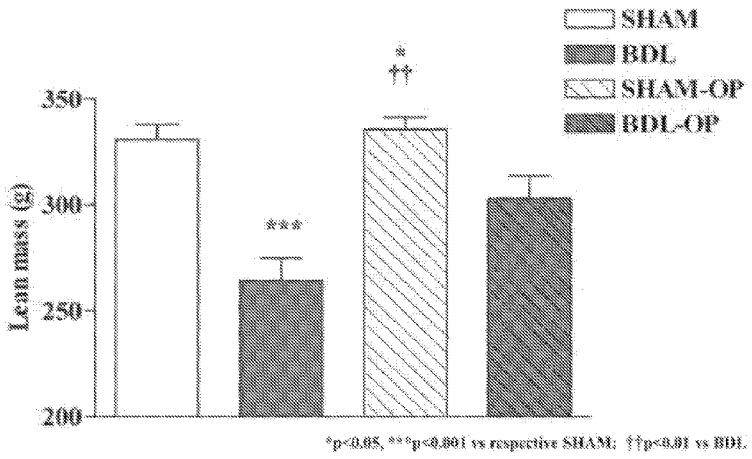
Published:

— *with international search report (Art. 21(3))*

WO 2017/031131 A1

(54) Title: TREATMENT AND PREVENTION OF MUSCLE LOSS USING L-ORNITHINE IN COMBINATION WITH AT LEAST ONE OF PHENYLACETATE AND PHENYLBUTYRATE

Figure 3E



(57) Abstract: Disclosed herein are methods of treating and preventing muscle loss using ornithine in combination with at least one of phenyl acetate and phenylbutyrate.

TREATMENT AND PREVENTION OF MUSCLE LOSS USING L-ORNITHINE IN COMBINATION WITH AT LEAST ONE OF PHENYLACETATE AND PHENYLBUTYRATE

RELATED APPLICATIONS

[0001] The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/206466, filed on August 18, 2015. The content of this related application is herein expressly incorporated by reference in its entirety.

BACKGROUND

Field

[0002] The present application relates to the fields of pharmaceutical chemistry, biochemistry and medicine. One aspect relates to the treatment and/or prevention of muscle loss using ornithine in combination with at least one of phenylacetate and phenylbutyrate.

Description of the Related Art

[0003] Loss of muscle is often characterized by a deterioration of muscle quantity and quality. In patients with chronic liver diseases, numerous metabolic disturbances occur which can lead to complications that impact the clinical outcome. For example, loss of muscle in patients with chronic liver diseases can lead to a decrease in functional capacity and adversely affect survival, quality of life and outcome following liver transplantation.

[0004] Various prevention, treatment and management strategies for muscle loss are currently available depending upon the severity of the symptoms. There is a need for additional therapies for treating or preventing muscle loss.

SUMMARY

[0005] Disclosed herein is a method of treating a condition of muscle loss. In some embodiments, the method comprises administering ornithine in combination with at least one of phenylacetate and phenylbutyrate to a subject in need thereof, and thereby relieving the condition. In some embodiments, the method further comprises identifying a

subject suffering from a condition of muscle loss. In some embodiments, the subject has received liver transplantation.

[0006] Also disclosed herein is a method of preventing a condition of muscle loss. In some embodiments, the method comprises administering ornithine in combination with at least one of phenylacetate and phenylbutyrate to a subject in need thereof, and thereby preventing the condition. In some embodiments, the method further comprises identifying a subject is at the risk of developing a condition of muscle loss. In some embodiments, the subject is going to receive liver transplantation.

[0007] In some embodiments, the methods disclosed herein further comprises determining muscle weight, muscle circumference, lean muscle, body weight, ammonia level, function(s) of one or more liver enzymes, fat mass, lean mass, brain water content, locomotor activity, protein synthesis rate, or any combination thereof of the subject. The one or more liver enzymes can comprise, for example, albumin, bilirubin, aspartate aminotransferase, alanine aminotransferase, phosphatase alkaline, or any combination thereof. In some embodiments, the brain water content is frontal cortex water content. In some embodiments, at least one symptom of the condition of muscle loss is skeletal muscle loss. In some embodiments, at least one symptom of the condition of muscle loss is muscle mass loss. In some embodiments, the condition of muscle loss is caused by aging, disease, injury, inactivity, or any combination thereof. In some embodiments, the condition of muscle loss is sarcopenia, muscle atrophy, cachexia, muscular dystrophy, or any combination thereof. In some embodiments, the subject is suffering from chronic liver disease. In some embodiments, the chronic liver disease is cirrhosis.

[0008] In some embodiments, the treatment and/or prevention of the condition in the method disclosed herein is achieved by reducing blood ammonia, directly improving muscle metabolism, or a combination thereof.

[0009] In some embodiments, separate pharmaceutically acceptable salts of the ornithine and at least one of phenylacetate and phenylbutyrate are administered to the subject. In some embodiments, the at least one of phenylacetate and phenylbutyrate is administered as a sodium phenylacetate or sodium phenylbutyrate. In some embodiments, the ornithine is administered as a free monomeric amino acid or physiologically acceptable salt thereof. In

some embodiments, the ornithine and phenylacetate is administered as ornithine phenylacetate.

[0010] In some embodiments, the administration is oral, intravenous, intraperitoneal, intragastric, or intravascular administration. In some embodiments, the administration is intravenous administration. In some embodiments, the administration is oral administration.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] **Figure 1** is a schematic illustration of how the sham and BDL rats used in the study described in Example 4 were generated.

[0012] **Figures 2A-C** show muscle weight, muscle circumference and lean muscle of sham and BDL rats.

[0013] **Figures 3A-H** show body weight, ammonia level, liver enzyme function, fat mass, lean mass, frontal cortex water content, locomotor activity, and muscle protein fractional synthesis rate (FSR) in all four experimental groups (including sham, BDL, sham-OP, and BDL-OP groups)

DETAILED DESCRIPTION

[0014] In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented here. It will be readily understood that the aspects of the present disclosure, as generally described herein, can be arranged, substituted, combined, and designed in a wide variety of different configurations, all of which are explicitly contemplated and make part of this disclosure.

Definitions

[0015] As used herein, a "subject" refers to an animal that is the object of treatment, observation or experiment. "Animals" include cold- and warm-blooded vertebrates and invertebrates such as fish, shellfish, reptiles and, in particular, mammals. "Mammal"

includes, without limitation, mice; rats; rabbits; guinea pigs; dogs; cats; sheep; goats; cows; horses; primates, such as monkeys, chimpanzees, and apes, and, in particular, humans.

[0016] As used herein, a "patient" refers to a subject that is being treated by a medical professional, such as a Medical Doctor (*i.e.* Doctor of Allopathic medicine or Doctor of Osteopathic medicine) or a Doctor of Veterinary Medicine, to attempt to cure, or at least ameliorate the effects of, a particular disease or disorder or to prevent the disease or disorder from occurring in the first place.

[0017] As used herein, "administration" or "administering" refers to a method of giving a dosage of a pharmaceutically active ingredient to a vertebrate.

[0018] As used herein, a "dosage" refers to the combined amount of the active ingredients (e.g., ornithine and phenylacetate, or ornithine and phenylbutyrate).

[0019] As used herein, a "unit dosage" refers to an amount of therapeutic agent administered to a patient in a single dose.

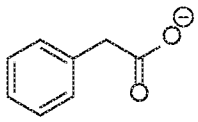
[0020] As used herein, a "daily dosage" refers to the total amount of therapeutic agent administered to a patient in a day.

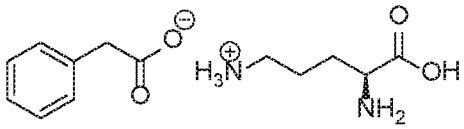
[0021] As used herein, "therapeutically effective amount" or "pharmaceutically effective amount" is meant an amount of therapeutic agent, which has a therapeutic effect. The dosages of a pharmaceutically active ingredient which are useful in treatment are therapeutically effective amounts. Thus, as used herein, a therapeutically effective amount means those amounts of therapeutic agent which produce the desired therapeutic effect as judged by clinical trial results and/or model animal studies.

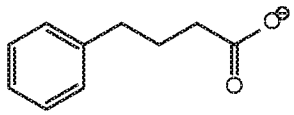
[0022] As used herein, a "therapeutic effect" relieves, to some extent, one or more of the symptoms of a disease or disorder. For example, a therapeutic effect may be observed by a reduction of the subjective discomfort that is communicated by a subject (*e.g.*, reduced discomfort noted in self-administered patient questionnaire).

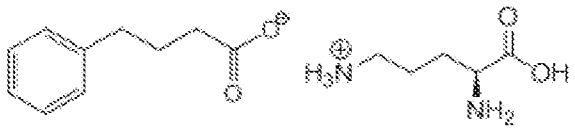
[0023] "Treat," "treatment," or "treating," as used herein refers to administering a compound or pharmaceutical composition to a subject for prophylactic and/or therapeutic purposes. The term "prophylactic treatment" refers to treating a subject who does not yet exhibit symptoms of a disease or condition, but who is susceptible to, or otherwise at risk of, a particular disease or condition, whereby the treatment reduces the likelihood that the patient

will develop the disease or condition. The term "therapeutic treatment" refers to administering treatment to a subject already suffering from a disease or condition.

[0024] The term "phenylacetate" as used herein, refers to the anionic form of phenylacetic acid with the following chemical structure:  .

[0025] The term "L-ornithine phenylacetate" as used herein, refer to a compound consisting of L-ornithine cation and phenylacetate anion. It has the following chemical structure:  .

[0026] The term "phenylbutyrate" as used herein, refers to the anionic form of phenylbutyric acid with the following chemical structure:  .

[0027] The term "L-ornithine phenylbutyrate" as used herein, refer to a compound consisting of L-ornithine cation and phenylbutyrate anion. It has the following chemical structure:  .

Abbreviations

[0028] BDL=bile duct ligation;

[0029] OP=ornithine, phenylacetate;

Muscle Loss

[0030] Muscle loss is a condition of deterioration of muscle quantity and quality. Non-limiting symptoms of muscle loss can be loss or reduction of muscle mass, loss or reduction of lean muscle, loss or reduction of muscle weight, loss or reduction of muscle circumference, loss or reduction of fat mass, loss or reduction of lean mass, loss or reduction of muscle function, loss or reduction of muscle strength, loss or reduction of mobility, weight loss, reduction in muscle protein fractional synthesis rate (FSR), or any combination thereof.

In some embodiments, at least one symptom of the condition of muscle loss is muscle mass loss or skeletal muscle loss. In some embodiment, at least one symptom of the condition of muscle loss is weight loss. In some embodiment, at least one symptom of the condition of muscle loss is loss or reduction in lean mass, loss or reduction of muscle circumference, or reduction in muscle protein fractional synthesis rate (FSR). There are a variety of causes for muscle loss. For example, the muscle loss can be caused by aging, disease (for example cancer and liver diseases), inactivity, injury (for example liver transplantation), or any combination thereof.

[0031] Some non-limiting examples of causes for muscle loss include age (e.g., age-related reduction in nerve cells responsible for sending signals from the brain to the muscles to initiate movement); a decrease in the concentration of some hormones, including but not limited to, growth hormone, testosterone, and insulin-like growth factor; a decrease in the body's ability to synthesize protein; inadequate intake of calories and/or protein to sustain muscle mass; and any combination thereof. In some embodiments, the condition of muscle loss is sarcopenia, muscle atrophy, cachexia, muscular dystrophy, muscle wasting, or any combination thereof. In patients having sarcopenia, the patients display a deterioration of muscle quantity and quality, leads to a decrease in functional capacity, adversely affecting survival, quality of life and outcome following liver transplantation. Cirrhotic patients with sarcopenia have higher ammonia levels. Without being bound by any particular theory, it is believed that the relationship between sarcopenia and hyperammonemia is bi-directional: (1) sarcopenia may reduce the subject's capacity to reduce ammonia via muscle in cirrhosis, and (2) the toxic effect of ammonia possibly affects the muscle.

[0032] Muscle loss can be a symptom or a result of an underlying condition (e.g., liver disorder), and therefore a subject may have muscle loss that is associated with a one or more conditions. In some embodiments, the muscle loss is associated with a liver disease. Non-limiting examples of liver disease include intrahepatic cholestasis (alagille syndrome, biliary liver cirrhosis), fatty liver (alcoholic fatty liver, reye syndrome), hepatic vein thrombosis, hepatolentricular degeneration, hepatomegaly, liver abscess (amebic liver abscess), liver cirrhosis (alcoholic, biliary and experimental), alcoholic liver diseases (fatty liver, hepatitis, cirrhosis), parasitic (hepatic echinococcosis, fascioliasis, amebic liver

abscess), jaundice (hemolytic, hepatocellular, and cholestatic), cholestasis, portal hypertension, liver enlargement, ascites, hepatitis (alcoholic hepatitis, animal hepatitis, chronic hepatitis (autoimmune, hepatitis B, hepatitis C, hepatitis D, drug induced), toxic hepatitis, viral human hepatitis (hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E), Wilson's disease, granulomatous hepatitis, secondary biliary cirrhosis, hepatic encephalopathy, varices, primary biliary cirrhosis, primary sclerosing cholangitis, hepatocellular adenoma, hemangiomas, bile stones, liver failure (hepatic encephalopathy, acute liver failure), and liver neoplasms (angiomyolipoma, calcified liver metastases, cystic liver metastases, epithelial tumors, fibrolamellar hepatocarcinoma, focal nodular hyperplasia, hepatic adenoma, hepatobiliary cystadenoma, hepatoblastoma, hepatocellular carcinoma, hepatoma, liver cancer, liver hemangioendothelioma, mesenchymal hamartoma, mesenchymal tumors of liver, nodular regenerative hyperplasia, benign liver tumors (Hepatic cysts [Simple cysts, Polycystic liver disease, Hepatobiliary cystadenoma, Choledochal cyst], Mesenchymal tumors [Mesenchymal hamartoma, Infantile hemangioendothelioma, Hemangioma, Peliosis hepatis, Lipomas, Inflammatory pseudotumor, Miscellaneous], Epithelial tumors [Bile duct epithelium (Bile duct hamartoma, Bile duct adenoma), Hepatocyte (Adenoma, Focal nodular hyperplasia, Nodular regenerative hyperplasia)], malignant liver tumors [hepatocellular, hepatoblastoma, hepatocellular carcinoma, cholangiocellular, cholangiocarcinoma, cystadenocarcinoma, tumors of blood vessels, angiosarcoma, Karposi's sarcoma, hemangioendothelioma, other tumors, embryonal sarcoma, fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, carcinosarcoma, teratoma, carcinoid, squamous carcinoma, primary lymphoma]), peliosis hepatis, erythrohepatic porphyria, hepatic porphyria (acute intermittent porphyria, porphyria cutanea tarda), Zellweger syndrome).

[0033] In some embodiments, the muscle loss is associated with a chronic liver disease, for example hepatitis or cirrhosis. For example, loss of muscle mass which is characterized by a deterioration of muscle quantity and quality is frequently observed in patients suffering from cirrhosis. Cirrhosis is characterized by numerous metabolic disturbances which lead to complications that impact the clinical outcome. In some instances, the loss of muscle mass leads to decreased functional capacity adversely affecting survival,

quality of life, and outcome following liver transplantation. Hyperammonemia can be a major complication of cirrhosis. Without being bound by any particular theory, it is believed that the toxic effect of ammonia can affect muscle, for example result in muscle loss. A condition of muscle loss can be, but is not necessarily, associated with liver diseases (e.g., chronic liver diseases).

[0034] Muscle loss can be determined by various conventional methods, for example measuring muscle size (for example circumference of the rectus femoris) by techniques such as ultrasound, measuring muscle resistance to an electrical current using electric impedance myography (EIM), measuring change in body weight, measuring muscle mass, measure lean mass or fat mass, measuring locomotor activity, measuring skeletal muscle fiber number, measuring muscle cross-sectional area (CSA), measuring fractional synthesis of protein (FSR) in muscle (e.g., with D_2O), tracking lean body mass (LBM), or any combination thereof. In some embodiments, muscle loss can be measured by tracking the lean body mass (LBM) of a subject over time.

Treatment and Prevention of Muscle Loss

[0035] Some embodiments disclosed herein include methods of treating or preventing a condition of muscle loss by co-administering to a subject in need thereof ornithine in combination with phenylacetate and/or phenylbutyrate. Some such embodiments include therapeutic treatment, and some embodiments include prophylactic treatment.

[0036] The subject in need thereof can be a patient who is suffering from a condition of muscle loss or a subject that is suspect of or at the risk of developing a condition of muscle loss. The subject may have, or may not have, symptoms of liver diseases (for example, acute liver failure or acute liver decompensation). In some embodiments, the subject does not have hyperammonemia. In some embodiments, the subject has hyperammonemia. In some embodiments, the subject does not have hepatic encephalopathy (HE). In some embodiments, the subject has HE. In some embodiments, the subject has a liver disease but is not exhibiting any significant symptoms of liver disease.

[0037] The methods disclosed herein can also comprise identifying a subject who is suffering from a condition of muscle loss or a subject that is suspect of or at the risk of

developing a condition of muscle loss; and co-administering to the subject ornithine in combination with phenylacetate and/or phenylbutyrate. In some embodiments, the methods disclosed herein include acquiring knowledge of the presence of a condition of muscle loss in a subject or the risk/potential of developing a condition of muscle loss in a subject; and co-administering to the subject ornithine in combination with phenylacetate and/or phenylbutyrate.

[0038] Change in muscle loss, for example attenuation or acceleration of muscle loss can be detected, for example, by detecting loss in muscle mass, detecting change in body weight, detecting change in muscle lean mass and/or fat mass, determining change in locomotor activity, detecting change in muscle fiber number, detecting change in muscle cross-sectional area, or any combination thereof of the subject.

[0039] Some embodiments disclosed herein provide methods of treating or preventing a condition of muscle loss by co-administering to a subject in need thereof ornithine in combination with phenylacetate and/or phenylbutyrate. Some embodiments can include identifying a subject as having or at risk for developing a condition of muscle loss (e.g., sarcopenia, muscle atrophy, cachexia, or muscular dystrophy) prior to administering the ornithine in combination with phenylacetate and/or phenylbutyrate.

[0040] By "co-administration," it is meant that the two or more agents may be found in the patient's bloodstream at the same time, regardless of when or how they are actually administered. In one embodiment, the agents are administered simultaneously. In one such embodiment, administration in combination is accomplished by combining the agents in a single dosage form. In another embodiment, the agents are administered sequentially. In one embodiment the agents are administered through the same route, such as orally. In another embodiment, the agents are administered through different routes, such as one being administered orally and another being administered i.v.

[0041] In some embodiments, the co-administration is useful to reduce blood ammonia level, which treat or reduce the likelihood of muscle loss. In some embodiments, muscle loss is attenuated or prevented in patients with existing chronic liver disease such as cirrhosis by the administration of the combination. Thus, in some embodiments, the

combination is administered to a patient having chronic liver disease also having a condition of muscle loss.

[0042] While not being bound by any particular theory, in some embodiments, the co-administration of ornithine with phenylacetate and/or phenylbutyrate (e.g., ornithine phenylacetate (OP)) prevents or relieves the condition of portal hypertension through effects on muscle metabolism. In some embodiments, reducing muscle metabolism results in the treating or prevention of the condition of muscle loss. In some embodiments, the co-administration of ornithine with phenylacetate and/or phenylbutyrate (e.g., ornithine phenylacetate (OP)) lowers blood ammonia attenuate muscle mass loss in cirrhotic patients.

[0043] In some embodiments, the methods and composition disclosed herein can prevent or reduce loss of muscle mass (including but not limited to loss of lean muscle mass). For example, the methods and composition may prevent muscle mass loss (including but not limited to loss of lean muscle mass) from occurring. In some embodiments, the rate of muscle mass loss is reduced in a patient receiving or received treatment by at least, or at least about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% as compared to the patients received no treatment. In some embodiments, the methods and composition reduce the rate of loss of muscle mass in a patient by, or by about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values as compared to the patients received no treatment. As another example, the methods and composition may reduce the final muscle mass loss (including but not limited to final loss in lean muscle mass). In some embodiments, the final muscle mass loss in the patient receiving or received treatment is at most, or at most about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the final muscle mass loss in patients received no treatment. In some embodiments, the final muscle mass loss in the patient receiving or received treatment is, or is about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values, of the final muscle mass loss in patients received no treatment.

[0044] In some embodiments, the methods and composition disclosed herein can prevent or reduce loss of muscle weight. For example, the methods and composition may prevent muscle weight loss from occurring. In some embodiments, the rate of muscle weight loss in a patient receiving or received treatment is reduced by at least, or at least about, 5%,

10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% as compared to the patients received no treatment. In some embodiments, the methods and composition reduce the rate of loss of muscle weight in the patient receiving or received treatement by, or by about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values as compared to patients received no treatment. As another example, the methods and composition may reduce the final muscle weight loss. In some embodiments, the final muscle weight loss in the patient receiving or received treatment is at most, or at most about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the final muscle weight loss in patients received no treatment. In some embodiments, the final muscle weight loss in the patient receiving or received treatment is, or is about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values, of the final muscle weight loss in patients received no treatment.

[0045] In some embodiments, the methods and composition disclosed herein can prevent or reduce loss of muscle circumference. For example, the methods and composition may prevent muscle circumference loss from occurring. In some embodiments, the rate of muscle circumference loss in a patient receiving or received treatment is reduced by at least, or at least about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% as compared to the patients received no treatment. In some embodiments, the methods and composition reduce the rate of muscle circumference loss in a patient receiving or received treatment by, or by about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values as compared to the patients received no treatment. As another example, the methods and composition may reduce the final muscle circumference loss. In some embodiments, the final muscle circumference loss in the patient receiving or received treatment is at most, or at most about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the final muscle circumference loss in patients received no treatment. In some embodiments, the final muscle circumference loss in the patient receiving or received treatment is, or is about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values, of the final muscle circumference loss in patients received no treatment.

[0046] In some embodiments, the methods and composition disclosed herein can prevent or reduce loss of muscle strength. For example, the methods and composition may prevent muscle strength loss from occurring. In some embodiments, the rate of muscle strength loss in a patient receiving or received treatment is reduced by at least, or at least about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% as compared to the patients received no treatment. In some embodiments, the methods and composition reduce the rate of muscle strength loss in a patient receiving or received treatment by, or by about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values as compared to patients received no treatment. As another example, the methods and composition may reduce the final muscle strength loss. In some embodiments, the final muscle strength loss in the patient receiving or received treatment is at most, or at most about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the final muscle strength loss in patients received no treatment. In some embodiments, the final muscle strength loss in the patient receiving or received treatment is, or is about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values, of the final muscle strength loss in patients received no treatment.

[0047] In some embodiments, the methods and composition disclosed herein can prevent or reduce mobility loss. For example, the methods and composition may prevent mobility loss from occurring. In some embodiments, the rate of mobility loss in a patient receiving or received treatment is reduced by at least, or at least about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% as compared to the patients received no treatment. In some embodiments, the methods and composition reduce the rate of mobility loss in the patient receiving or received treatment by, or by about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values as compared to the patients received no treatment. As another example, the methods and composition may reduce the final mobility loss. In some embodiments, the final mobility loss in the patient receiving or received treatment is at most, or at most about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the final mobility loss in patients received no treatment. In some embodiments, the final mobility loss in the patient receiving or received treatment is, or is about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or

a range between any two of these values, of the final mobility loss in patients received no treatment.

[0048] In some embodiments, the methods and composition disclosed herein can prevent or reduce weight loss. For example, the methods and composition may prevent weight loss from occurring. In some embodiments, the rate of weight loss in a patient receiving or received treatment is reduce by at least, or at least about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% as compared to patients received no treatment. In some embodiments, the methods and composition reduce the rate of weight loss in a patient receiving or received treatment by, or by about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values as compared to patients received no treatment. As another example, the methods and composition may reduce the final weight loss. In some embodiments, the final weight loss in the patient receiving or received treatment is at most, or at most about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the final weight loss in patients received no treatment. In some embodiments, the final weight loss in the patient receiving or received treatment is, or is about, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values, of the final weight loss in patients received no treatment.

[0049] In some embodiments, the methods and composition disclosed herein can prevent reduction in muscle protein FSR or reduce the rate of reduction in muscle protein FSR. For example, the methods and composition may prevent the reduction in muscle protein FSR from occurring. In some embodiments, the rate of reduction in muscle protein FSR in a patient receiving or received treatment is reduced by at least, or at least about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% as compared to patients received no treatment. In some embodiments, the methods and composition reduce the rate of reduction in muscle protein FSR in a patient receiving or received treatment by, or by about, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or a range between any two of these values as compared to the patients received no treatment.

Salts

[0050] In some embodiments, the ornithine and phenylacetate or phenylbutyrate are administered as pharmaceutically acceptable salts. The term "pharmaceutically acceptable

salt" refers to salts that retain the biological effectiveness and properties of a compound and, which are not biologically or otherwise undesirable for use in a pharmaceutical. In many cases, the compounds disclosed herein are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto. Pharmaceutically acceptable acid addition salts can be formed with inorganic acids and organic acids. Inorganic acids from which salts can be derived include, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Organic acids from which salts can be derived include, for example, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like. Pharmaceutically acceptable salts can also be formed using inorganic and organic bases. Inorganic bases from which salts can be derived include, for example, bases that contain sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like; particularly preferred are the ammonium, potassium, sodium, calcium and magnesium salts. In some embodiments, treatment of the compounds disclosed herein with an inorganic base results in loss of a labile hydrogen from the compound to afford the salt form including an inorganic cation such as Li^+, Na^+, K^+, Mg^{2+} and Ca^{2+} and the like. Organic bases from which salts can be derived include, for example, primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, basic ion exchange resins, and the like, specifically such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. Many such salts are known in the art, as described in WO 87/05297 published September 11, 1987 (incorporated by reference herein in its entirety).

[0051] In some embodiments, ornithine is administered as the ornithine HCl salt. In some embodiments, phenylacetate or phenylbutyrate is administered as their sodium salts. In some embodiments, ornithine and phenylacetate or phenylbutyrate are administered as salts of each other (e.g., ornithine phenylacetate).

Pharmaceutical Compositions and Methods of Administration

[0052] The ornithine (e.g., L-ornithine) and phenylacetate or phenylbutyrate may be administered separately or in a single dosage form. In one embodiment, the combination is administered as the ornithine phenylacetate salt or as a solution of the ornithine phenylacetate salt.

[0053] Different forms of composition of ornithine in combination with at least one of phenylacetate (or phenyl acetate salts) and phenylbutyrate have been described in U.S. Patent Publication Nos. US2008/0119554 and US2010/0280119, which are hereby incorporated by reference in their entireties. In some embodiments, ornithine and phenylacetate is present and/or administered as ornithine phenyl acetate or physiologically acceptable salt thereof. In some embodiments, ornithine is present and/or administered as a free monomeric amino acid or physiologically acceptable salt thereof In some embodiments, at least one of phenylacetate and phenylbutyrate is present and/or administered as a sodium phenylacetate or sodium phenylbutyrate. In some embodiments, a physiologically acceptable salt of ornithine and a physiologically acceptable salt of at least one of phenylacetate and phenylbutyrate are administered to the subject.

[0054] As disclosed herein, the ornithine and the phenylacetate and/or phenylbutyrate can be formulated for administration in a pharmaceutical composition comprising a physiologically acceptable surface active agents, carriers, diluents, excipients, smoothing agents, suspension agents, film forming substances, coating assistants, or a combination thereof. In some embodiments, the ornithine and the phenylacetate and/or phenylbutyrate are formulated for administration with a pharmaceutically acceptable carrier or diluent. The ornithine and the phenylacetate and/or phenylbutyrate can be formulated as a medicament with a standard pharmaceutically acceptable carrier(s) and/or excipient(s) as is routine in the pharmaceutical art. The exact nature of the formulation will depend upon several factors including the desired route of administration. Typically, ornithine and the phenylacetate and/or phenybutyrate are formulated for oral, intravenous, intragastric, intravascular or intraperitoneal administration. Standard pharmaceutical formulation techniques may be used, such as those disclosed in Remington's The Science and Practice of

Pharmacy, 21st Ed., Lippincott Williams & Wilkins (2005), incorporated herein by reference in its entirety.

[0055] The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. In addition, various adjuvants such as are commonly used in the art may be included. Considerations for the inclusion of various components in pharmaceutical compositions are described, e.g., in Gilman et al. (Eds.) (1990); Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., Pergamon Press, which is incorporated herein by reference in its entirety.

[0056] Some examples of substances, which can serve as pharmaceutically-acceptable carriers or components thereof, are sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose; powdered tragacanth; malt; gelatin; talc; solid lubricants, such as stearic acid and magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma; polyols such as propylene glycol, glycerine, sorbitol, mannitol, and polyethylene glycol; alginic acid; emulsifiers, such as the TWEENS; wetting agents, such sodium lauryl sulfate; coloring agents; flavoring agents; tableting agents, stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline; and phosphate buffer solutions.

[0057] The choice of a pharmaceutically-acceptable carrier to be used in conjunction with the subject compound is basically determined by the way the compound is to be administered.

[0058] The compositions described herein are preferably provided in unit dosage form. As used herein, a "unit dosage form" is a composition containing an amount of a compound that is suitable for administration to an animal, preferably mammal subject, in a single dose, according to good medical practice. The preparation of a single or unit dosage form however, does not imply that the dosage form is administered once per day or once per

course of therapy. Such dosage forms are contemplated to be administered once, twice, thrice or more per day and may be administered as infusion over a period of time (e.g., from about 30 minutes to about 2-6 hours), or administered as a continuous infusion, and may be given more than once during a course of therapy, though a single administration is not specifically excluded. The skilled artisan will recognize that the formulation does not specifically contemplate the entire course of therapy and such decisions are left for those skilled in the art of treatment rather than formulation.

[0059] The compositions useful as described above may be in any of a variety of suitable forms for a variety of routes for administration, for example, for oral, nasal, rectal, topical (including transdermal), ocular, intracerebral, intracranial, intrathecal, intra-arterial, intravenous, intramuscular, or other parental routes of administration. The skilled artisan will appreciate that oral and nasal compositions include compositions that are administered by inhalation, and made using available methodologies. Depending upon the particular route of administration desired, a variety of pharmaceutically-acceptable carriers well-known in the art may be used. Pharmaceutically-acceptable carriers include, for example, solid or liquid fillers, diluents, hydrotropies, surface-active agents, and encapsulating substances. Optional pharmaceutically-active materials may be included, which do not substantially interfere with the inhibitory activity of the compound. The amount of carrier employed in conjunction with the compound is sufficient to provide a practical quantity of material for administration per unit dose of the compound. Techniques and compositions for making dosage forms useful in the methods described herein are described in the following references, all incorporated by reference herein: Modern Pharmaceutics, 4th Ed., Chapters 9 and 10 (Banker & Rhodes, editors, 2002); Lieberman *et al.*, Pharmaceutical Dosage Forms: Tablets (1989); and Ansel, Introduction to Pharmaceutical Dosage Forms 8th Edition (2004).

[0060] Various oral dosage forms can be used, including such solid forms as tablets, capsules, and granules. Tablets can be compressed, tablet triturates, enteric-coated, sugar-coated, film-coated, or multiple-compressed, containing suitable binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, flow-inducing agents, and melting agents. Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules, and effervescent

preparations reconstituted from effervescent granules, containing suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, melting agents, coloring agents and flavoring agents.

[0061] The pharmaceutically-acceptable carriers suitable for the preparation of unit dosage forms for peroral administration is well-known in the art. Tablets typically comprise conventional pharmaceutically-compatible adjuvants as inert diluents, such as calcium carbonate, sodium carbonate, mannitol, lactose and cellulose; binders such as starch, gelatin and sucrose; disintegrants such as starch, alginic acid and croscarmelose; lubricants such as magnesium stearate, stearic acid and talc. Glidants such as silicon dioxide can be used to improve flow characteristics of the powder mixture. Coloring agents, such as the FD&C dyes, can be added for appearance. Sweeteners and flavoring agents, such as aspartame, saccharin, menthol, peppermint, and fruit flavors, are useful adjuvants for chewable tablets. Capsules typically comprise one or more solid diluents disclosed above. The selection of carrier components depends on secondary considerations like taste, cost, and shelf stability, which are not critical, and can be readily made by a person skilled in the art.

[0062] Peroral compositions also include liquid solutions, emulsions, suspensions, and the like. The pharmaceutically-acceptable carriers suitable for preparation of such compositions are well known in the art. Typical components of carriers for syrups, elixirs, emulsions and suspensions include ethanol, glycerol, propylene glycol, polyethylene glycol, liquid sucrose, sorbitol and water. For a suspension, typical suspending agents include sodium carboxymethyl cellulose, AVICEL RC-591, tragacanth and sodium alginate; typical wetting agents include lecithin and polysorbate 80; and typical preservatives include methyl paraben and sodium benzoate. Peroral liquid compositions may also contain one or more components such as sweeteners, flavoring agents and colorants disclosed above.

[0063] Other compositions useful for attaining systemic delivery of the subject compounds include sublingual, buccal and nasal dosage forms. Such compositions typically comprise one or more of soluble filler substances such as sucrose, sorbitol and mannitol; and binders such as acacia, microcrystalline cellulose, carboxymethyl cellulose and hydroxypropyl methyl cellulose. Glidants, lubricants, sweeteners, colorants, antioxidants and flavoring agents disclosed above may also be included.

[0064] For topical use, creams, ointments, gels, solutions or suspensions, etc., containing the compound disclosed herein are employed. Topical formulations may generally be comprised of a pharmaceutical carrier, co-solvent, emulsifier, penetration enhancer, preservative system, and emollient.

[0065] For intravenous administration, the compounds and compositions described herein may be dissolved or dispersed in a pharmaceutically acceptable diluent, such as a saline or dextrose solution. Suitable excipients may be included to achieve the desired pH, including but not limited to NaOH, sodium carbonate, sodium acetate, HCl, and citric acid. In various embodiments, the pH of the final composition ranges from 2 to 8, or preferably from 4 to 7. Antioxidant excipients may include sodium bisulfite, acetone sodium bisulfite, sodium formaldehyde, sulfoxylate, thiourea, and EDTA. Other non-limiting examples of suitable excipients found in the final intravenous composition may include sodium or potassium phosphates, citric acid, tartaric acid, gelatin, and carbohydrates such as dextrose, mannitol, and dextran. Further acceptable excipients are described in Powell, et al., Compendium of Excipients for Parenteral Formulations, *PDA J Pharm Sci and Tech* **1998**, *52* 238-311 and Nema et al., Excipients and Their Role in Approved Injectable Products: Current Usage and Future Directions, *PDA J Pharm Sci and Tech* **2011**, *65* 287-332, both of which are incorporated herein by reference in their entirety. Antimicrobial agents may also be included to achieve a bacteriostatic or fungistatic solution, including but not limited to phenylmercuric nitrate, thimerosal, benzethonium chloride, benzalkonium chloride, phenol, cresol, and chlorobutanol.

[0066] The compositions for intravenous administration may be provided to caregivers in the form of one more solids that are reconstituted with a suitable diluent such as sterile water, saline or dextrose in water shortly prior to administration. In other embodiments, the compositions are provided in solution ready to administer parenterally. In still other embodiments, the compositions are provided in a solution that is further diluted prior to administration. In embodiments that include administering a combination of a compound described herein and another agent, the combination may be provided to caregivers as a mixture, or the caregivers may mix the two agents prior to administration, or the two agents may be administered separately.

[0067] In non-human animal studies, applications of potential products are commenced at higher dosage levels, with dosage being decreased until the desired effect is no longer achieved or adverse side effects disappear. The dosage may range broadly, depending upon the desired effects and the therapeutic indication. Typically, dosages may be between about 0.1 mg/kg and 4000 mg/kg body weight, preferably between about 80 mg/kg and 1600 mg/kg body weight. Alternatively dosages may be based and calculated upon the surface area of the patient, as understood by those of skill in the art.

[0068] Depending on the severity and responsiveness of the condition to be treated, dosing can also be a single administration of a slow release composition, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved. The amount of a composition to be administered will, of course, be dependent on many factors including the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician. The compound or combination of compounds disclosed herein may be administered orally or via injection at a dose from 0.1 mg/kg to 4000 mg/kg of the patient's body weight per day. The dose range for adult humans is generally from 1 g to 100 g/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of the compound or combination of compounds disclosed herein which is effective at such dosage or as a multiple of the same, for instance, units containing 1 g to 60 g (for example, from about 5 g to 20 g, from about 10 g to 50 g, from about 20 g to 40 g, or from about 25 g to 35 g). The precise amount of compound administered to a patient will be the responsibility of the attendant physician. However, the dose employed will depend on a number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity. Also, the route of administration may vary depending on the condition and its severity. A typical dose of ornithine, or of phenylacetate or phenylbutyrate can be from 0.02 g to 1.25 g per kg of body weight, for example from 0.1 g to 0.5 g per kg of body weight, depending on such parameters. In some embodiments, a dosage of ornithine, or of phenylacetate or phenylbutyrate can be from 1 g to 100 g, for example, from 10 g to 80 g, from 15 g to 60 g, from 20 g to 40 g, or from 25 g to 35 g. In some embodiments, the ornithine and phenylacetate/phenylbutyrate can be administered in a weight ratio from 10:1 to 1:10, for

example, from 5:1 to 1:5, from 4:1 to 1:4, from 3:1 to 1:3, from 2:1 to 1:2, or about 1:1. A physician will be able to determine the required dosage of ornithine and of phenylacetate or phenylbutyrate for any particular subject.

[0069] The exact formulation, route of administration and dosage for the pharmaceutical compositions of the compound or combination of compounds disclosed herein can be chosen by the individual physician in view of the patient's condition. (See, e.g., Fingl et al. 1975, in "The Pharmacological Basis of Therapeutics," which is hereby incorporated herein by reference, with particular reference to Ch. 1). Typically, the dose range of the composition administered to the patient can be from about 0.1 to about 4000 mg/kg of the patient's body weight. The dosage may be a single one or a series of two or more given in the course of one or more days, as is needed by the patient. In instances where human dosages for compounds have been established for at least some condition, the present disclosure will use those same dosages, or dosages that are between about 0.1% and about 5000%, more preferably between about 25% and about 1000% of the established human dosage. Where no human dosage is established, as will be the case for newly-discovered pharmaceutical compounds, a suitable human dosage can be inferred from ED_{50} or ID_{50} values, or other appropriate values derived from in vitro or in vivo studies, as qualified by toxicity studies and efficacy studies in animals.

[0070] It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity or organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

[0071] Although the exact dosage will be determined on a drug-by-drug basis, in most cases, some generalizations regarding the dosage can be made. In cases of

administration of a pharmaceutically acceptable salt, dosages may be calculated as the free base. In some embodiments, the composition is administered 1 to 4 times per day. Alternatively the compositions of the compound or combination of compounds disclosed herein may be administered by continuous intravenous infusion, preferably at a dose of each active ingredient up to 100 g per day. As will be understood by those of skill in the art, in certain situations it may be necessary to administer the compound disclosed herein in amounts that exceed, or even far exceed, the above-stated, preferred dosage range in order to effectively and aggressively treat particularly aggressive diseases or infections. In some embodiments, the compound or combination of compounds disclosed herein will be administered for a period of continuous therapy, for example for a week or more, or for months or years.

[0072] In some embodiments, the dosing regimen of the compound(s) or combination of compounds disclosed herein is administered for a period of time, which time period can be, for example, from at least about 1 week to at least about 4 weeks, from at least about 4 weeks to at least about 8 weeks, from at least about 4 weeks to at least about 12 weeks, from at least about 4 weeks to at least about 16 weeks, or longer. The dosing regimen of the compound(s) or combination of compounds disclosed herein can be administered three times a day, twice a day, daily, every other day, three times a week, every other week, three times per month, once monthly, substantially continuously or continuously.

Examples

[0073] Embodiments of the present application are disclosed in further detail in the following examples, which are not in any way intended to limit the scope of the present disclosure.

Example 1

In vivo effect in BDL rats

[0074] Chronic liver disease (CLD) was induced in rats following 6-week bile-duct ligation (BDL). Four experimental groups were tested; 1) Sham; 2) BDL; 3) Sham + OP; and 4) BDL + OP. One week following BDL, rats were orally administered (gavage) OP (1g/kg) daily for 5 weeks. Body weight, fat and lean mass (EchoMRI), blood ammonia,

cerebral edema (specific gravity method), fractional synthesis of protein (FSR) in muscle (with D2O) and locomotor activity (day/night) were measured.

[0075] At the end of the 6-weeks experiment, BDL rats demonstrated a 4-fold increase in blood ammonia vs Sham-operated controls. This increase was reduced by 40% in OP-treated BDL rats. BDL rats gained less body weight compared to sham-operated controls (body weight of 360.2g ± 13.6 vs 476.8g ± 10.38; $p<0.001$) which was accompanied with a lower gain of lean mass and a lower muscle FSR. OP-treated BDL rats showed a significant increase in body weight (429.6g ± 117.9; $p<0.001$ vs BDL) with a significant higher lean mass (303.1g ± 10.7 in BDL+OP vs 264.4g ± 10.5 in BDL, $p<0.01$). Fat mass remained unchanged between the treated and untreated BDL groups. OP treatment normalized brain water content in BDL rats. In contrast, OP-treatment reduced muscle FSR in SHAM animals, but not in BDL rats. Locomotor activity in BDL rats was reduced compared with sham-operated controls but no significant change was found between BDL+OP and SHAM+OP.

[0076] These results demonstrate efficient ammonia-lowering effect, as well as a protective effect on the development of brain edema and muscle mass loss, of an oral formulation of OP. In addition, these data supports the use of ornithine phenylacetate in the treatment (including prevention) of muscle loss.

Example 2

Treatment of Sarcopenia in Rats

[0077] This example is to determine whether treatment with L-ornithine phenylacetate combinations (OP) decreases age-related muscle loss in rats.

[0078] The Fisher 344×Brown Norway (FBN) rat model has been recommended by the National Institute on Aging (NIA) for age-related research. In some instances, the rats suffer from chronic liver disease. Muscle mass, fiber number, and muscle cross-sectional area (CSA) in young (e.g., 5 months), middle age (e.g., 18 months), and old (e.g., 36 months) FBN hybrid rats are measured. Significant muscle mass loss, a reduction in muscle CSA, and muscle fiber loss are expected in, for example, the quadriceps muscles of the aged rat.

[0079] OP is administered, for example orally, to young, middle age, and old FBN hybrid rats. It is expected that the administration of OP is effective in reducing muscle mass loss in middle age and/or old FBN hybrid rats.

Example 3

Treatment of Cachexia in Rats

[0080] This example is to determine whether treatment with L-ornithine phenylacetate combinations (OP) treat cachexia in rats.

[0081] A rat model of cachexia, for example the $Apc^{Min/+}$ rats, is used. In some instances, the rats suffer from chronic liver disease. Muscle mass, fiber number, and muscle cross-sectional area (CSA) of the $Apc^{Min/+}$ rats are measured before and after being administered with OP. It is expected that the administration of OP is effective in reducing muscle mass loss in the $Apc^{Min/+}$ rats.

Example 4

Prevention of Muscle Mass Loss in Cirrhotic Rats

[0082] In the study described in this Example, 6-week bile-duct ligated (BDL) rat model was used. To generate the animal model, cirrhosis was induced in male Sprague-Dawley rats (200-225 g) (Charles River, St-Constant, QC) following bile-duct ligation. The characteristics of the BDL rats are: jaundice, ascites, liver dysfunction, brain edema, hyperammonemia, and minimal HE. As shown in Figures 2A-C, the BDL rats also showed loss in grastrocnemius muscle mass and descrease in circumference.

[0083] As previously described, rats were anaesthetized with isoflurane, and the common bile duct ligated and resected under a dissecting microscope. Sham-operated control rats, matched for weight, were similarly anaesthetized; a laparotomy was performed and the bile duct was isolated (Rose et al. Gastroenterology 117:640–644 (1999); Bosoi et al. Hepatology 53:1995–2002 (2011); Bosoi et al. Free Radic Biol Med 52:1228–1235 (2012)). Rats were maintained under controlled conditions (22°C, 12 h:12 h dark-light cycle) with free access to their food and water. One week following the BDL surgery, rats were treated orally daily with ornithine phenylacetate (OP; 1g/kg) by gavage for 5 weeks. Experiments were

conducted following the guidelines of the Canadian Council on Animal Care. Four experimental groups of animals were tested: (1) sham; (2) BDL, (3) sham + OP, and (4) BDL + OP.

[0084] Body weight of the rats was measured every day for the 6 week after BDL surgery (including the 5-week treatment) using an electronic scale. As shown in Figure 3A, BDL rats gained less body weight compared to sham-operated controls ($p<0.001$). At 6 weeks, OP-treated BDL rats showed a significant increase in body weight ($P<0.05$ vs BDL rats).

[0085] 6-week post BDL surgery, blood ammonia and liver enzymes of the rats were measured. Plasmatic ammonia, albumin, bilirubin, aspartate aminotransferase, alanine aminotransferase, phosphatase alkaline levels in SHAM, BDL and BDL treated with OP were measured using routine biochemistry techniques. To measuring ammonia, commercial kit based on the reaction of α-ketoglutarate and reduced nicotinamide adenine dinucleotide phosphate in the presence of L-glutamate dehydrogenase was used. The results for blood ammonia are shown in Figure 3B which shows that ammonia increased 4-fold in BDL rats vs. sham-operated rats and a significant increase was observed in OP-treated BDL rats ($p<0.01$ vs. BDL rats). The results of liver enzymes are shown in Figure 3C.

[0086] Body mass composition in terms of lean and fat mass was also assessed in conscious rats (full body) by *in vivo* scanning and magnetic resonance imaging (EchoMRI 100® Body Composition Analyzer) 6 weeks after the surgeries, according to the manufacturer's protocol. The instrument for composition analysis creates contrast between soft tissues by taking advantage of the differences in relaxation times of the hydrogen proton spins in different environments. Radio pulses cause protons to spin and emit radio signals which are then received and analysed. The amplitude, duration, and spatial distribution of these signals are related to properties of the material scanned. The high contrast between fat, muscle tissue, and free water is further enhanced by application of define composed radio pulses sequences similarly as described in Nixon et al. Obesity (Silver Spring) 18:1652–1659 (2010). The results are shown in Figure 3D (fat mass) and Figure 3E (lean mass). As shown in Figure 3E, BDL rats demonstrated a lower gain of lean mass compared to sham-operated controls. OP-treated BDL rats showed a significant higher lean mass ($p<0.01$ vs BDL rats).

As shown in Figure 3D, fat mass decreased in BDL rats compared to sham-operated controls and remained unchanged between the treated and untreated BDL groups.

[0087] Brain water content of the animals was measured using the sensitive densitometry technique, as previously described in Bosoi et al, 2012. Briefly, after the animal was sacrificed frontal cortex was freshly dissected at 4°C and cut into 2 mm3 pieces. Tissue pieces were placed in density gradient columns and equilibrium point was recorded after 2 minutes. Columns were made with different kerosene and bromobenzene mixtures and precalibrated with K_2SO_4 solutions of known densities. 8 samples measurements were averaged in each rat. Water content was calculated based on tissue density, according to the formula described by Marmarou et al., J Neurosurg. 49(4):530–537 (1978). The results of brain edema are shown in Figure 3F. As shown in Figure 3F, frontal cortex water content significantly increased in BDL rats ($p<0.05$ vs SHAM) and normalized following OP treatment.

[0088] Locomotor activity of the animals was assessed using an infrared beam computerized auto-track system (Accuscan). Rats were individually placed in plexiglas cages for 6 hours before beginning to record activity. Distance travelled during the night (active) and day (inactive) period was recorded for 24 hours. As shown in Figure 3G, locomotor activity in BDL rats was reduced compared with sham-operated controls ($p<0.05$) but no significant change was found between SHAM and BDL OP-treated rats.

[0089] Rate of protein synthesis was quantified as the fractional and absolute protein synthesis rates in the dissected and homogenized muscle and other organs including the brain (frontal cortex), heart, intestine, kidney and liver, using the modified phenylalanine tracer pulse method described in Zhang et al. Am J Physiol Endocrinol Metab 283:E753-764 (2002) and Dasarathy et al. J Hepatol 54:915–921 (2011). In brief, rats were given a small dose (0.5 mg/100 g body weight) of L-[ring-$^2H_5$]phenylalanine ip at t = 0 minute, L-[1-^{13}C]Phenylalanine ip at t = 30 minutes and L-[15N]Phenylalanine ip at t = 60 minutes. At t = 65 minutes, the rats were killed and blood and tissue collected. The calculation of the fractional protein synthesis was done by using the enrichment in tissue protein samples of L-[ring-$^2H_5$]phenylalanine, divided by the average enrichment in plasma (from area under the curve calculation of the curve, constructed from the three different phenylalanine isotopes).

The enrichment of phenylalanine in plasma and tissue hydrolysates was measured by liquid chromatography coupled to mass spectrometry (LC-MS/MS) as described in Engelen et al. J Cyst Fibros 12:445–453 (2013) and Luiking et al. Clin Sci 128:57–67 (2015). The results are shown in Figure 3H (FSR: fractional protein synthesis rate). As shown in Figure 3H, BDL rats demonstrated a lower muscle FSR compared to sham-operated controls, and OP-treatment reduced muscle FSR in sham-operated animals, but not in BDL rats.

[0090] In the statistical analysis, data were expressed as mean ± standard error of the mean (SEM). Significance of difference was tested with unpaired t test or ANOVA followed by Bonferroni post-test using GraphPad Prism4 (La Jolla, CA, USA). Probability values of $p < 0.05$ were considered statistically significant.

[0091] This example shows that the oral OP formulation efficiently lowers ammonia, preserves muscle mass and functions, improves locomotor activity, and protects against the development of brain edema in rats with cirrhosis.

[0092] In at least some of the previously described embodiments, one or more elements used in an embodiment can interchangeably be used in another embodiment unless such a replacement is not technically feasible. It will be appreciated by those skilled in the art that various other omissions, additions and modifications may be made to the methods and structures described above without departing from the scope of the claimed subject matter. All such modifications and changes are intended to fall within the scope of the subject matter, as defined by the appended claims.

[0093] With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity.

[0094] It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (*e.g.*, bodies of the appended claims) are generally intended as "open" terms (*e.g.*, the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). It will be

further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases "at least one" and "one or more" to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles "a" or "an" limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases "one or more" or "at least one" and indefinite articles such as "a" or "an" (*e.g.*, "a" and/or "an" should be interpreted to mean "at least one" or "one or more"); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (*e.g.*, the bare recitation of "two recitations," without other modifiers, means at least two recitations, or two or more recitations). Furthermore, in those instances where a convention analogous to "at least one of A, B, and C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (*e.g.*, " a system having at least one of A, B, and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where a convention analogous to "at least one of A, B, or C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (*e.g.*, " a system having at least one of A, B, or C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or "B" or "A and B."

[0095] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0096] As will be understood by one skilled in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

[0097] While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

[0098] All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited herein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differ from or contradict this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

WHAT IS CLAIMED IS:

1. A method of treating a condition of muscle loss, comprising administering ornithine in combination with at least one of phenylacetate and phenylbutyrate to a subject in need thereof, and thereby relieving the condition.

2. The method of claim 1, further comprising identifying a subject suffering from a condition of muscle loss.

3. The method of claim 2, wherein the subject has received liver transplantation.

4. A method of preventing a condition of muscle loss, comprising administering ornithine in combination with at least one of phenylacetate and phenylbutyrate to a subject in need thereof, and thereby preventing the condition.

5. The method of claim 4, further comprising identifying a subject is at the risk of developing a condition of muscle loss.

6. The method of claim 5, wherein the subject is going to receive liver transplantation.

7. The method of any one of claims 1-6, further comprising determining muscle weight, muscle circumference, lean muscle, body weight, ammonia level, function(s) of one or more liver enzymes, fat mass, lean mass, brain water content, locomotor activity, protein synthesis rate, or any combination thereof of the subject.

8. The method of claim 7, wherein the one or more liver enzymes comprise albumin, bilirubin, aspartate aminotransferase, alanine aminotransferase, phosphatase alkaline, or any combination thereof.

9. The method of claim 7, wherein the brain water content is frontal cortex water content.

10. The method of any one of claims 1-9, at least one symptom of the condition of muscle loss is skeletal muscle loss.

11. The method of any one of claims 1-9, at least one symptom of the condition of muscle loss is muscle mass loss.

12. The method of any one of claims 1-9, wherein the condition of muscle loss is caused by aging, disease, injury, inactivity, or any combination thereof.

13. The method of any one of claims 1-9, wherein the condition of muscle loss is sarcopenia, muscle atrophy, cachexia, muscular dystrophy, or any combination thereof.

14. The method of any one of claims 1-9, wherein the subject is suffering from chronic liver disease.

15. The method of claim 14, wherein the chronic liver disease is cirrhosis.

16. The method of any one of claims 1-15, wherein the treatment and prevention of the condition is achieved by reducing blood ammonia, directly improving muscle metabolism, or a combination thereof.

17. The method of any one of claims 1-16, wherein separate pharmaceutically acceptable salts of the ornithine and at least one of phenylacetate and phenylbutyrate are administered to the subject.

18. The method of claim 17, wherein the at least one of phenylacetate and phenylbutyrate is administered as a sodium phenylacetate or sodium phenylbutyrate.

19. The method of any one of claims 1-18, wherein the ornithine is administered as a free monomeric amino acid or physiologically acceptable salt thereof.

20. The method of any one of claims 1-16, wherein the ornithine and phenylacetate is administered as ornithine phenylacetate.

21. The method of any one of claims 1-20, wherein the administration is oral, intravenous, intraperitoneal, intragastric, or intravascular administration.

22. The method of any one of claims 1-20, wherein the administration is intravenous administration.

23. The method of any one of claims 1-20, wherein the administration is oral administration.

Figure 1

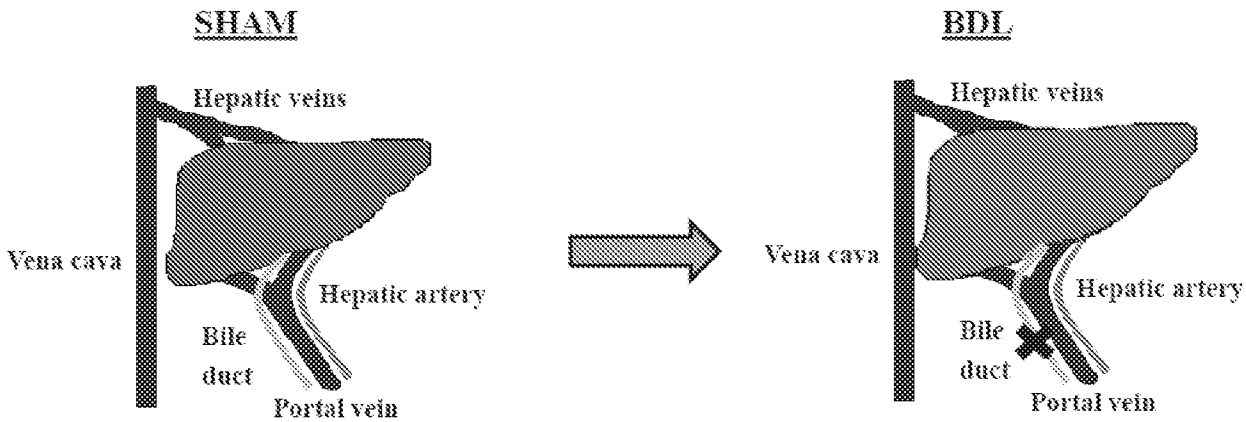

SHAM
Hepatic veins
Vena cava
Hepatic artery
Bile duct
Portal vein
BDL
Hepatic veins
Vena cava
Hepatic artery
Bile duct
Portal vein

Figure 2A

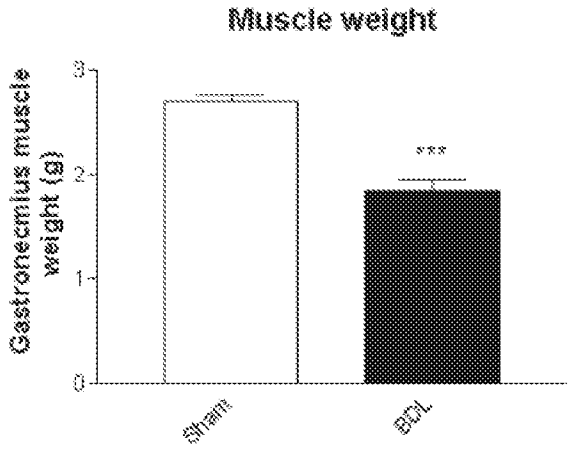


Figure 2B

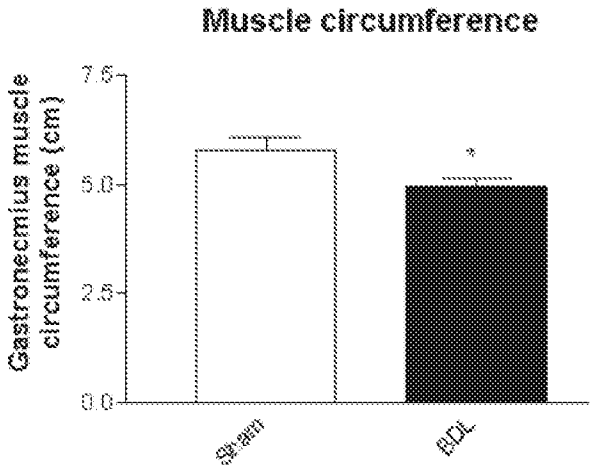
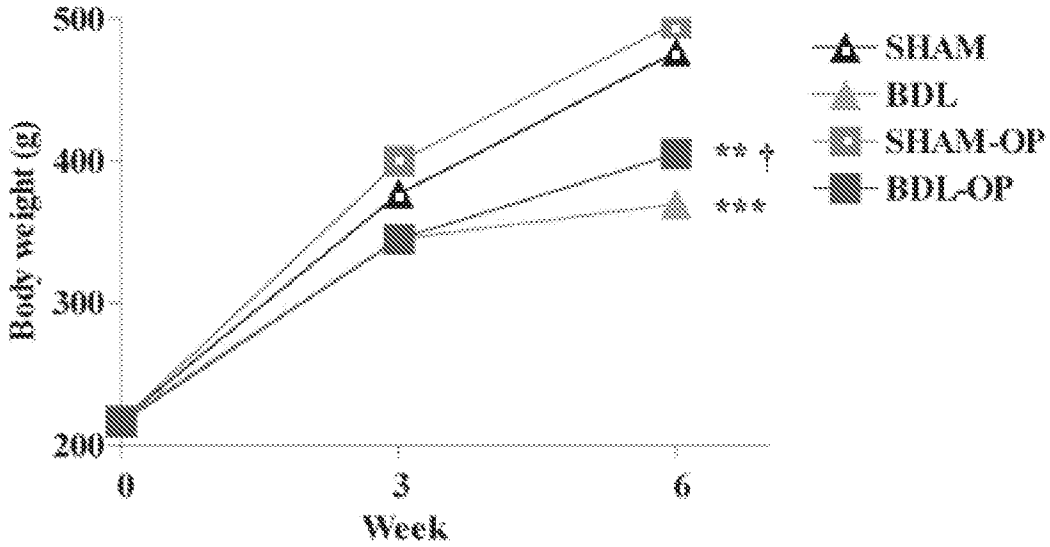


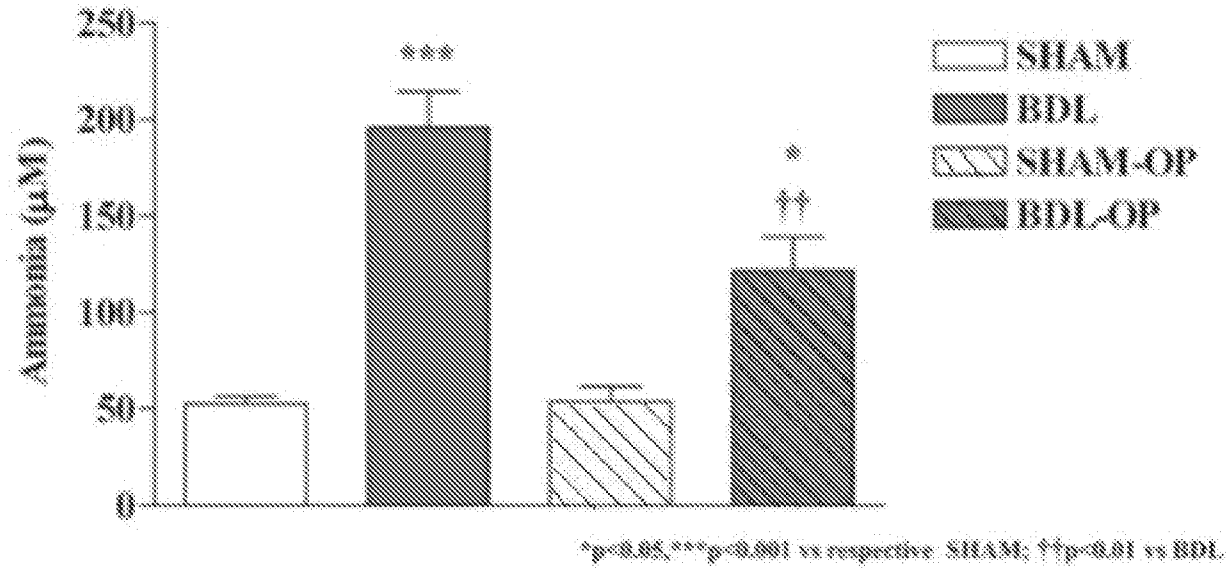
Figure 2C

Figure 3A



**p<0.01, ***p<0.001 vs respective SHAM; †p<0.05, vs BDL

Figure 3B



*p<0.05,***p<0.001 vs respective SHAM; ††p<0.01 vs BDL

Figure 3C

	SHAM	BDL	SHAM-OP	BDL-OP
Albumin (g/L)	19 ± 1	<10 ± 0***	17 ± 1	11 ± 1***
Bilirubin (μmol/L)	4 ± 2	178 ± 26***	4 ± 1	155 ± 38***
Aspartate Aminotransferase (AST) (U/L)	113 ± 151	472 ± 243***	65 ± 17	386 ± 143***
Alanine Aminotransferase (ALT) (U/L)	43 ± 5	75 ± 17**	48 ± 19	79 ± 24**
Alkaline Phosphatase (U/L)	241 ± 19	546 ± 143***	213 ± 42	544 ± 116***

**p<0.01, ***p<0.001 vs respective SHAM

Figure 3D

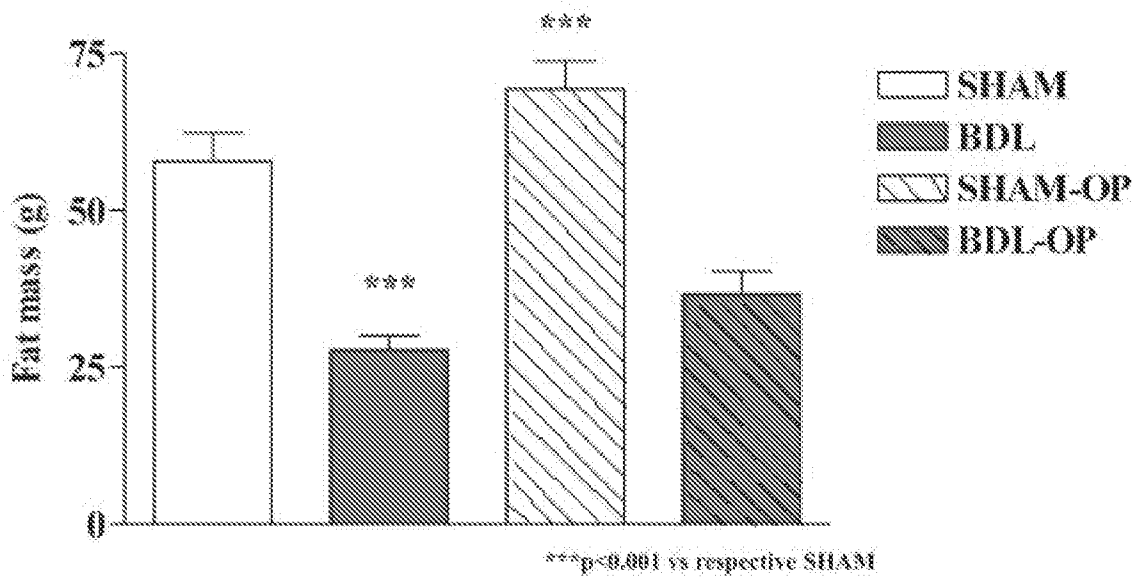


Figure 3E

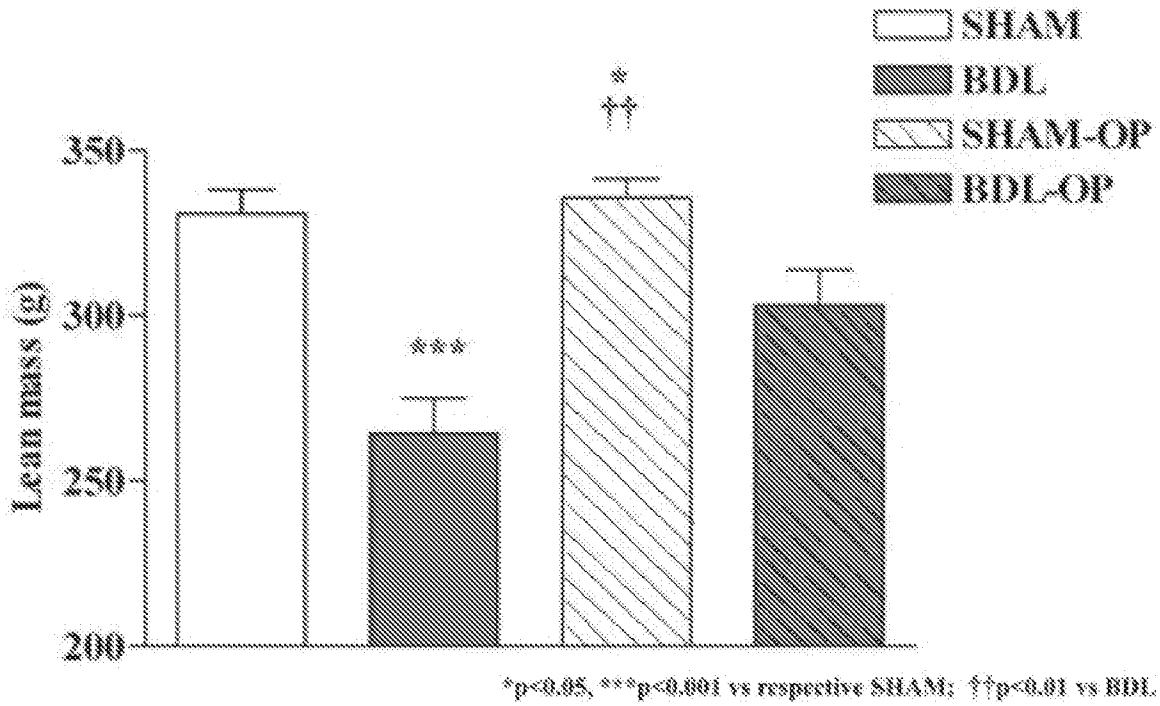


Figure 3F

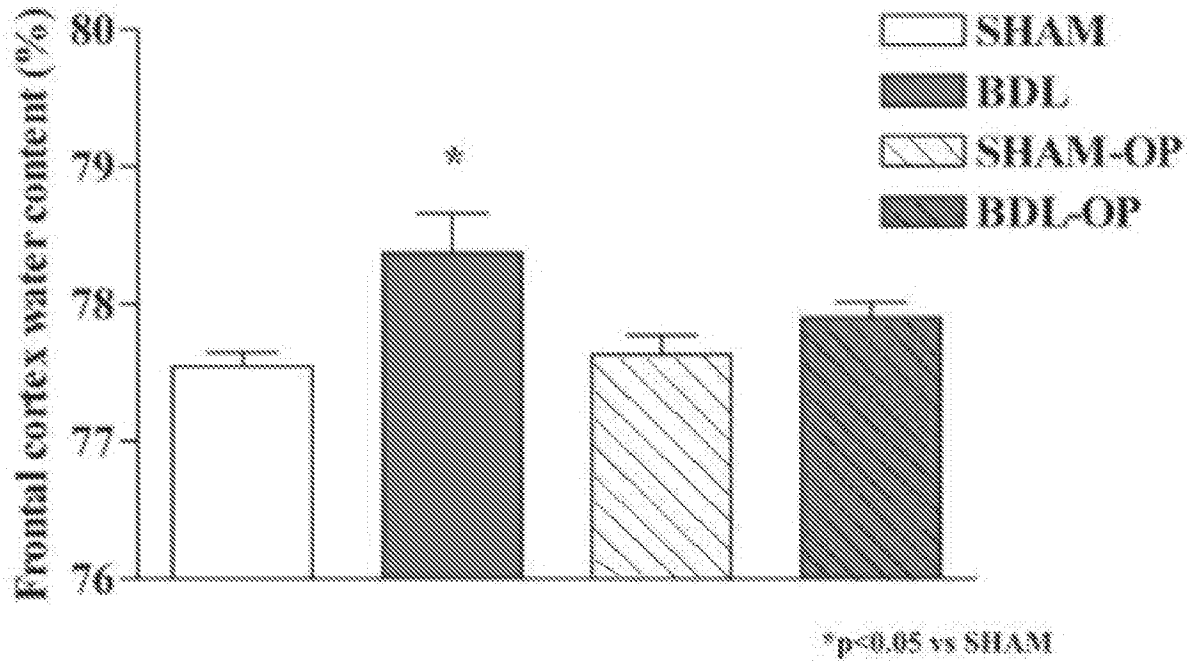


Figure 3G

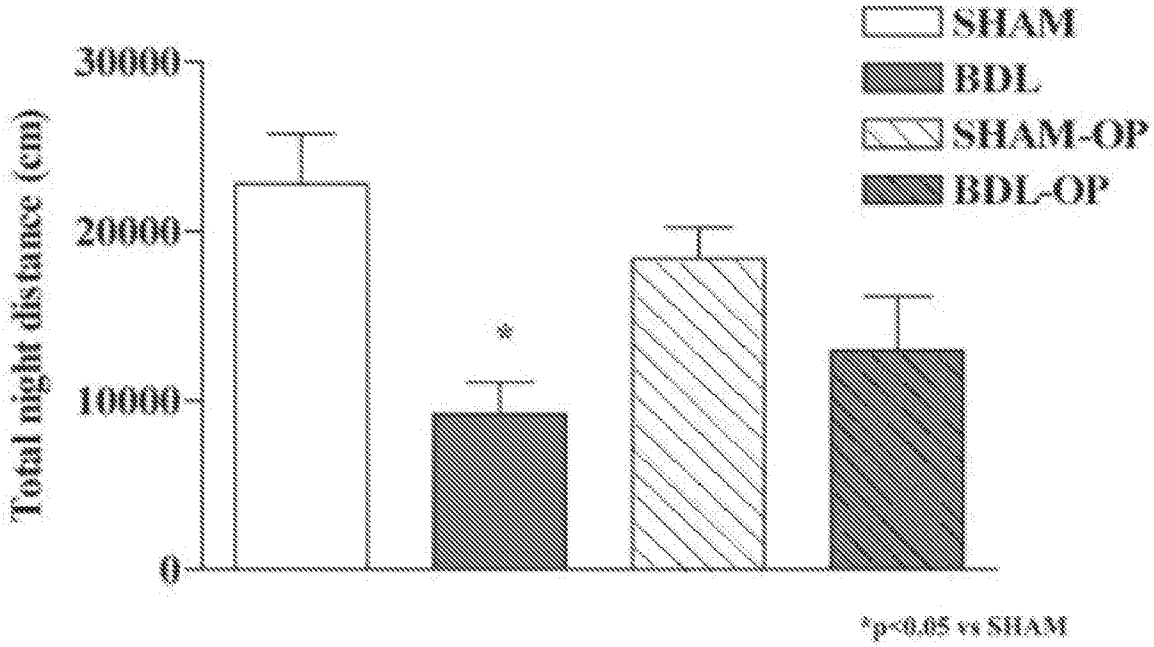
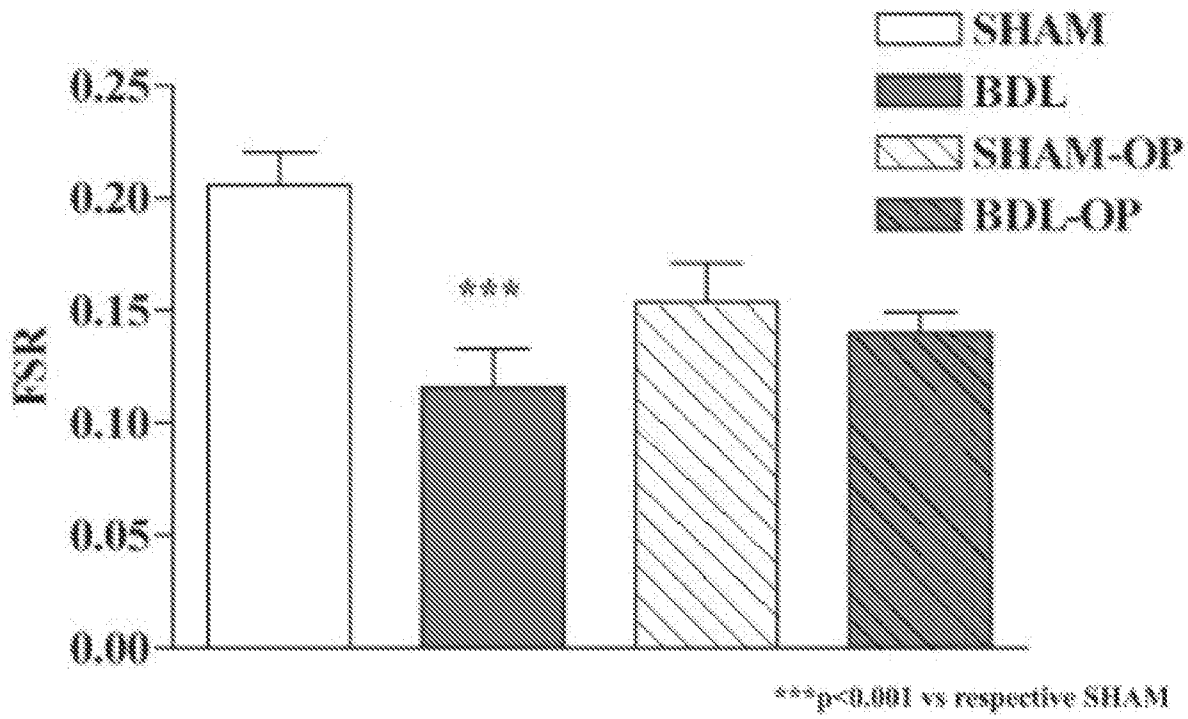


Figure 3H



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/047211

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/192 (2006.01) A61K 31/198 (2006.01) A61P 1/16 (2006.01)

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)

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)

Databases: MEDLINE, CAPLUS, EMBASE, WPIAP, EPODOC, Esp@cenet, Pubmed, and internal databases provided by IP Australia.

Keywords: ORNITHINE, PHENYLACETATE, PHENYLBUTYRATE, MUSCLE, SARCOPENIA, CACHEXIA and similar terms, as well as the Applicant's and Inventors names.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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

"E" earlier application or patent 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	Date of mailing of the international search report
7 November 2016	07 November 2016
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Christina van Broekhoven AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262833196

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/US2016/047211**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/144498 A2 (UCL BUSINESS PLC et al.) 16 December 2010 see examples and claims	1-23
X Y	WO 2006/056794 A1 (UCL BIOMEDICA PLC) 01 June 2006 see examples and claims see examples and claims	1-23 1-23
A	Qiu, J. et al. 2012 "Hyperammonemia-mediated autophagy in skeletal muscle contributes to sarcopenia of cirrhosis", American J. Physiol. Endocrinol. Metab. Vol. 303, pp. E983-E993 see abstract and discussion	1-23
A	Qiu, J. et al. 2013 "Hyperammonemia in cirrhosis induces transcriptional regulation of myostatin by an NF-κB–mediated mechanism", PNAS, Vol. 110, no. 45, pp.18162-18167 see abstract and significance	1-23
Y	"Brain Muscle Axis During Treatment of Hepatic Encephalopathy With L-ornithine L-aspartate", ClinicalTrials.gov Identifier: NCT01847651 https://clinicaltrials.gov/ct2/show/NCT01847651 Retrieved: 27-10-16 see Purpose and Detailed Description	1-23
A	Walrand, S. 2010 "Ornithine Alpha-Ketoglutarate : Could It Be A New Therapeutic Option For Sarcopenia?" The Journal of Nutrition, Health & Aging, Vol. 14, no. 7, pp. 570-577	1-23
A	Lucero, C. et al. 2015 "The Role of Sarcopenia and Frailty in Hepatic Encephalopathy Management", Clin. Liver Dis. Vol. 19, pp. 507-528	1-23

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/047211**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2010/144498 A2	16 December 2010	WO 2010144498 A2	16 Dec 2010
		AU 2010258888 A1	02 Feb 2012
		AU 2010258888 B2	07 Aug 2014
		CA 2764587 A1	16 Dec 2010
		CN 102625699 A	01 Aug 2012
		CN 102625699 B	20 Aug 2014
		CN 104434894 A	25 Mar 2015
		EA 201171396 A1	30 Oct 2012
		EP 2440200 A2	18 Apr 2012
		EP 2440200 B1	27 Aug 2014
		EP 2799067 A1	05 Nov 2014
		HK 1174264 A1	06 Mar 2015
		HK 1203419 A1	30 Oct 2015
		JP 2012529523 A	22 Nov 2012
		JP 5749255 B2	15 Jul 2015
		JP 2015163635 A	10 Sep 2015
		KR 20120047891 A	14 May 2012
		MX 2011013129 A	14 Mar 2012
		NZ 596916 A	27 Sep 2013
		NZ 615091 A	27 Mar 2015
		SG 176675 A1	30 Jan 2012
		US 2012157526 A1	21 Jun 2012
		ZA 201108988 B	29 Apr 2015
WO 2006/056794 A1	01 June 2006	WO 2006056794 A1	01 Jun 2006
		AU 2005308622 A1	01 Jun 2006
		AU 2005308622 B2	02 Aug 2012
		CA 2589261 A1	01 Jun 2006
		CN 101102816 A	09 Jan 2008
		CN 101102816 B	07 Dec 2011
		CN 102512408 A	27 Jun 2012
		CN 102512408 B	25 Nov 2015
		EA 200701146 A1	28 Feb 2008
		EA 011716 B1	28 Apr 2009
		EA 200900105 A1	28 Aug 2009
		EA 018007 B1	30 Apr 2013

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/047211**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		EP 1824563 A1	29 Aug 2007
		EP 1824563 B1	10 Feb 2010
		EP 2153870 A1	17 Feb 2010
		EP 2153870 B1	25 Dec 2013
		EP 2319581 A1	11 May 2011
		EP 2319581 B1	25 Feb 2015
		HK 1141471 A1	29 Aug 2014
		IL 183401 A	30 Sep 2013
		JP 2008521784 A	26 Jun 2008
		JP 5116479 B2	09 Jan 2013
		JP 2012246294 A	13 Dec 2012
		JP 5612030 B2	22 Oct 2014
		KR 20070100721 A	11 Oct 2007
		KR 101380446 B1	01 Apr 2014
		MX 2007006171 A	08 Oct 2007
		NO 20073254 A	23 Aug 2007
		NZ 555870 A	25 Sep 2009
		SG 158073 A1	29 Jan 2010
		US 2008119554 A1	22 May 2008
		US 8389576 B2	05 Mar 2013
		US 2012259016 A1	11 Oct 2012
		ZA 200704950 B	25 Sep 2008

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.