COMPOSITIONS AND METHODS FOR THE REDUCTION OR PREVENTION OF HEPATIC STEATOSIS

[0001] This application claims the benefit to U.S. Provisional Application No. 61/945,412, filed on February 27, 2014, which is incorporated herein by reference in its entirety.

INCORPORATION BY REFERENCE

[0002] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference. In case of any inconsistency between the incorporated by reference publications and the instant specification, the instant specification will control.

BACKGROUND OF THE INVENTION

[0003] Hepatic steatosis, also sometimes referred to as fatty liver disease, is a condition generally characterized by an abnormal retention of lipids in cells of the liver. Hepatic steatosis affects millions of people worldwide. For example, the prevalence of fatty liver disease has been estimated to range from 10-24% in various countries around the globe. Fatty liver disease can have various causes. For example, non-alcoholic fatty liver disease (NAFLD) generally refers to a spectrum of hepatic lipid disorders characterized by hepatic steatosis with no known secondary cause. NAFLD can be subcategorized into (a) nonalcoholic fatty liver (NAFL), defined as the presence of steatosis in the absence of histological evidence of hepatocellular injury, and (b) nonalcoholic steatohepatitis (NASH), hepatic steatosis accompanied by hepatocyte injury and inflammation; NASH may occur with or without fibrosis, but may progress to fibrosis and cirrhosis. NAFLD is generally associated with energy metabolism pathologies, including obesity, dyslipidemia, diabetes and metabolic syndrome. The prevalence of NAFLD is high. Prevalence in the general population is estimated at 20%, with prevalence of NASH estimated to be 3-5%. There is an estimated ~70% prevalence of NAFLD among patients with obesity or diabetes, and an estimated prevalence of ~50% prevalence of NAFLD among patients with dyslipidemias. However, there are presently no approved pharmaceuticals for the treatment of NAFLD/NASH.

[0004] Sirtuins are highly conserved protein deacetylases and/or ADP-ribosyltransferases that have been shown to extend lifespan in lower model organisms, such as yeast, *C. elegans*,

and drosophila. In mammals, sirtuins have been shown to act as metabolic sensors, responding to environmental signals to coordinate the activity of genes that regulate multiple energy homeostasis pathways. For example, studies have shown that sirtuin activation mimics the effects of caloric restriction, an intervention demonstrated to significantly extend lifespan, and activates genes that improve glucose homeostasis and the conversion of fat to energy by fatty acid oxidation.

[0005] The sirtuin pathway may be defined as any pathway incorporating or converging upon pathways mediated by phosphodiesterases (PDEs). PDEs are enzymes that interact with cyclic adenosine monophosphates (cAMPs) and cyclic guanosine monophosphates (cGMPs). The PDE family of enzymes comprises multiple subclasses, including PDE 1-11 in humans. Inhibitors of these phosphodiesterases can prevent the inactivation of cAMPs and cGMPs, and can have a variety of different physiological effects. The PDE inhibitors can be selective, by preferentially inhibiting one PDE subclass as compared to another subclass, or non-selective, which have a substantially lower degree of selectivity for individual PDE subclasses. Sildenafil is an example of a selective PDE inhibitor that has shown selective inhibition of PDE 5. Sildenafil is a pharmaceutically active agent that has been used to treat pulmonary hypertension, erectile dysfunction, and altitude sickness.

SUMMARY OF THE INVENTION

[0006] The present invention generally relates to regulation of fat accumulation in cells and/or tissue. In some embodiments, the present invention provides for compositions, methods, and kits for reducing, treating, or preventing hepatic steatosis in a subject in need thereof.The present invention generally relates to regulation of fat accumulation in cells and/or tissue. In some embodiments, the present invention provides for compositions, methods, and kits for reducing, treating, or preventing hepatic steatosis in a subject in need thereof.

[0006a] In one aspect, there is provided the use of a composition comprising:

a) an amount of leucine in the form of a free amino acid and/or metabolite thereof, wherein the metabolite is β-hydroxy β-methylbutyrate (HMB) or keto-isocaproic acid (KIC); and
b) an amount of metformin, and
c) an amount of sildenafil,

in the manufacture of a medicament for reducing non-alcoholic steatohepatitis (NASH).

[0006b] In one aspect, there is provided the use of a composition comprising

a. an amount of leucine in the form of a free amino acid and/or metabolite thereof, wherein the metabolite is β-hydroxy β-methylbutyrate (HMB) or keto-isocaproic acid (KIC); and
b. an amount of metformin,
c. an amount of sildenafil,

in the manufacture of a medicament for preventing non-alcoholic steatohepatitis (NASH) in a subject exhibiting a propensity to NASH, wherein said composition is to be administered prior to manifestation of a hepatic steatosis symptom.

[0007] The invention provides a method for reducing hepatic steatosis in a subject in need thereof comprises administering to the subject: (a) an amount of leucine in the form of a free amino acid and/or metabolite thereof; and (b) an amount of a sirtuin pathway activator, wherein the administering of (a) and (b) reduces hepatic steatosis to a greater extent than administration of (a) alone, thereby reducing hepatic steatosis in the subject. The invention also provides a method of reducing hepatic steatosis in a subject in need thereof, comprising administering to the subject:(a) an amount of leucine in the form of a free amino acid and/or metabolite thereof, and (b) an amount of a PDE-5 specific inhibitor, wherein the

administering of a) and b) reduces hepatic steatosis to a greater extent than administration of (a) alone, thereby reducing hepatic steatosis in the subject. In some embodiments, the subject has been diagnosed with said hepatic steatosis. In some embodiments, the subject exhibits a non-alcoholic fatty liver disease (NAFLD). In some embodiments, the NAFLD is selected from the group consisting of non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH) and NASH-related cirrhosis. In some embodiments, the subject exhibits one or more symptoms of hepatic steatosis selected from the group consisting of weakness, fatigue, unexplained weight loss, ache and jaundice. In some embodiments, the reduction in hepatic steatosis is characterized by a reduction in hepatic liver vacuole number or size or density. In some embodiments, the method can further comprising administering to the subject (c) a second sirtuin pathway activator.

[0008] The invention also provides a method of preventing hepatic steatosis in a subject exhibiting a propensity to hepatic steatosis, comprising administering to the subject prior to manifestation of a hepatic steatosis symptom: (a) an amount of leucine in the form of a free amino acid and/or metabolite thereof, and (b) an amount of a sirtuin pathway activator, wherein the administering of (a) and (b) reduces hepatic steatosis to a greater extent than administration of (a) alone, thereby preventing hepatic steatosis in the subject. The invention also provides a method of preventing hepatic steatosis in a subject exhibiting a propensity to hepatic steatosis, comprising administering to the subject prior to manifestation of a hepatic steatosis symptom: (a) an amount of leucine in the form of a free amino acid and/or metabolite thereof, and (b) an amount of a PDE-5 specific inhibitor, wherein the administering of (a) and (b) reduces hepatic steatosis to a greater extent than administration of (a) alone, thereby preventing hepatic steatosis in the subject. In some embodiments, the method can further comprising administering to the subject (c) a second sirtuin pathway activator.

[0009] The invention also provides a method of reducing hepatic steatosis in a subject in need thereof. The method comprises administering to the subject a composition comprising: (a) an amount of leucine in the form of a free amino acid; (b) an amount of metformin; and (c) an amount of sildenafil. In some embodiments, the administering of (a), (b) and (c) reduces hepatic steatosis to a greater extent than administration of (a) alone, thereby reducing hepatic steatosis in the subject.

[0010] The invention also provides a method of preventing hepatic steatosis in a subject exhibiting a propensity to hepatic steatosis, comprising administering to the subject prior to

manifestation of a hepatic steatosis symptom a composition comprising: (a) an amount of leucine in the form of a free amino acid; (b) an amount of metformin; and (c) an amount of sildenafil. In some embodiments, the administering of (a), (b) and (c) reduces hepatic steatosis to a greater extent than administration of (a) alone, thereby preventing hepatic steatosis in the subject. In some embodiments, the method can further comprise administering to the subject a second sirtuin pathway activator.

[0011] In some embodiments of any of the foregoing methods, the subject is administered periodically over a course of at least 2 months. In some embodiments, the hepatic steatosis is evidenced by accumulation of fat in hepatic cells detectable by one or more methods selected from the group consisting of ultrasonography, computed tomography (CT), magnetic resonance imaging, measurement of serum alanine transaminase and aspartate transaminase, and biopsy. In some embodiments, the reduction of hepatic steatosis in the subject is evidenced by a reduction in hepatic fat detectable by one or more methods selected from the group consisting of ultrasonography, computed tomography (CT), magnetic resonance imaging measurement of serum alanine transaminase and aspartate transaminase, and biopsy. In some embodiments, the leucine metabolite thereof is β-hydroxy β-methylbutyrate (HMB) or keto-isocaproic acid (KIC).

[0012] In some embodiments, the amount of leucine administered to the subject is about 0.25-3 g/day. In some embodiments, the amount of leucine administered to the subject is about 0.25-3 g. In some embodiments, the amount of leucine metabolite administered to the subject is about 0.2-3 g/day. In some embodiments, the amount of leucine metabolite administered to the subject is about 0.2-3 g. In some embodiments, a weight (wt) % of the leucine in the composition, excluding fillers, is 50-95 wt%. In some embodiments, a wt% of the leucine metabolite in the composition, excluding fillers, is 50-95 wt%. In some embodiments, a wt% of the sirtuin pathway activator in the composition, excluding fillers, is 5-50 wt%. In some embodiments, the molar ratio of leucine to sirtuin pathway activator is at least about 20. In some embodiments, component (a) is hydroxymethylbutyrate and component (b) is metformin. In some embodiments, (a) is leucine existing in form of a free amino acid and component (b) is metformin.

[0013] In some embodiments, the sirtuin pathway activator is a sirtuin activator. In some embodiments, the sirtuin activator is resveratrol. In some embodiments, the sirtuin activator is a polyphenol selected from the group consisting of chlorogenic acid, resveratrol, caffeic acid, cinnamic acid, ferulic acid, piceatannol, ellagic acid, epigallocatechin gallate, grape

seed extract, and any analog thereof. In some embodiments, the amount of resveratrol administered to the subject is 0.5-100 mg/day. In some embodiments, the amount of chlorogenic acid, caffeic acid, cinnamic acid, ferulic acid, piceatannol, ellagic acid, epigallocatechin gallate, or grape seed extract, or any analog thereof administered to the subject is 0.5 – 500 mg/day.

[0014] In some embodiments, the PDE-5 specific inhibitor is selected from the group consisting of icariin, sildenafil, tadalafil, vardenafil, avanafil, lodenafil, mirodenafil and udenafil. In some embodiments, the amount of icariin administered to the subject is 1-2000 mg/day. In some embodiments, the amount of sildenafil administered to the subject is 0.05-100 mg/day. In some embodiments, the amount of tadalafil administered to the subject is 0.01-20 mg/day. In some embodiments, the amount of vardenafil administered to the subject is 0.01-20 mg/day. In some embodiments, the amount of avanafil administered to the subject is 1-200 mg/day. In some embodiments, the amount of lodenafil administered to the subject is 1-200 mg/day. In some embodiments, the amount of mirodenafil administered to the subject is 1-100 mg/day. In some embodiments, the amount of udenafil administered to the subject is 1-200 mg/day. In some embodiments, the amount of zaprinast administered to the subject is 1-2000 mg/day.

[0015] In some embodiments, the sirtuin pathway activator is an AMPK activator. In some embodiments, AMPK activator is a biguanide. In some embodiments, the biguanide is metformin. In some embodiments, the amount of metformin is a therapeutic amount of metformin. In some embodiments, the therapeutic amount of metformin is about 1 g/day to about 2.55 g/day. In some embodiments, the therapeutic amount of metformin is about 0.5 g to about 1.25 g. In some embodiments, the amount of metformin is a sub-therapeutic amount of metformin. In some embodiments, the sub-therapeutic amount of metformin is about 20-1000 mg metformin/day. In some embodiments, the sub-therapeutic amount of metformin is about 10-500 mg metformin.

[0016] In some embodiments, the sirtuin pathway activator is a PGC-1α activator. In some embodiments, the PGC-1α activator is a thiazolidinedione. In some embodiments, the thiazolidinedione is selected from the group consisting of rosiglitazone and pioglitazone. In some embodiments, the amount of rosiglitazone administered is 0.1 – 4 mg. In some embodiments, the amount of pioglitazone administered is 0.1 – 45 mg. In some embodiments, the amount of sirtuin pathway activator is a sub-therapeutic amount.

[0017] In some embodiments, the method further comprises administering to the subject a second sirtuin pathway activator. In some embodiments, the sirtuin pathway activator comprises a biguanide and the second sirtuin pathway activator comprises a sirtuin activator. In some embodiments, the sirtuin pathway activator comprises a sirtuin activator and the second sirtuin pathway activator comprises a thiazolidinedione. In some embodiments, the sirtuin pathway activator comprises a sirtuin activator and the second sirtuin pathway activator comprises a PDE5-specific inhibitor. In some embodiments, the sirtuin pathway activator comprises a biguanide and the second sirtuin pathway activator comprises a thiazolidinedione. In some embodiments, the sirtuin pathway activator comprises a biguanide and the second sirtuin pathway activator comprises a PDE5-specific inhibitor. In some embodiments, the sirtuin pathway activator comprises a thiazolidinedione and the second sirtuin pathway activator comprises a PDE5-specific inhibitor.

[0018] In some embodiments, the amounts of (a) and (b) are co-administered. In some embodiments, the amounts of (a) and (b) are administered simultaneously as a single composition. In some embodiments, the amounts of (a) and (b) are administered sequentially. In some embodiments, all of the amounts are administered sequentially within 15 minutes, 60 minutes or 2 hours. In some embodiments, all components of the composition are administered 15 minutes, 60 minutes or 2 hours. In some embodiments, the method comprises administering (a) and (b) 1, 2, 3, 4, 5 or more times per day. In some embodiments, the method comprises administering (a) and (b) 3 times daily and the liver mass of the subject is decreased by 25% within 6 weeks. In some embodiments, the composition is substantially free of one or more free amino acids selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

[0019] In some other embodiments, the present invention relates to a composition comprising: (a) an amount of leucine in the form of a free amino acid; (b) an amount of metformin; and (c) an amount of sildenafil. In some embodiments, the amount of leucine is between about 50-95 wt% of the total wt of (a), (b), and (c), the amount of metformin is between about 5-50 wt% of the total wt of (a), (b), and (c), and the amount of sildenafil is between about 0.01-1 wt% of the total wt of (a), (b), and (c). In some embodiments, the composition is formulated as a unit dose comprising about 900-1200 mg of leucine, about

200-550 mg of metformin, and about 0.1 to 10 mg of sildenafil. In some embodiments, the composition is administered twice a day.

INCORPORATION BY REFERENCE

[0020] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0022] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawing(s) of which:

[0023] FIG. 1 depicts a sirtuin pathway.

[0024] FIG. 2 depicts effects of chronic high fat diet on liver mass in C57Bl/6 mice.

[0025] FIG. 3 depicts effects of chronic high fat diet on hepatic lipid accumulation in C57Bl/6 mice.

[0026] FIG. 4 depicts effects of administration of leucine, administration of therapeutic amounts of metformin, and administration of various combinations of low dose leucine, resveratrol, and metformin on liver mass following a chronic high fat diet.

[0027] FIG. 5 depicts effects of administration of leucine, administration of therapeutic amounts of metformin, and administration of various combinations of low dose leucine, resveratrol, and metformin on hepatic lipid accumulation following a chronic high fat diet.

[0028] FIG. 6 depicts effects of administration of leucine, administration of therapeutic amounts of metformin, and administration of low dose leucine and metformin on liver mass following a chronic high fat diet.

[0029] FIG 7 depicts effects of administration of leucine, administration of therapeutic amounts of metformin, and administration of low dose leucine and metformin on liver triglyceride content following a chronic high fat diet.

[0030] FIG. 8 depicts effects of administration of leucine, administration of therapeutic amounts of metformin, and administration of low dose leucine and metformin on histological evidence of hepatic lipid accumulation following a chronic high fat diet.

[0031] FIG 9 depicts effects of administration of leucine, administration of therapeutic amounts of metformin, and administration of low dose leucine and metformin on hepatic expression of lipogenic genes.

[0032] FIG 10 depicts effects of administration of leucine, administration of therapeutic amounts of metformin, and administration of low dose leucine and metformin on hepatic expression of inflammatory genes.

[0033] FIG. 11 depicts effects of administration of therapeutic amounts of metformin, and administration of low dose metformin and HMB on liver mass following a chronic high fat diet.

[0034] FIG. 12 depicts effects of administration of therapeutic amounts of metformin, and administration of low dose metformin and HMB on hepatic lipid accumulation following a chronic high fat diet.

[0035] FIG. 13 depicts effects of administration of low dose leucine and icariin on liver mass following a chronic high fat diet.

[0036] FIG. 14 depicts effects of administration of low dose leucine and icariin on hepatic lipid accumulation following a chronic high fat diet.

[0037] FIG 15 depicts effects of administration of low dose leucine and icariin on hepatic expression of fatty acid oxidation genes.

[0038] FIG 16 depicts effects of administration of low dose leucine and icariin on hepatic expression of inflammatory genes.

[0039] FIG 17 depicts effects of administration of low dose leucine and icariin circulating levels of C-reactive protein.

[0040] FIG 18 depecits effects of subtherapeutic amounts of leucine, metformin and sildenafil on fat oxidation (upper panel) and palmitate-induced triglyceride accumulation (lower panel) in HepG2 hepatocytes.

[0041] FIG 19 depicts effects of two-weeks administration of low dose leucine, metformin and sildenafil on circulating levels of alanine amino transferase (ALT) following a chronic atherogenic diet.

[0042] FIG 20 depicts effects of six-weeks administration of low dose leucine, metformin and sildenafil on circulating levels of alanine amino transferase (ALT) following a chronic atherogenic diet.

[0043] FIG 21 depicts effects of six-weeks administration of low dose leucine, metformin and sildenafil on liver mass, normalized to body weight.

DETAILED DESCRIPTION OF THE INVENTION

[0044] Several aspects of the invention are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or with other methods. Unless stated otherwise, the present invention is not limited by the illustrated ordering of acts or events, as some acts may occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the present invention. The concentration of various components in the disclosed compositions are exemplary and not meant to be limited to the recited concentration per se.

[0045] As used herein, the singular forms "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

[0046] The terms "determining", "measuring", "evaluating", "assessing," "assaying," "interrogating," and "analyzing" are used interchangeably herein to refer to any form of measurement, and include determining if an element is present or not. These terms include both quantitative and/or qualitative determinations. Assessing may be relative or absolute.

[0047] As used herein, the term "subject" or "individual" includes mammals. Non-limiting examples of mammals include humans and mice, including transgenic and non-transgenic mice. The methods described herein can be useful in both human therapeutics, pre-clinical, and veterinary applications. In some embodiments, the subject is a mammal, and in some embodiments, the subject is human. Other mammals include, and are not limited to, apes, chimpanzees, orangutans, monkeys; domesticated animals (pets) such as dogs, cats, guinea pigs, hamsters, mice, rats, rabbits, and ferrets; domesticated farm animals such as cows, buffalo, bison, horses, donkey, swine, sheep, and goats; or exotic animals typically found in zoos, such as bear, lions, tigers, panthers, elephants, hippopotamus, rhinoceros,

giraffes, antelopes, sloth, gazelles, zebras, wildebeests, prairie dogs, koala bears, kangaroo, pandas, giant pandas, hyena, seals, sea lions, and elephant seals.

[0048] As used herein, "agent" or "biologically active agent" refers to a biological, pharmaceutical, or chemical compound or other moiety. Non-limiting examples include simple or complex organic or inorganic molecule, a peptide, a protein, a peptide nucleic acid (PNA), an oligonucleotide (including e.g., aptomer and polynucleotides), an antibody, an antibody derivative, antibody fragment, a vitamin derivative, a carbohydrate, a toxin, a branched chain amino acid in free amino acid form or metabolite thereof, or a chemotherapeutic compound. Various compounds can be synthesized, for example, small molecules and oligomers (e.g., oligopeptides and oligonucleotides), and synthetic organic compounds based on various core structures. In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

[0049] The terms "administer", "administered", "administers" and "administering" are defined as the providing a composition to a subject via a route known in the art, including but not limited to intravenous, intraarterial, oral, parenteral, buccal, topical, transdermal, rectal, intramuscular, subcutaneous, intraosseous, transmucosal, or intraperitoneal routes of administration. In certain embodiments of the subject application, oral routes of administering a composition may be preferred.

[0050] As used herein, the terms "co-administration", "administered in combination with" and their grammatical equivalents are meant to encompass administration of the invention composition and additional therapeutic agent to a single subject. Co-administration can encompass administration of two or more agents to an animal so that both agents and/or their metabolites are present in the subject at the same time. Co-administration can encompass treatment regimens in which the composition and additional therapeutic agent are administered by the same or different route of administration or at the same or different times. Co-administration includes simultaneous administration in separate compositions, administration at different times in separate compositions, or administration in a composition in which both agents are present. Co-administration can include simultaneous administration of the agents in separate compositions, administration at different times in separate compositions, and/or administration in a single composition comprising each of the agents to be co-administered.

[0051] The term "effective amount" or "therapeutically effective amount" refers to that amount of a compound described herein that is sufficient to effect the intended application including but not limited to disease treatment, as defined below. The therapeutically effective amount may vary depending upon the intended application (in vitro or in vivo), or the subject and disease condition being treated, e.g., the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The term also applies to a dose that will induce a particular response in target cells, e.g., reduction of proliferation or down regulation of activity of a target protein. The specific dose will vary depending on the particular compounds chosen, the dosing regimen to be followed, whether it is administered in combination with other compounds, timing of administration, the tissue to which it is administered, and the physical delivery system in which it is carried.

[0052] The term "energy metabolism," as used herein, refers to the transformation of energy that accompanies biochemical reactions in the body, including cellular metabolism and mitochondrial biogenesis. Energy metabolism can be quantified using the various measurements described herein, for example and without limitations, weight-loss, fat-loss, insulin sensitivity, fatty acid oxidation, glucose utilization, triglyceride content, Sirt 1 expression level, AMPK expression level, oxidative stress, and mitochondrial biomass.

[0053] The term "isolated", as applied to the subject components, for example a PDE 5 inhibitor, including but not limited to sildenafil and icariin, leucine and leucine metabolites (such as HMB), and resveratrol, refers to a preparation of the substance devoid of at least some of the other components that may also be present where the substance or a similar substance naturally occurs or is initially obtained from. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichment of the embodiments of this invention are increasingly more preferred. Thus, for example, a 2-fold enrichment is preferred, 10-fold enrichment is more preferred, 100-fold enrichment is more preferred, 1000-fold enrichment is even more preferred. A substance can also be provided in an isolated state by a process of artificial assembly, such as by chemical synthesis.

[0054] A "modulator" of a pathway refers to a substance or agent which modulates the activity of one or more cellular proteins mapped to the same specific signal transduction

pathway. A modulator may augment or suppress the activity and/or expression level or pattern of a signaling molecule. A modulator can activate a component in a pathway by directly binding to the component. A modulator can also indirectly activate a component in a pathway by interacting with one or more associated components. The output of the pathway can be measured in terms of the expression or activity level of proteins. The expression level of a protein in a pathway can be reflected by levels of corresponding mRNA or related transcription factors as well as the level of the protein in a subcellular location. For instance, certain proteins are activated by translocating in or out of a specific subcellular component, including but not limited to nucleus, mitochondria, endosome, lysosome or other membraneous structure of a cell. The output of the pathway can also be measured in terms of physiological effects, such as mitochondrial biogenesis, fatty acid oxidation, or glucose uptake.

[0055] An "activator" refers to a modulator that influences a pathway in a manner that increases the pathway output. Activation of a particular target may be direct (e.g. by interaction with the target) or indirect (e.g. by interaction with a protein upstream of the target in a signaling pathway including the target).

[0056] The term "selective inhibition" or "selectively inhibit" as referred to a biologically active agent refers to the agent's ability to preferentially reduce the target signaling activity as compared to off-target signaling activity, via direct or interact interaction with the target.

[0057] The term "substantially free", as used herein, refers to compositions that have less than about 10%, less than about 5%, less than about 1%, less than about 0.5%, less than 0.1% or even less of a specified component. For example a composition that is substantially free of non-branched chain amino acids may have less than about 1% of the non-branched chain amino acid lysine. For example, substantially free of a non-branched chain amino acid can be evidenced by less than 1% of the non-branched chain amino acid when compared to the rest of the amino acids in a given composition.

[0058] A "sub-therapeutic amount" of an agent, an activator or a therapy is an amount less than the effective amount for that agent, activator or therapy, but when combined with an effective or sub-therapeutic amount of another agent or therapy can produce a desired result, due to, for example, synergy in the resulting efficacious effects, and/or reduced side effects. A sub-therapeutic amount of the agent or component can be such that it is an amount below which would be considered therapeutic. For example, FDA guidelines can suggest a specified level of dosing to treat a particular condition, and a sub-therapeutic amount would be any

level that is below the FDA suggested dosing level. The sub-therapeutic amount can be about 1, 5, 10, 15, 20, 25, 30, 35, 50, 75, 90, or 95% less than the amount that is considered to be a therapeutic amount. The therapeutic amount can be assessed for individual subjects, or for groups of subjects. The group of subjects can be all potential subjects, or subjects having a particular characteristic such as age, weight, race, gender, or physical activity level.

[0059] A "synergistic" or "synergizing" effect can be such that the one or more effects of the combination compositions are greater than the one or more effects of each component alone at a comparable dosing level, or they can be greater than the predicted sum of the effects of all of the components at a comparable dosing level, assuming that each component acts independently. The synergistic effect can be about, or greater than about 10, 20, 30, 50, 75, 100, 110, 120, 150, 200, 250, 350, or 500% or even more than the effect on a subject with one of the components alone, or the additive effects as measured when each of the components when administered individually. The effect can be any of the measurable effects described herein.

[0060] The terms "free amino acid form" or "individual amino acid form", as used herein, can refer to amino acids that are not bound to other amino acids, for example, by peptide bonds. For example, "free" or "individual" leucine refers to leucine not bound to other amino acids by peptide bonds.

OVERVIEW

[0061] The invention provides methods, compositions, and kits for reducing or preventing hepatic steatosis in a subject in need thereof. For example, the invention provides a method of reducing hepatic steatosis in a subject in need thereof, comprising administering to the subject a composition comprising an amount of a sirtuin pathway activator such as, e.g., metformin. Another exemplary method of reducing or preventing hepatic steatosis comprises administering (a) an amount of a branched chain amino acid and/or metabolite thereof existing in form of a free amino acid, and (b) an additional agent. In some embodiments, the branched chain amino acid and/or metabolite thereof is leucine. In some embodiments, the additional agent is one or more sirtuin pathway activators. In some embodiments, the additional agent is metformin, and/or a PDE inhibitor, e.g. sildenafil. In some embodiments, co-administration of such the branched chain amino acid and/or metabolite thereof and the additional agent reduces or prevents hepatic steatosis to a greater extent than administration of any one of the agents alone. In some embodiments, co-administration of the branched chain amino acid in free amino acid form (or metabolite thereof) and the additional agent has

a synergistic effect, e.g., reduces or prevents hepatic steatosis to a greater extent than an additive effect of administering the branched chain amino acid in free amino acid form (or metabolite thereof) alone and administering the additional agent alone.

Assessment of hepatic steatosis

[0062] Any of the invention methods described herein can include an assessment of hepatic steatosis in a subject. For example, assessment of hepatic steatosis in a subject can be used to determine if a subject is in need of reduction of hepatic steatosis. Assessment of hepatic steatosis in a subject can be used to determine if and/or to what extent hepatic steatosis is prevented or reduced in a subject. Hepatic steatosis can be assessed by any means known to those of skill in the art or otherwise described herein. Hepatic steatosis in a subject can be evidenced, e.g., by an accumulation of fat in the liver of the subject (e.g., by an accumulation of fat in hepatic cells of the subject). Accumulation of fat in the liver can be indicated by several means, for example, by ultrasonography, computed tomography (CT), magnetic resonance imaging, measurement of serum alanine transaminase and aspartate transaminase, measurement of liver size or weight, or biopsy.

[0063] Ultrasonography methods for assessing liver can be as known to those of skill in the art or otherwise described herein. Ultrasonography assessment of hepatic steatosis (e.g., fat accumulation in liver) can comprise use of conventional B-mode ultrasonography. Assessment of various hepatic ultrasonography parameters can be used for the assessment of hepatic steatosis. Exemplary ultrasonography parameters for the assessment of hepatic steatosis include, but are not limited to (1) parenchymal brightness, (2) liver-to-kidney contrast, (3) deep beam attenuation,(4) bright vessel walls, and (5) gallbladder wall definition. Assessment of such ultrasonography parameters can be used to calculate an ultrasonographic steatosis score (USS). USS can be calculated, e.g., as follows: absent (score 0) steatosis was defined as normal liver echotexture; mild (score 1) steatosis as slight and diffuse increase in fine parenchymal echoes with normal visualization of diaphragm and portal vein borders; moderate (score 2) steatosis as moderate and diffuse increase in fine echoes with slightly impaired visualization of portal vein borders and diaphragm; and severe (score 3) steatosis as fine echoes with poor or no visualization of portal vein borders, diaphragm, and posterior portion of the right lobe.

[0064] CT methods for assessing liver can be any known to those of skill in the art or otherwise described herein. CT images can be assessed by, e.g., a radiologist. CT images of the liver of a subject can be assessed by, e.g., measuring density of regions of interest in the

images. Regions of interest within images can be selected so as not to contain blood vessels or other artifacts (e.g., motion artifacts). Density of regions of interest in a CT image can be measured in Hounsfield units (HU). Normal liver tissue can have a HU measurement of 40-60 HU. By contrast, fat typically has a lower density. For example, fat can have an HU measurement of, e.g., about -100 to about -500. Hepatic steatosis can be evidenced by an HU measurement less than 40 HU. Hepatic steatosis can be evidenced by an HU measurement that is between -500 and 40 HU, for example, an HU measurement that is -500-1 HU, -100-10 HU, 0-20 HU, 5-30 HU, or 20-39.9 HU. A subject can be diagnosed with hepatic steatosis is the subject exhibits an HU measurement of 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23,22, 21, 20, 19, 18, 17, 16, 15, 14, 13,12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0, or less than 0 HU. Hepatic steatosis can be evidenced by a difference in HU measurement between spleen and liver (e.g., HU_{spleen} - HU_{liver}) For example, hepatic steatosis can be evidenced if HU_{spleen} - HU_{liver} is greater than 0, for example, if HU_{spleen} - HU_{liver} is between 1-10, 10-20, or more than 20. In some embodiments, a difference in HU measurement between spleen and liver of 18.5 is used to diagnose hepatic steatosis.

[0065] MRI methods for assessing liver can be any known to those of skill in the art or otherwise described herein. Exemplary methods of using MRI to determine steatosis, e.g., hepatic steatosis, are described in US Patent Application Pub. No. 20050215882, which is hereby incorporated by reference.

[0066] Hepatic steatosis can be evidenced by measurement of serum alanine transaminase (ALT) and/or aspartate transaminase levels. Methods of measuring serum alanine transaminase and/or aspartate transaminase levels can be any known to those of skill in the art or otherwise described herein. Hepatic steatosis can be indicated by an increase in serum ALT levels as compared to a control subject without hepatic steatosis. In some cases, hepatic steatosis can be indicated by an increase in serum ALT levels and serum aspartate transaminase levels as compared to a control subject without hepatic steatosis. In some cases, hepatic steatosis can be indicated by a aspartate transaminase to alanine transaminase ratio that is greater than one.

[0067] Hepatic steatosis can be evidenced by measurement of liver weight and/or size. Methods of measuring liver weight and/or size can be any known to those of skill in the art or otherwise described herein. Hepatic steatosis can be indicated by an increase in liver weight and/or size as compared to a control subject without hepatic steatosis. In some embodiments, an increase in liver weight/size by 10% or more, 15% or more, 20%, or more, 25% or more,

30%, or more, 35% or more, 40% or more, 45% or more, 50%, or more, 55% or more, 60%, or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90%, or more, 95% or more, 100%, or more than 100% as compared to a control subject without hepatic steatosis. can indicate hepatic steatosis in a subject.

[0068] Hepatic steatosis can be evidenced by tissue biopsy. A liver biopsy sample can be obtained by any means known to those of skill in the art, for example, by needle biopsy. The sample can be processed by any means known to those of skill in the art or otherwise described herein. The sample can be fixed (e.g., with formalin) or may be unfixed. The sample may be snap-frozen. For example, the sample may be sectioned into thin sections. The sections may be stained, e.g., with hematoxylin and eosin. Accumulation of fat in the liver can be evidenced by appearance of vacuoles which are filled with lipids such as, by way of example only, triglycerides. Such vacuoles can be appear to be optically "empty", since fats can dissolve during histological tissue processing. Accordingly, levels of hepatic steatosis can be determined by measuring the number, size, or density of hepatic lipid vacuoles.

[0069] Levels of hepatic steatosis can be determined using any of the assessment methods described herein. Levels of hepatic steatosis can be quantified, by way of nonlimiting example only, as a percentage of fat accumulation (e.g., fat content) in the liver. In some embodiments, hepatic steatosis is scored according to a 0-3 score, with 0= <5% fat accumulation in the liver, 1=5%-33% fat accumulation in the liver, 2=33%-66% fat accumulation in the liver, and 3= >66% fat accumulation in the liver. Liver fat content can be assessed by any means known to those of skill in the art, including, e.g., by proton magnetic resonance spectroscopy, by biopsy, or by any other methods described herein.

Reduction of hepatic steatosis in a subject in need thereof

[0070] Administration and/or co-administration of any of the compounds described herein can reduce hepatic steatosis in a subject in need thereof. Exemplary subjects in need of hepatic steatosis reduction can include subjects which are diagnosed with hepatic steatosis. Any of the methods described herein for assessment of hepatic steatosis, or otherwise known in the art, may be used for the diagnosis of hepatic steatosis in a subject. For example, a subject may be diagnosed with hepatic steatosis if the subject exhibits a hepatic fat content of 5% or higher, a hepatic fat content of 10% or higher, a hepatic fat content of 20% or higher, a hepatic fat content of 30% or higher, a hepatic fat content of 40% or higher, a hepatic fat content of 50% or higher, a hepatic fat content of 60% or higher, or a hepatic fat content of

70% or higher. A subject can be diagnosed with stage 1 hepatic steatosis if the subject exhibits 5%-33% fat accumulation in liver. A subject can be diagnosed with stage 2 hepatic steatosis if the subject exhibits 33%-66% fat accumulation in liver. A subject can be diagnosed with stage 3 hepatic steatosis if the subject exhibits over 66% fat accumulation in liver.

[0071] The subject may exhibit a non-alcoholic fatty liver disease (NAFLD). Diagnosis of NAFLD can comprise a diagnosis of hepatic steatosis. Diagnosis of NAFLD can further comprise determination of an average daily amount of alcohol consumption by the subject. In some cases, NAFLD is diagnosed in a subject if the subject is diagnosed with hepatic steatosis and is determined to consume an average of less than 20 grams of alcohol per day (e.g., an average of less than 25 ml alcohol/day). NAFLD in a subject may progress to non-alcoholic steatohepatitis (NASH).

[0072] The subject may exhibit NASH. Diagnosis of NASH can comprise a diagnosis of NAFLD. Diagnosis of NASH can further comprise a determination of inflammation in the liver of a subject concurrent with hepatic steatosis. NASH can be diagnosed in a subject, for example, upon detection of hepatic fat accumulation (steatosis) and one or more of the following liver conditions: inflammation, ballooning degeneration of hepatocytes (sometimes with identifiable Mallory bodies), glycogenated hepatocyte nuclei, and pericellular fibrosis. Pericellular fibrosis can be identfied by trichome strain. Pericellular fibrosis can exhibit upon trichrome staining, for example, a characteristic "chicken wire" pattern. NASH in a subject may progress to cirrhosis.

[0073] A subject in need of hepatic steatosis reduction can be suffering from a symptom of hepatic steatosis. Exemplary symptoms include, but are not limited to fatigue, malaise, unexplained weight loss, weakness, lack of appetite, nausea, appearance of small, red spider veins under the skin, easy bruising, jaundice, internal bleeding (e.g., bleeding from engorged veins in the esophagus or intestines), loss of sex drive, ascites, itching, edema, mental confusion, and pain or ache (e.g., pain or ache of the upper right abdomen). In some embodiments, the subject is asymptomatic.

[0074] Reduction of hepatic steatosis can be determined by comparison to a control subject and/or control population. Hepatic steatosis in a subject can be considered reduced if any one or more of hepatic fat content, as measured by any of the methods described herein, liver size or weight, liver vacuole number, size of a liver vacuole, liver vacuole density, serum alanine transaminase (ALT) and/or aspartate transaminase levels in the subject are

reduced as compared to a control subject and/or control population. A control subject can be an individual that has not been administered one or more compounds described herein. Likewise, a control population can encompass a plurality of individuals that have not been administered one or more compounds described herein. The control subject can be a subject having hepatic steatosis, that is not administered one or more compounds described herein. The control subject can be a different subject.

[0075] It is not necessary for the control subject to be a different individual from said subject. For example, the control subject can be the same subject at an earlier time point, for example, prior to receiving a first dose of any of the compounds described herein. In some embodiments, a level of hepatic steatosis in the subject following administration of one or more compounds described herein is compared to a level of hepatic steatosis in the subject prior to first administration of the one or more compounds.

[0076] An exemplary method of assessing hepatic steatosis reduction in a subject in need thereof can comprise measuring a level of hepatic steatosis in the subject or in a biological sample derived from the subject at a first time point. The first time point may be a time point prior to administration of one or more compounds described herein. The method may further comprise measuring a level of hepatic steatosis in the subject or in a biological sample derived from the subject at a second time point. The second time point may follow administration of the one or more compounds described herein. The level measured at the second time point may be compared to the level measured at the first time point to determine whether reduction has occurred. Reduction of hepatic steatosis can indicate clinical efficacy of the administration of the one or more compounds described herein. The method may further comprise administering additional doses of the one or more compounds described herein if the level of hepatic steatosis in the subject is reduced.

[0077] Practice of any one of the methods of the invention can reduce hepatic steatosis by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more than 99%. For example, practice of any one of the methods of the invention can reduce hepatic steatosis by 5-20%, 10-40%, 30-60%, 40-80%, 60-95%, or 75-99%. In some embodiments, administration of any combination of agents described herein reduces hepatic steatosis in about six weeks or less. Administration of any combination of agents described herein can, for example, reduce liver weight by 25% in about six weeks. Administration of any combination of agents described

herein can, by way of example only, reduce liver vacuole number, size, and/or density by over 25%, over 50%, over 75%, or over 90% in about six weeks.

Prevention of hepatic steatosis

[0078] Administration and/or co-administration of any of the compounds described herein can reduce hepatic steatosis in a subject in need thereof. Exemplary subjects in need of hepatic steatosis reduction can include subjects exhibiting a propensity for, or having a high risk of developing, hepatic steatosis.

[0079] A number of environmental and genetic risk factors have been found to increase propensity of a subject to develop hepatic steatosis. A subject can exhibit a propensity for developing hepatic steatosis if the subject exhibits any combination of risk factors described herein. A greater number of risk factors exhibited by a subject can indicate a higher propensity for developing hepatic steatosis, as compared to a subject that exhibits a lower number of risk factors. It is understood that a subject can be considered to exhibit a propensity for developing hepatic steatosis if the subject exhibits even one, two, three, or more of the risk factors described herein.

[0080] An exemplary risk factor for developing hepatic steatosis is obesity. Accordingly, a subject can be considered to exhibit an increased propensity to develop hepatic steatosis if the subject is obese. Obesity in a subject can be assessed using a body mass index (BMI) measurement. A subject's BMI can be calculated by dividing the subject's body weight (in kg) by the square of the subject's height (m2). A subject can be considered obese if the subject exhibits a BMI that is over 25 kg/m2, over 26kg/m2, over 27 kg/m2, over 28 kg/m2, over 29 kg/m2, over 30 kg/m2, over 31 kg/m2, over 32 kg/m2, over 33 kg/m2, over 34 kg/m2, over 35 kg/m2, over 36 kg/m2, over 37 kg/m2, over 38 kg/m2, over 39 kg/m2, or over 40 kg/m2. A subject can also be considered to exhibit a propensity to develop hepatic steatosis if the subject exhibits abdominal obesity. Abdominal obesity can be assessed by measuring the circumference of the subject's waist. For example, if the subject is an adult male, the subject can be considered to exhibit abdominal obesity if the subject exhibits a waist circumference of 102 cm or greater. For other example, if the subject is an adult female, the subject can be considered to exhibit abdominal obesity if the subject exhibits a waist circumference of 88 cm or greater.

[0081] Another exemplary risk factor for developing hepatic steatosis is high blood pressure. Accordingly, a subject can be considered to exhibit an increased propensity to develop hepatic steatosis if the subject exhibits high blood pressure. Blood pressure in a

subject can be assessed by measuring a systolic and/or diastolic blood pressure (bp) in the subject. A subject can be considered to exhibit high blood pressure if the subject exhibits a systolic bp that is 120 or higher, 121 or higher, 122 or higher, 123 or higher, 124 or higher, 125 or higher, 126 or higher, 127 or higher, 128 or higher, 129 or higher, 130 or higher, 131 or higher, 132 or higher, 133 or higher, 134 or higher, 135 or higher, 136 or higher, 137 or higher, 138 or higher, 139 or higher, or 140 or higher. A subject can be considered to exhibit high blood pressure if the subject exhibits a diastolic bp that is 80 or higher, 81 or higher, 82 or higher, 83 or higher, 84 or higher, 85 or higher, 86 or higher, 87 or higher, 88 or higher, 89 or higher, 90 or higher, 91 or higher, 92 or higher, 93 or higher, 94 or higher, 95 or higher, 96 or higher, 97 or higher, 98 or higher, 99 or higher, or 100 or higher.

[0082] Another exemplary risk factor for developing hepatic steatosis is high cholesterol. Accordingly, a subject can be considered to exhibit an increased propensity to develop hepatic steatosis if the subject exhibits high cholesterol. Cholesterol in a subject can be assessed by measuring total blood cholesterol levels in the subject. A subject can be considered to exhibit high cholesterol if the subject exhibits, e.g., over 200 mg/dl total blood cholesterol, over 210 mg/dl total blood cholesterol, over 220 mg/dl total blood cholesterol, over 230 mg/dl total blood cholesterol, or over 240 mg/dl total blood cholesterol. A subject can be considered to exhibit high cholesterol if the subject exhibits, 200-220 mg/dl total blood cholesterol, 220-240 mg/dl total blood cholesterol, or 240 mg/dl total blood cholesterol or higher. Cholesterol in a subject can also be assessed by measuring blood low density lipoprotein (LDL) levels in the subject. A subject can be considered to exhibit high cholesterol if the subject exhibits, e.g., 130 mg/dl LDL or higher, 135 mg/dl LDL or higher, 140 mg/dl LDL or higher, 145 mg/dl LDL or higher, 150 mg/dl LDL or higher, 155 mg/dl LDL or higher, 160 mg/dl LDL or higher, 165 mg/dl LDL or higher, 170 mg/dl LDL or higher, 175 mg/dl LDL or higher, 180 mg/dl LDL or higher, 185 mg/dl LDL or higher, or 190 mg/dl LDL or higher. Cholesterol in a subject can also be assessed by measuring blood high density lipoprotein (HDL) levels in the subject. A subject can be considered to exhibit high cholesterol if the subject exhibits, e.g.,60 mg/dl HDL or lower, 55 mg/dl HDL or lower, 50 mg/dl HDL or lower, 45 mg/dl HDL or lower, or 40 mg/dl HDL or lower.

[0083] Another exemplary risk factor for developing hepatic steatosis is insulin resistance. Accordingly, a subject can be considered to exhibit an increased propensity to develop hepatic steatosis if the subject exhibits insulin resistance. Insulin resistance in a subject can be assessed by any means known in the art or otherwise described herein. By way of

example only, insulin resistance may be assessed by measuring Homeostatic Assessment of Insulin Resistance ($HOMA_{IR}$) in the subject. $HOMA_{IR}$ can be determined by any means known in the art, for example, by the following equation: $HOMA_{IR}$ = Insulin (uU/mL) X glucose (mM)]/22.5. The $HOMA_{IR}$ value increases with increasing insulin resistance, and values above (2.6) are generally considered to be insulin resistant. A subject can be considered to exhibit insulin resistance if the subject exhibits a $HOMA_{IR}$ above the the 75th percentile, or a $HOMA_{IR}$ value of over 2.6. Insulin resistance may also be assessed by measuring fasting serum insulin levels in the subject. A subject may be considered to exhibit insulin resistance if the subject exhibits a fasting serum insulin level of 60 pmol/L or higher. Insulin resistance may also be assessed by measuring a quantitative insulin sensitivity check index (QUICKI) in the subject. QUICKI can be determined by the following equation: QUICKI = 1/(log(fasting insulin μU/mL) + log(fasting glucose mg/dL)). A subject may be considered to exhibit insulin resistance if the subject exhibits a QUICKI of 0.30 or less. For other example, insulin resistance can be determined by measuring blood glucose levels in a subject, in some cases over a period of time, following administration of a bolus of insulin. Subjects with insulin resistance typically exhibit an attenuated drop in blood glucose levels following insulin administration, as compared to subject without insulin resistance.

[0084] Another exemplary risk factor for developing hepatic steatosis is diabetes. Accordingly, a subject can be considered to exhibit an increased propensity to develop hepatic steatosis if the subject has or is diagnosed with diabetes. The diabetes can be Type I or Type II diabetes. Diabetes may be diagnosed by any means known to those of skill in the art, or otherwise described herein. For example, a subject may be diagnosed with diabetes if the subject exhibits high fasting plasma glucose levels (e.g., 126 mg/dl or higher). A subject may be diagnosed with diabetes if the subject exhibits high plasma glucose levels following administration of a bolus of glucose (e.g., in a glucose tolerance test). For example, a subject may be diagnosed with diabetes if the subject exhibits 200 mg/dl plasma glucose or higher two hours after administration of a 75 g bolus of glucose.

[0085] Genetics may be a risk factor for developing hepatic steatosis. For example, males of Indian, Asian, and/or Mexican descent may have a higher risk of developing hepatic steatosis. Accordingly, a subject can be considered to exhibit an increased propensity to develop hepatic steatosis if the subject is a male of African, Asian, and/or Mexican descent. Genetic polymorphisms may also be associated with an increased propensity to develop hepatic steatosis. For example, a subject may be considered to exhibit an increased

propensity to develop hepatic steatosis if the subject exhibits a T455C or C482T polymorphism in the APOC3 gene.

[0086] Certain medications increase risk for developing hepatic steatosis. Such medications which increase risk for developing hepatic steatosis include, but are not limited to oral corticosteroids (e.g., prednisone, hydrocortisone, among others), synthetic estrogens (e.g., Premarin, Ortho-Est, tamoxifen, among others), amiodarone (Cordarone, Pacerone), diltiazem, anti-retroviral drugs such as, e.g., indinavir, and methotrexate. Accordingly, a subject may be considered to exhibit an increased propensity to develop hepatic steatosis if the subject has taken any of the medications described herein as increasing risk for developing hepatic steatosis.

[0087] In some embodiments, the subject exhibiting a propensity for developing hepatic steatosis has not yet developed hepatic steatosis. For example, the subject may not have exhibited a symptom of hepatic steatosis. For other example, the subject may not exhibit increased fat content of the liver. In some embodiments, the subject has not been diagnosed with hepatic steatosis. In some embodiments, the subject does not exhibit an increase in serum ALT levels as compared to a control subject without hepatic steatosis.

[0088] Practice of any one of the methods of the invention can prevent or reduce occurrence of hepatic steatosis in at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more than 99% of treated subjects. For example, practice of any one of the methods of the invention can prevent hepatic steatosis or reduce occurrence of hepatic steatosis by 5-20%, 10-40%, 30-60%, 40-80%, 60-95%, or 75-99%. In some embodiments, administration of any combination of agents described herein for about, less than about, or greater than about 1, 2, 3, 4, 5, 6, 7, 8 weeks or 2, 4, 6, 12 or 24 months and such administration prevents or reduces occurrence of hepatic steatosis in administered subjects. Prevention of hepatic steatosis can be determined by comparison to a reference subject and/or reference population. Hepatic steatosis in a subject can be considered to be prevented or to have reduced occurence if any one or more of hepatic fat content, as measured by any of the methods described herein, liver size or weight, liver vacuole number, size of a liver vacuole, liver vacuole density, serum alanine transaminase (ALT) and/or aspartate transaminase levels in the subject does not increase or increases to a lesser extent as compared to a reference subject and/or reference population. The reference subject and/or reference population can be another subject or population of subjects exhibiting a comparable propensity for developing hepatic steatosis

who has not developed hepatic steatosis, and who is not treated with one or more compounds described herein.

Exemplary compounds

[0089] The invention provides compounds for the reduction and/or prevention of hepatic steatosis. For example, co-administration of a branched chain amino acid in free amino acid form or metabolite thereof, and an additional agent can reduce and/or prevent hepatic steatosis in a subject. The additional agent can be a sirtuin pathway activator and/or a PDE inhibitor, such as a PDE5 inhibitor.

Branched chain amino acids

[0090] Branched chain amino acids may have aliphatic side chains with a branch carbon atom that is bound to two or more other atoms. The other atoms may be carbon atoms. Examples of branched chain amino acids include leucine, isoleucine, and valine. Branched chain amino acids may also include other compounds, such as 4-hydroxyisoleucine. Such branched chain amino acids may be administered to a subject in free amino acid form. In some embodiments, the branched chain amino acid in free amino acid form is leucine in free amino acid form. In some embodiments, a composition comprising a branched chain amino acid in free amino acid form is substantially free of one or more, or all of non-branched chain amino acids. In some embodiments, the compositions are substantially free of one or more, or all of non-branched chain amino acids in free amino acid form. For example, the composition can be substantially free of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, and/or tyrosine. The composition can be substantially free of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, and/or tyrosine in free amino acid form. In some embodiments, the composition is substantially free of isoleucine and/or valine in free form.

[0091] In some embodiments, a method described herein can include administration of a salt, derivative, metabolite, catabolite, anabolite, precursor, and/or analog of any branched chain amino acids. The metabolite can be a metabolite of leucine, such as HMB. Metabolites of branched chain amino acids can include hydroxymethylbutyrate (HMB), α-hydroxyisocaproic acid, and keto-isocaproic acid (KIC), keto isovalerate, and keto isocaproate. Non-limiting exemplary anabolites of branched chain amino acids can include

glutamate, glutamine, threonine, α-ketobytyrate, α-aceto-α-hydroxy butyrate, α,β-dihydroxy-β-methylvalerate, α-keto-β-methylvalerate, α,β-dihydroxy isovalerate, and α-keto isovalerate.

[0092] In certain embodiments of the invention, any of the compositions to be administered to a subject can be formulated such that they do not contain (or exclude) one or more amino acids selected from the group consisting of lysine, glutamate, proline, arginine, valine, isoleucine, aspartic acid, asparagine, glycine, threonine, serine, phenylalanine, tyrosine, histidine, alanine, tryptophan, methionine, glutamine, taurine, carnitine, cystine and cysteine. Any of the compositions disclosed herein can be formulated such that they do not contain (or exclude) one or more free amino acids selected from the group consisting of lysine, glutamate, proline, arginine, valine, isoleucine, aspartic acid, asparagine, glycine, threonine, serine, phenylalanine, tyrosine, histidine, alanine, tryptophan, methionine, glutamine, taurine, carnitine, cystine and cysteine. In some cases, a composition does not contain any non-branched chain amino acids. In some cases, a composition does not contain any non-branched chain amino acids in free amino acid form. The mass or molar amount of a non-branched chain amino acid in a composition can be less than 0.01, 0.1, 0.5, 1, 2, or 5 % of the total composition. The mass or molar amount of a non-branched chain amino acid in free amino acid form can be less than 0.01, 0.1, 0.5, 1, 2, or 5 % of the total composition. The mass or molar amount of any branched-chain amino acid or metabolite thereof, aside from leucine or its metabolites can be less than 0.01, 0.1, 0.5, 1, 2, or 5 % of the total composition. The mass or molar amount of any branched-chain amino acid in free amino acid form or metabolite thereof, aside from leucine or its metabolites can be less than 0.01, 0.1, 0.5, 1, 2, or 5 % of the total composition.

Exemplary sirtuin pathway activators

[0093] Sirtuin pathway activators can include any agents which activate one or more components of a sirtuin pathway. The sirtuin pathway includes, without limitation, signaling molecules such as, Sirt1, Sirt3, and AMPK. The output of the pathway can be determined by the expression level and/or the activity of the pathway and/or a physiological effect. In some embodiments, activation of the Sirt1 pathway includes stimulation of PGC1-α and/or subsequent stimulation of mitochondrial biogenesis and fatty acid oxidation. An increase or activation of a sirtuin pathway can be observed by an increase in the activity of a pathway component protein. For example, the protein can be Sirt1, PGC1-α, AMPK, Epac1, Adenylyl cyclase, Sirt3, or any other proteins and their respective associated proteins along the

signaling pathway depicted in FIG. 1 (Park et. al., "Resveratrol Ameliorates Aging-Related Metabolic Phenotypes by Inhibiting cAMP Phosphodiesterases," Cell 148, 421-433 February 3, 2012. Non-limiting examples of physiological effects that can serve as measures of sirtuin pathway output include mitochondrial biogenesis, fatty acid oxidation, glucose uptake, palmitate uptake, oxygen consumption, carbon dioxide production, weight loss, heat production, visceral adipose tissue loss, respiratory exchange ratio, insulin sensitivity, inflammation marker level, vasodilation, browning of fat cells, and irisin production. Examples of indicia of browning of fat cells include, without limitation, increased fatty acid oxidation, and expression of one or more brown-fat-selective genes (e.g. Ucp1, Cidea, Prdm16, and Ndufs1). In some embodiments, changes in one or more physiological effects that can serve as measures of sirtuin pathway output are induced by increasing irisin production.

[0094] An increase in mitochondrial biogenesis can be evidenced by an increase in the formation of new mitochondria and/or by an increase in mitochondrial functions, such as increased fatty acid oxidation, increased heat generation, increased insulin sensitivity, increased in glucose uptake, increased in vasodilation, decreased in weight, decreased in adipose volume, and decreased inflammatory response or markers in a subject.

[0095] In some embodiments the sirtuin pathway activator is a sirtuin activator. The sirtuin activator can be a Sirt1 activator, a Sirt2 activator, and/or Sirt3 activator. Sirt1 activity can be determined by measuring deacetylation of a substrate, which can be detected using a fluorophore. An increase in sirt1, sirt2, or sirt3 is observed by applying a corresponding substrate in a deacylation assay conducted in vitro. The substrate for measuring SIRT1 activity can be any substrate known in the art (for example a peptide containing amino acids 379-382 of human p53 (Arg-His-Lys-Lys[Ac]). The substrate for measuring SIRT3 activity can be any substrate known in the art (for example a peptide containing amino acids 317-320 of human p53 (Gln-Pro--Lys-Lys[Ac])).

[0096] Exemplary sirtuin activators can include those described in Howitz et al. (2003) Nature 425: 191 and include, for example, resveratrol (3,5,4′-Trihydroxy-trans-stilbene), butein (3,4,2′,4′-Tetrahydroxychalcone), piceatannol (3,5,3′,4′-Tetrahydroxy-trans-stilbene), isoliquiritigenin (4,2′,4′-Trihydroxychalcone), fisetin (3,7,3′,4′-Tetrahyddroxyflavone), quercetin (3,5,7,3′,4′-Pentahydroxyflavone), Deoxyrhapontin (3,5-Dihydroxy-4′-methoxystilbene 3-O-β-D-glucoside); trans-Stilbene; Rhapontin (3,3′,5-Trihydroxy-4′-methoxystilbene 3-O-β-D-glucoside); cis-Stilbene; Butein (3,4,2′,4′-Tetrahydroxychalcone);

3,4,2′4′6′-Pentahydroxychalcone; Chalcone; 7,8,3′,4′-Tetrahydroxyflavone; 3,6,2′,3′-Tetrahydroxyflavone; 4′-Hydroxyflavone; 5,4′-Dihydroxyflavone 5,7-Dihydroxyflavone; Morin (3,5,7,2′,4′- Pentahydroxyflavone); Flavone; 5-Hydroxyflavone; (−)-Epicatechin (Hydroxy Sites: 3,5,7,3′,4′); (−)-Catechin (Hydroxy Sites: 3,5,7,3′,4′); (−)-Gallocatechin (Hydroxy Sites: 3,5,7,3′,4′,5′) (+)-Catechin (Hydroxy Sites: 3,5,7,3′,4′); 5,7,3′,4′,5′-pentahydroxyflavone; Luteolin (5,7,3′,4′-Tetrahydroxyflavone); 3,6,3′,4′-Tetrahydroxyflavone; 7,3′,4′,5′-Tetrahydroxyflavone; Kaempferol (3,5,7,4′-Tetrahydroxyflavone); 6-Hydroxyapigenin (5,6,7,4′-Tetrahydoxyflavone); Scutellarein); Apigenin (5,7,4′-Trihydroxyflavone); 3,6,2′,4′-Tetrahydroxyflavone; 7,4′-Dihydroxyflavone; Daidzein (7,4′-Dihydroxyisoflavone); Genistein (5,7,4′-Trihydroxyflavanone); Naringenin (5,7,4′-Trihydroxyflavanone); 3,5,7,3′,4′-Pentahydroxyflavanone; Flavanone; Pelargonidin chloride (3,5,7,4′-Tetrahydroxyflavylium chloride); Hinokitiol (b-Thujaplicin; 2-hydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one); L-(+)-Ergothioneine ((S)-a-Carboxy-2,3-dihydro-N,N,N-trimethyl-2-thioxo-1H-imidazole-4-ethanaminium inner salt); Caffeic Acid Phenyl Ester; MCI-186 (3-Methyl-1-phenyl-2-pyrazolin-5-one); HBED (N,N′-Di-(2-hydroxybenzyl) ethylenediamine-N,N′-diacetic acid-H2O); Ambroxol (trans-4-(2-Amino-3,5-dibromobenzylamino) cyclohexane-HCl; and U-83836E ((−)-2-((4-(2,6-di-1-Pyrrolidinyl-4-pyrimidinyl)-1-piperzainyl)methyl)-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol.2HCl). Analogs and derivatives thereof can also be used.

[0097] In some embodiments the sirtuin pathway activator is an AMPK pathway activator. AMPK activity can be determined by any means known in the art, such as, e.g., measuring AMPK phosphorylation via an ELISA assay or by Western blot. The AMPK pathway activator can be a biguanide. Examples of biguanides include and are not limited to metformin, buformin, phenformin, proguanil or the like.

[0098] In some embodiments, the sirtuin pathway activator (e.g., AMPK pathway activator) is a polyphenol. Exemplary polyphenols include, e.g., chlorogenic acid, resveratrol, caffeic acid, piceatannol, ellagic acid, epigallocatechin gallate (EGCG), stilbene, hydroxycinnamic acid, grape seed extract, or any analog thereof. In some embodiments, the sirtuin pathway activator is resveratrol, an analog thereof, or a metabolite thereof. For example, the activator can be pterostilbene or a small molecule analog of resveratrol. Examples of small molecule analogs of resveratrol are described in U.S. Patent Application Nos. 20070014833, 20090163476, and 20090105246, which are incorporated herein by reference in its entirety.

[0099] The polyphenol can be a substantially homogeneous population of polyphenols. The polyphenol can be one type of polyphenol, wherein the composition can exclude all other types of polyphenols. In some embodiments, an invention method comprises administration one type of polyphenol, and exclude all other types of polyphenols. In some embodiments, an invention method comprises administration of two, three, or four types of polyphenols, and exclude all other types of polyphenols. In some embodiments, an invention method comprises administration of 1, 2, 3, or 4 types of polyphenols and less than 0.1, 0.5, 1, or 2% of any other types of polyphenols.

[00100] Sirtuin pathway activators can also include PDE inhibitors. PDE inhibitors can include non-specific PDE inhibitors. PDE inhibitors can be naturally occurring or non-naturally occurring (e.g. manufactured), and may be provided in the form of a natural source comprising the PDE inhibitor, or an extract thereof (e.g. purified). Examples of non-specific PDE inhibitors include, but are not limited to, caffeine, theophylline, theobromine, 3-isobutyl-1- methylxanthine (IBMX), pentoxifylline (3,7-dihydro-3,7-dimethyl-l-(5oxohexyl)-lH-purine-2, 6-dione), aminophylline, paraxanthine, and salts, derivatives, metabolites, catabolites, anabolites, precursors, and analogs thereof. Non-limiting examples of natural sources of PDE inhibitors include coffee, tea, guarana, yerba mate, cocoa, and chocolate (e.g. dark chocolate).

[00101] Sirtuin pathway activators can include irisin, quinic acid, cinnamic acid, ferulic acid, fucoxanthin, rosiglitazone, or any analog thereof. Sirtuin-pathway activators can also include isoflavones, pyroloquinoline (PQQ), quercetin, L-carnitine, lipoic acid, coenzyme Q10, pyruvate, 5-aminoimidazole-4-carboxamide ribotide (ALCAR), bezfibrate, oltipraz, and/or genistein.

[00102] Sirtuin pathway activators can agents that stimulate expression of the Fndc5, PGC1-α, or UCP1. The expression can be measured in terms of the gene or protein expression level. Alternatively, the sirtuin pathway activator can be irisin. Methods for increasing the level of irisin are described in Boström et al., "A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis," Nature, Jan 11, 2012.

[00103] Sirtuin pathway activators can include thiazolidinediones. Exemplary thiazolidinediones include, e.g., rosiglitazaone, pioglitazone, troglitazone, and any analogs thereof.

Exemplary PDE inhibitors

[00104] One or more methods of the invention can further comprise administering to a subject a PDE inhibitor. A PDE inhibitor can act as a sirtuin pathway activator. The PDE inhibitor can be selective or non-selective. The PDE inhibitor can exhibit selective inhibition to a PDE subclass, for example PDE 5. Examples of selective PDE inhibitors include inhibitors to PDE 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. A non-selective PDE inhibitor can be one that does not distinguish among sub-classes of phosphodiesterases. In addition, some non-selective PDE inhibitors may interact with more than one metabolic pathway. For examine, some non-selective PDE inhibitors may be xanthine derivatives and serve as adenosine antagonists and have unknown interactions with other metabolic pathways. Selective PDE inhibitors can be PDE inhibitors that exhibit preferential interaction with a selected PDE. For example, a PDE inhibitor can have a strong interaction with PDE 5, and very little interaction with other PDE sub-classes.

[00105] PDE inhibitors can be naturally occurring or non-naturally occurring (e.g. manufactured), and may be provided in the form of a natural source comprising the PDE inhibitor, or an extract thereof (e.g. purified). In some embodiments, the PDE inhibitor is a non-specific PDE inhibitor. Examples of non-specific PDE inhibitors include, but are not limited to, caffeine, theophylline, theobromine, 3-isobutyl-1- methylxanthine (IBMX), pentoxifylline (3,7-dihydro-3,7-dimethyl-l-(5oxohexyl)-lH-purine-2, 6-dione), aminophylline, paraxanthine, and salts, derivatives, metabolites, catabolites, anabolites, precursors, and analogs thereof. The PDE inhibitor can be sourced from a natural source of PDE inhibitors. Non-limiting examples of natural sources of PDE inhibitors include coffee, tea, guarana, yerba mate, cocoa, and chocolate (e.g. dark chocolate).

[00106] Any agents that selectively and negatively regulate a PDE subclass, such as PDE 5, expression or activity can be used as selective PDE inhibitors in the compositions and methods of the invention.

[00107] For example, a selective PDE inhibitor alternatively can be an agent that exhibits a 50% inhibitory concentration (IC50) with respect to a PDE subclass, such as PDE 5, that is at least at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 1000-fold, at least 10,000-fold lower than the inhibitor's IC50 with respect to one, two, three, or more other PDE subclasses. In some embodiment, a selective PDE inhibitor can be an agent that exhibits a 50% inhibitory concentration (IC50) with respect to a PDE subclass, such as PDE 5, that is at least at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least

1000-fold, at least 10,000-fold, or more, lower than the inhibitor's IC50 with respect to all other PDE subclasses.

[00108] In one aspect, IC50 is a determination of the concentration at which 50% of a given PDE is inhibited in a cell-based assay. IC50 determinations can be accomplished using any conventional techniques known in the art. In general, an IC50 can be determined by measuring the activity of a given enzyme in the presence of a range of concentrations of the inhibitor under study. The experimentally obtained values of enzyme activity then are plotted against the inhibitor concentrations used. The concentration of the inhibitor that shows 50% enzyme activity (as compared to the activity in the absence of any inhibitor) is taken as the "IC50" value. Analogously, other inhibitory concentrations can be defined through appropriate determinations of activity. For example, in some settings it can be desirable to establish a 90% inhibitory concentration, i.e., IC90, etc.

[00109] Methods for measuring selectivity of PDE inhibitors are described in "Phosphodiesterase-5 Gln-817 is critical for cGMP, vardenafil, or sildenafil affinity: its orientation impacts cGMP but not cAMP affinity" by Zoraghi (2006) and "Cyclic Nucleotide Phosphodiesterases: Molecular Regulation to Clinical Use" by Bender (2006) which are incorporated herein in its entirety by reference.

[00110] The subject biologically active agent may inhibit PDE activity with an IC50 value of about 100 nM or less, preferably about 50 nM, about 25 nM, about 10 nM, about 5nM, about 1 nM, 100 pM, 50 pM, 25 pM, 10 pM, 1 pM, or less, as ascertained in a cell-based assay or an in vitro kinase assay.

[00111] In some embodiments, the PDE inhibitor is a PDE1 inhibitor such as nimodipine, vinopocetine, and IC224. The PDE 1 inhibitor can interact with PDE1, which is a Ca2+/calmodulin-regulated phosphodiesterase that serves to degrade both cAMP and cGMP. The vinopocetine can be derived from periwinkle extract, and it can serve as a cerebrovascularvasodilator. Vinopocetine can be in the form of a dietary supplement.

[00112] In other embodiments, the PDE inhibitor is a PDE3 inhibitor such as meribendan, arinone and cilostamide. The PDE inhibitor can be a PDE4 inhibitor, such as apremilast, mesembrine, ibudilast, piclamilast, luteolin, roflumilast, cilomilast, diazepam, rolipram and YM796. The PDE inhibitor can be a PDE4 inhibitor, such as rolipram and YM796. The PDE4 inhibitor can interact with PDE 4, which is a cAMP-specific phosphodiesterase that predominates in immune cells.

[00113] In some embodiments, the PDE inhibitor is a PDE5 specific inhibitor. icariin, sildenafil, tadalafil, vardenafil, avanafil, iodenafil, mirodenafil, udenafil, and zaprinast In some embodiments, the PDE5 inhibitor is icariin. In some embodiments, the PDE5 inhibitor is sildenafil. In some embodiments, the PDE5 inhibitor is tadalafil. In some embodiments, the PDE5 inhibitor is vardenafil. In some embodiments, the PDE5 inhibitor is avanafil. In some embodiments, the PDE5 inhibitor is iodenafil. In some embodiments, the PDE5 inhibitor is mirodenafil. In some embodiments, the PDE5 inhibitor is udenafil. In some embodiments, the PDE5 inhibitor is zaprinast. The PDE 5 inhibitor can interact with PDE 5, which is a cGMP-specific PDE. Increases in cGMP signaling can increase mitochondrial biogenesis both in vitro and in vivo. A PDE 5 inhibitor can increase nitric oxide signaling and be an effective vasodilator. Other examples of PDE 5 inhibitors are described in U.S. Patent No. 5,250,534 and 6,469,012, which are each incorporated by reference in their entirety.

[00114] In some embodiments, a PDE inhibitor is administered in place of or in addition to resveratrol or other sirtuin pathway activator. In some embodiments, compositions comprising one or more components described herein comprise a PDE inhibitor in place of or in addition to resveratrol or other sirtuin pathway activator.

[00115] A method of reducing and/or preventing hepatic steatosis in a subject can comprise co-administering to the subject (a) a sirtuin pathway activator, (b) a branched chain amino acid in free amino acid form or metabolite thereof, and optionally (c) a PDE inhibitor. In some embodiments, the sirtuin pathway activator is an AMPK activator. In some embodiments, the AMPK activator is a biguanide. In particular embodiments, the biguanide is metformin. In some embodiments, the branched chain amino acid in free amino acid form is leucine. In some embodiments, the PDE inhibitor is a PDE5 inhibitor. Exemplary PDE5 inhibitors are described herein. The PDE5 inhibitor can be, for example, icariin. The PDE5 inhibitor can be any one of sildenafil, tadalafil, vardenafil, udenafil, or zaprinast. In some embodiments, metformin is co-administered with a metabolite of leucine. In particular embodiments, the metabolite of leucine is HMB. In particular embodiments, the metabolite of leucine is KIC. In some embodiments, more than one sirtuin pathway activator is co-administered with a branched chain amino acid in free amino acid form or metabolite thereof (e.g., co-administered with leucine in free amino acid form, HMB, or KIC). For instance, a method of the invention may comprise co-administering a biguanide, a polyphenol, and a branched chain amino acid in free amino acid form or metabolite thereof. For example, a method of the invention can comprise co-administering to a subject metformin, resveratrol,

and leucine in free amino acid form. A method of the invention can comprise co-administering to a subject metformin, resveratrol, and HMB. A method of the invention can comprise co-administering to a subject metformin, resveratrol, and KIC. In some embodiments, a method of the invention can comprise co-administering to a subject metformin, leucine, and sildenafil.

[00116] Another method of reducing and/or preventing hepatic steatosis in a subject can comprise co-administering to the subject (a) a PDE inhibitor, and (b) a branched chain amino acid in free amino acid form or a metabolite thereof. In some embodiments, the branched chain amino acid in free amino acid form is leucine. In some embodiments, a metabolite of leucine is co-administered. The metabolite can be, e.g., HMB or KIC. In some embodiments, the PDE inhibitor is a PDE5 inhibitor. Exemplary PDE5 inhibitors are described herein. The PDE5 inhibitor can be, for example, icariin. The PDE5 inhibitor can be any one of sildenafil, tadalafil, vardenafil, udenafil, or zaprinast.

[00117] In some embodiments, the particular choice of compounds depends upon the diagnosis of the attending physicians and their judgment of the condition of the individual and the appropriate treatment protocol. The compounds are optionally administered concurrently (e.g., simultaneously, essentially simultaneously or within the same treatment protocol) or sequentially, depending upon the nature of the disease, disorder, or condition, the condition of the individual, and the actual choice of compounds used. In certain instances, the determination of the order of administration, and the number of repetitions of administration of each therapeutic agent during a treatment protocol, is based on an evaluation of the disease being treated and the condition of the individual.

[00118] In some embodiments, therapeutically-effective dosages vary when the drugs are used in treatment combinations. Methods for experimentally determining therapeutically-effective dosages of drugs and other agents for use in combination treatment regimens are described in the literature.

[00119] In some embodiments of the combination therapies described herein, dosages of the co-administered compounds vary depending on the type of co-drug employed, on the specific drug employed, on the disease or condition being treated and so forth. In addition, when co-administered with one or more biologically active agents, the compound provided herein is optionally administered either simultaneously with the biologically active agent(s), or sequentially. In certain instances, if administered sequentially, the attending physician will

decide on the appropriate sequence of therapeutic compound described herein in combination with the additional therapeutic agent.

[00120] The multiple therapeutic agents are optionally administered in any order or even simultaneously. If simultaneously, the therapeutic agents are optionally provided in a single, unified form, or in multiple forms (by way of example only, either as a single pill or as two separate pills). In certain instances, one of the therapeutic agents is optionally given in multiple doses. In other instances, both are optionally given as multiple doses. If not simultaneous, the timing between the multiple doses is any suitable timing, e.g, from more than zero weeks to less than four weeks. In addition, the combination methods, compositions and formulations are not to be limited to the use of only two agents; the use of multiple therapeutic combinations is also envisioned (including two or more compounds described herein).

[00121] In certain embodiments, a dosage regimen to treat, prevent, or ameliorate the condition(s) for which relief is sought, is modified in accordance with a variety of factors. These factors include the disorder from which the subject suffers, as well as the age, weight, sex, diet, and medical condition of the subject. Thus, in various embodiments, the dosage regimen actually employed varies and deviates from the dosage regimens set forth herein.

[00122] In some embodiments, the pharmaceutical agents that make up the combination therapy described herein are provided in a combined dosage form or in separate dosage forms intended for substantially simultaneous administration. In certain embodiments, the pharmaceutical agents that make up the combination therapy are administered sequentially, with either therapeutic compound being administered by a regimen calling for two-step administration. In some embodiments, two-step administration regimen calls for sequential administration of the active agents or spaced-apart administration of the separate active agents. In certain embodiments, the time period between the multiple administration steps varies, by way of non-limiting example, from a few minutes to several hours, depending upon the properties of each pharmaceutical agent, such as potency, solubility, bioavailability, plasma half-life and kinetic profile of the pharmaceutical agent.

[00123] In various other embodiments, compositions for use in practicing one or more methods of the invention are formulated such that they do not contain (or exclude) one or more of the following ingredients: caffeine, green tea extract or extracts from guarana seed or guarana plants. Compositions can also be formulated such that they are substantially free of high glycemic index carbohydrate, such as, e.g., simple carbohydrates, including sugars such

as but not limited to sucrose, glucose, dextrose, maltose, fructose, and galactose, among others.

Dosing amounts

[00124] The amount of pharmaceutical agent, or any other component used in a combination composition described herein, can be an amount that is therapeutically effective. The amount of pharmaceutical agent, or any other component used in a combination composition described herein, can be an amount that is sub-therapeutic. In some embodiments, using sub-therapeutic amounts of an agent or component can reduce the side-effects of the agent. Use of sub-therapeutic amounts can still be effective, particularly when used in synergy with other agents or components.

[00125] A sub-therapeutic amount of the agent or component can be such that it is an amount below which would be considered therapeutic. For example, FDA guidelines can suggest a specified level of dosing to treat a particular condition, and a sub-therapeutic amount would be any level that is below the FDA suggested dosing level. The sub-therapeutic amount can be about 1, 5, 10, 15, 20, 25, 30, 35, 50, 75, 90, or 95% less than the amount that is considered to be a therapeutic amount. The therapeutic amount can be assessed for individual subjects, or for groups of subjects. The group of subjects can be all potential subjects, or subjects having a particular characteristic such as age, weight, race, gender, or physical activity level.

[00126] In the case of metformin, a physician suggested starting dose can be 1000 mg daily, with subject specific dosing having a range of 500 mg to a maximum of 2550 mg daily (metformin hydrochloride extended-release tablets label www.accessdata.fda.gov/drugsatfda_docs/label/2008/021574s010lbl.pdf). The particular dosing for a subject can be determined by a clinician by titrating the dose and measuring the therapeutic response. The therapeutic dosing level can be determined by measuring fasting plasma glucose levels and measuring glycosylated hemoglobin. A sub-therapeutic amount can be any level that would be below the recommended dosing of metformin. For example, if a subject's therapeutic dosing level is determined to be 700 mg daily, a dose of 600 mg would be a sub-therapeutic amount. Alternatively, a sub-therapeutic amount can be determined relative to a group of subjects rather than an individual subject. For example, if the average therapeutic amount of metformin for subjects with weights over 300 lbs is 2000 mg, then a sub-therapeutic amount can be any amount below 2000 mg. In some embodiments, the dosing can be recommended by a healthcare provider including, but not limited to a patient's

physician, nurse, nutritionist, pharmacist, or other health care professional. A health care professional may include a person or entity that is associated with the health care system. Examples of health care professionals may include surgeons, dentists, audiologists, speech pathologists, physicians (including general practitioners and specialists), physician assistants, nurses, midwives, pharmaconomists/pharmacists, dietitians, therapists, psychologists, physical therapists, phlebotomists, occupational therapists, optometrists, chiropractors, clinical officers, emergency medical technicians, paramedics, medical laboratory technicians, radiographers, medical prosthetic technicians social workers, and a wide variety of other human resources trained to provide some type of health care service.

[00127] The invention provides for compositions that can comprise any combination of agents, such as leucine, metabolites of leucine, such as HMB or KIC, PDE inhibitors (e.g., PDE5 inhibitors such as, by way of example only, sildenafil, icariin), sirtuin pathway activators such as, e.g., AMPK activators, polyphenols such as, e.g., resveratrol, and sirtuin activators, that have been isolated from one or more sources. The agents can be isolated from natural sources or created from synthetic sources and then enriched to increase the purity of the components. For example, sildenafil can be created from a synthetic source and then enriched by one or more purification methods. Additionally, leucine (e.g., free leucine), can be isolated from a natural source and then enriched by one or more separations. The isolated and enriched components, such as, e.g., metformin, resveratrol, icariin, sildenafil, free leucine, HMB, and KIC can then be formulated for administration to a subject in any combination.

Dosage of branched chain amino acid

[00128] Any of the invention methods can comprise administering a dose of a branched chain amino acid in free amino acid form, and/or a dose of a metabolite thereof. The dose of the branched chain amino acid in free amino acid form, or metabolite thereof, can be a therapeutic dose. The dose of the branched chain amino acid in free amino acid form or metabolite thereof can be a sub-therapeutic dose. A sub-therapeutic dose of leucine in free amino acid form can be about, less than about, or more than about 0.25 – 3.0 g (e.g. 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, or more g). A sub-therapeutic dose of leucine in free amino acid form can be about, less than about, or more than about 0.25 – 3.0 g/day (e.g. 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, or more g/day). In some embodiments, the method comprises administering less than 3.0 g leucine in free amino acid form per day. A sub-therapeutic dose of HMB can be about, less than about, or more than about 0.05 – 3.0 g (e.g.

0.05, 0.1, 0.2, 0.4, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, or more g). A sub-therapeutic dose of HMB can be about, less than about, or more than about 0.05 – 3.0 g/day (e.g. 0.05, 0.1, 0.2, 0.4, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, or more g/day). A sub-therapeutic dose of KIC can be about, less than about, or more than about 0.1 – 3.0 g (e.g. 0.1, 0.2, 0.4, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, or more g). A sub-therapeutic dose of KIC can be about, less than about, or more than about 0.1 – 3.0 g/day (e.g. 0.1, 0.2, 0.4, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, or more g/day).

[00129] Any of the invention methods can comprise administering a dose of metformin. The dose of metformin can be a therapeutic dose. The therapeutic dose can be above 500 mg, above 600 mg, above 700 mg, above 800 mg, above 900 mg, or above 1000 mg (1 g). A therapeutic dose of metformin can be about 1 g, about 1.1 g, about 1.2 g, about 1.3 g, about 1.4 g, about 1.5 g, about 1.6 g, about 1.7 g, about 1.8 g, about 1.9 g, about 2 g, or more than 2 g. The therapeutic dose can be above 500 mg/day, above 600 mg/day, above 700 mg/day, above 800 mg/day, above 900 mg/day, above 1000 mg/day (1 g/day). A therapeutic dose of metformin can be about 1 g/day, about 1.1 g/day, about 1.2 g/day, about 1.3 g/day, about 1.4 g/day, about 1.5 g/day, about 1.6 g/day, about 1.7 g/day, about 1.8 g/day, about 1.9 g/day, about 2 g/day, or more than 2 g/day. The dose of metformin can be a sub-therapeutic dose. The sub-therapeutic dose of metformin can be about 1-1000 mg, about 5-500 mg, about 10-100 mg, about 30-90 mg, about 50-70 mg, or about 62.5 mg. The sub-therapeutic dose of metformin can be, e.g., 1, 5, 10, 15, 25, 50, 125, 250, or 500 mg. The dose of metformin can be a sub-therapeutic dose. The sub-therapeutic dose of metformin can be about 40-70, 25-1000 mg/day, about 50-500 mg/day, about 100-500 mg/day. The sub-therapeutic dose of metformin can be about, less than about, or greater than about 1, 5, 10, 15, 25, 50, 60 125, 250, or 500 mg/day. The metformin can be administered as a unit dose. The unit dose of metformin can be 1, 5, 10, 15, 25, 50, 62.5, 125 or 250 mg.

Dosage of polyphenols

[00130] Any of the invention methods can comprise administering a dose of a polyphenol, e.g., resveratrol. The dose may be administered daily. The dose can be a low dose, a medium dose, or a high dose. A low dose of resveratrol may comprise about, less than about, or more than about 0.5mg, 1 mg, 2.5mg, 5mg, 7.5mg, 10mg, 12.5mg, 15mg, 20mg, 25mg, 50mg, 75mg, 100mg, or more; a medium dose of resveratrol may comprise about, less than about, or more than about 20mg, 25mg, 50mg, 75mg, 100mg, 125mg, 150mg, 175mg, 200mg, 250mg, or more; and a high dose of resveratrol may comprise about, less than about, or more than about 150mg, 175mg, 200mg, 225mg, 250mg, 300mg, 350mg, 400mg, or more.

A daily low dose of resveratrol may comprise about, less than about, or more than about 0.5mg, 1 mg, 2.5mg, 5mg, 7.5mg, 10mg, 12.5mg, 15mg, 20mg, 25mg, 50mg, 75mg, 100mg, or more; a daily medium dose of resveratrol may comprise about, less than about, or more than about 20mg, 25mg, 50mg, 75mg, 100mg, 125mg, 150mg, 175mg, 200mg, 250mg, or more; and a daily high dose of resveratrol may comprise about, less than about, or more than about 150mg, 175mg, 200mg, 225mg, 250mg, 300mg, 350mg, 400mg, or more.

Dosage of thiazoladinediones

[00131] Any of the invention methods can comprise administering a dose of a thiazoladinedione. Exemplary thiazoladinediones are described herein. The dose of thiazoladinedione can be a therapeutic dose or a sub-therapeutic dose. The thiazoladinedione can be rosiglitazone. The dose of the rosiglitazone can be at least 100 μg. The dose of the rosiglitazone can be about or less than about 4 mg. The dose of the rosiglitazone can be 100 μg - 4 mg, can be 200 μg - 2 mg, can be 400 μg to 1000 μg. The thiazoladinedione can be pioglitazone. The dose of the pioglitazone can be at least 100 μg. The dose of the pioglitazone can be about or less than about 15 mg. The dose of the pioglitazone can be 100 μg - 45 mg, can be 200 μg - 10 mg, can be 400 μg to 5 mg, can be 500 μg to 1 mg.

Dosages of PDE inhibitor

[00132] In some embodiments, a composition comprises an amount of a selective PDE inhibitor (e.g., PDE-5 inhibitor including but not limited to sildenafil or icariin). The amount of a PDE inhibitor may be a subtherapeutic amount, and/or an amount that is synergistic with one or more other compounds in the composition or one or more of the compounds administered simultaneously or in close temporal proximity with the composition. In some embodiments, the PDE inhibitor is administered in a low dose, a medium dose, or a high dose, which describes the relationship between two doses, and generally do not define any particular dose range.

[00133] A dose of sildenafil can be about or less than about 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 40, 60, 80, or 100 mg of sildenafil. A dose of sildenafil can be about 0.05-100, 1-50, or 5-20 mg of sildenafil. A dose of icariin can be about or less than about 1, 10, 12.5, 20, 25, 50, 100, 150, 300, 400, 500, 750, 1000, 1500, or 2000 mg of icariin. A dose of icariin can be about 1-100, 5-60, or 10-30 mg of icariin. A dose of tadalafil can be about 0.01, 0.05, 0.1, 0.5, 1, 15, 2, 2.5, 5, 10, 15, or 20 mg. A dose of tadalafil can be about 0.1-50, 0.5-20, or 1-10 mg. A dose of vardenafil can be about 0.01, 0.05, 0.1, 0.5, 1, 15, 2, 2.5, 5, 10, 15, or 20 mg. A dose of vardenafil can be about 0.1-50, 0.5-20, or 1-10 mg. A dose of avanafil can be about 1, 10,

20, 50, 100, 150, 300, 400, 500, 750, 1000, 1500, or 2000 mg. A dose of avanafil can be about 1-100, 5-50, or 10-40 mg. A dose of lodenafil can be about 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 50, 80, 100, 150, 300, 400, 500, 750, 1000, 1500, or 2000 mg. A dose of lodenafil can be about 0.05-100, 1-50, or 5-20 mg. A dose of mirodenafil can be about 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 50, 80, 100, 150, 300, 400, 500, 750, 1000, 1500, or 2000 mg. A dose of mirodenafilcan be about 0.05-100, 1-50, or 5-20 mg. A dose of udenafil can be about 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 50, 80, 100, 150, 300, 400, 500, 750, 1000, 1500, or 2000 mg. A dose of udenafil can be about 0.05-100, 1-50, or 5-20 mg.

[00134] A daily dose of sildenafil can be about or less than about 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 40, 60, 80, or 100 mg of sildenafil. A daily dose of sildenafil can be about 0.05-100, 1-50, or 5-20 mg of sildenafil. A daily dose of icariin can be about or less than about 1, 10, 20, 25, 50, 100, 150, 300, 400, 500, 750, 1000, 1500, or 2000 mg of icariin. A daily dose of icariin can be about 1-100, 10-75, or 20-40 mg of icariin. A daily dose of tadalafil can be about 0.01, 0.05, 0.1, 0.5, 1, 15, 2, 2.5, 5, 10, 15, or 20 mg. A daily dose of tadalafil can be about 0.1-50, 0.5-20, or 1-10 mg. A daily dose of vardenafil can be about 0.01, 0.05, 0.1, 0.5, 1, 15, 2, 2.5, 5, 10, 15, or 20 mg. A daily dose of vardenafil can be about 0.1-50, 0.5-20, or 1-10 mg. A daily dose of avanafil can be about 1, 10, 20, 50, 100, 150, 300, 400, 500, 750, 1000, 1500, or 2000 mg. A daily dose of avanafil can be about 1-100, 5-50, or 10-40 mg. A daily dose of lodenafil can be about 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 50, 80, 100, 150, 300, 400, 500, 750, 1000, 1500, or 2000 mg. A daily dose of lodenafil can be about 0.05-100, 1-50, or 5-20 mg. A daily dose of mirodenafil can be about 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 50, 80, 100, 150, 300, 400, 500, 750, 1000, 1500, or 2000 mg. A daily dose of mirodenafilcan be about 0.05-100, 1-50, or 5-20 mg. A daily dose of udenafil can be about 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 50, 80, 100, 150, 300, 400, 500, 750, 1000, 1500, or 2000 mg. A daily dose of udenafil can be about 0.05-100, 1-50, or 5-20 mg.

[00135] Another aspect of the invention provides compositions comprising synergizing amounts of PDE-5 inhibitor, such as, e.g., sildenafil, icariin, tadalafil, vardenafil, avanafil, lodenafil, mirodenafil and udenafil, in combination with leucine, HMB, KIC, and/or resveratrol. Thus, one embodiment provides a composition comprising leucine in an amount of about 0.25 to about 3.0 g and sildenafil in an amount of about 0.05 to about 100 mg. Another embodiment provides a composition comprising HMB in an amount of 0.40 – 3.0 g and sildenafil in an amount of about 0.05 – 100 mg. Another embodiment provides for a composition comprising leucine in an amount of about 0.25 – about 3.0 g, HMB in an amount

of about 0.10 to about 3.0 g and sildenafil in an amount of about 0.05 - 100 mg. In compositions comprising a PDE inhibitor or methods comprising administration of a PDE inhibitor (separately from or concurrently with one or more other components), the PDE inhibitor may be provided in an amount that produces a peak plasma concentration of about, less than about, or more than about 0.1, 1, 5, 10, 25, 50, 100, 500, 1000, 2500, 5000, 10000, or more nM.

[00136] Thus, embodiments of a method of the invention comprise co-administering leucine in free amino acid form in an amount of about 0.25 to about 3.0 g/day and resveratrol in an amount of about 50 to about 500 mg/day. Other embodiments comprise co-administering HMB in an amount of 0.10 – 3.0 g/day and resveratrol in an amount of about 50 – 500 mg/day. Other embodiments comprise co-administering KIC in an amount of 0.20 – 3.0 g/day and resveratrol in an amount of about 50 – 500 mg/day (or 50 to 500 mg/day). Other embodiments comprise co-administering leucine in free amino acid form in an amount of about 0.5 – about 3.0 g/day, HMB in an amount of about 0.10 to about 3.0 g/day and resveratrol in an amount of about 50 to about 500 mg/day. Other embodiments comprise co-administering leucine in free amino acid form in an amount of about 0.5 – about 3.0 g/day, KIC in an amount of about 0.20 to about 3.0 g/day and resveratrol in an amount of about 50 to about 500 mg/day. Other embodiments comprise co-administering leucine in free amino acid form in an amount of about 0.25 – about 3.0 g/day, HMB in an amount of about 0.10 to about 3.0 g/day, KIC in an amount of about 0.20 to about 3.0 g/day, and resveratrol in an amount of about 50 to about 500 mg/day.

[00137] Some embodiments of a method of the invention comprise co-administering leucine in free amino acid form in an amount of about 0.25 to about 3.0 g/day and metformin in an amount of about 25 to about 500 mg/day. Other embodiments comprise co-administering HMB in an amount of 0.10 – 3.0 g/day and metformin in an amount of about 25 – 500 mg/day. Other embodiments comprise co-administering KIC in an amount of 0.20 – 3.0 g/day and metformin in an amount of about 25 – 500 mg/day. Other embodiments comprise co-administering leucine in free amino acid form in an amount of about 0.25 – about 3.0 g/day, HMB in an amount of about 0.40 to about 3.0 g/day and metformin in an amount of about 25 to about 500 mg/day. Other embodiments comprise co-administering leucine in free amino acid form in an amount of about 0.25 – about 3.0 g/day, KIC in an amount of about 0.20 to about 3.0 g/day and metformin in an amount of about 25 to about 500 mg/day. Other embodiments comprise co-administering leucine in free amino acid form

in an amount of about 0.25 – about 3.0 g/day, HMB in an amount of about 0.10 to about 3.0 g/day, KIC in an amount of about 0.20 to about 3.0 g/day, and metformin in an amount of about 25 to about 500 mg/day. In some embodiments, such methods further comprise co-administering resveratrol in an amount of about 50 to about 500 mg/day.

[00138] Some embodiments of a method of the invention comprise co-administering leucine in free amino acid form in an amount of about 0.25 to about 3.0 g/day and a PDE5 inhibitor in an amount of about 0.05 to about 2000 mg/day. Other embodiments comprise co-administering HMB in an amount of 0.10 – 3.0 g/day and a PDE5 inhibitor in an amount of about 0.05 to about 2000 mg/day. Other embodiments comprise co-administering KIC in an amount of 0.20 – 3.0 g/day and a PDE5 inhibitor in an amount of about 0.05 to about 2000 mg/day. Other embodiments comprise co-administering leucine in free amino acid form in an amount of about 0.25 – about 3.0 g/day, HMB in an amount of about 0.40 to about 3.0 g/day and a PDE5 inhibitor in an amount of about 0.05 to about 2000 mg/day. Other embodiments comprise co-administering leucine in free amino acid form in an amount of about 0.25 – about 3.0 g/day, KIC in an amount of about 0.20 to about 3.0 g/day and a PDE5 inhibitor in an amount of about 0.05 to about 2000 mg/day. Other embodiments comprise co-administering leucine in free amino acid form in an amount of about 0.25 – about 3.0 g/day, HMB in an amount of about 0.10 to about 3.0 g/day, KIC in an amount of about 0.20 to about 3.0 g/day, and a PDE5 inhibitor in an amount of about 0.05 to about 2000 mg/day. The PDE5 inhibitor can be any of the PDE5 inhibitors described herein. The daily dose of the PDE5 inhibitor can be as described herein. In some embodiments, such methods further comprise co-administering resveratrol in an amount of about 50 to about 500 mg/day or co-administering metformin in an amount of about 25 and about 500 mg/day.

[00139] Some embodiments of a method of the invention comprise co-administering leucine in free amino acid form in an amount of about 0.25 to about 3.0 g/day, metformin in an amount of about 25 to about 500 mg/day, and a PDE5 inhibitor in an amount of about 0.05 to about 2000 mg/day. In some embodiments, the PDE5 inhibitor is sildenafil. Other embodiments comprise co-administering HMB in an amount of 0.10 – 3.0 g/day, metformin in an amount of about 25 – 500 mg/day, and a PDE5 inhibitor, e.g. sildenafil, in an amount of about 0.05 to about 2000 mg/day. Other embodiments comprise co-administering KIC in an amount of 0.20 – 3.0 g/day, metformin in an amount of about 25 – 500 mg/day, and a PDE5 inhibitor, e.g. sildenafil, in an amount of about 0.05 to about 2000 mg/day. Other embodiments comprise co-administering leucine in free amino acid form in an amount of

about 0.25 – about 3.0 g/day, HMB in an amount of about 0.40 to about 3.0 g/day, metformin in an amount of about 25 to about 500 mg/day, and a PDE5 inhibitor, e.g. sildenafil, in an amount of about 0.05 to about 2000 mg/day. Other embodiments comprise co-administering leucine in free amino acid form in an amount of about 0.25 – about 3.0 g/day, KIC in an amount of about 0.20 to about 3.0 g/day, metformin in an amount of about 25 to about 500 mg/day, and a PDE5 inhibitor, e.g. sildenafil, in an amount of about 0.05 to about 2000 mg/day. Other embodiments comprise co-administering leucine in free amino acid form in an amount of about 0.25 – about 3.0 g/day, HMB in an amount of about 0.10 to about 3.0 g/day, KIC in an amount of about 0.20 to about 3.0 g/day, metformin in an amount of about 25 to about 500 mg/day, and a PDE5 inhibitor, e.g. sildenafil, in an amount of about 0.05 to about 2000 mg/day. In some embodiments, such methods further comprise co-administering resveratrol in an amount of about 50 to about 500 mg/day.

[00140] Any of the above agents can be administered in unit doses. Any of the above agents in the amounts described herein can be administered in unit doses. A unit dose can be a fraction of the daily dose, such as the daily dose divided by the number of unit doses to be administered per day. A unit dose can be a fraction of the daily dose that is the daily dose divided by the number of unit doses to be administered per day and further divided by the number of unit doses (e.g. tablets) per administration. The number of unit doses per administration may be about, less than about, or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. The number of doses per day may be about, less than about, or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. The number of unit doses per day may be determined by dividing the daily dose by the unit dose, and may be about, less than about, or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, 20, or more unit doses per day. For example, a unit dose can be about the daily dose or about 1/2, 1/3, 1/4, 1/5, 1/6, 1/7, 1/8, 1/9, 1/10 of the daily dose. A unit dose can be about one-third of the daily amount and administered to the subject three times daily. A unit dose can be about one-half of the daily amount and administered to the subject twice daily. A unit dose can be about one-fourth of the daily amount with two unit doses administered to the subject twice daily. In some embodiments, a unit dose comprises about, less than about, or more than about 50 mg resveratrol. In some embodiments, a unit dose comprises about, less than about, or more than about 50 mg metformin. In some embodiments, a unit dose comprises about, less than about, or more than about 550 mg leucine. In some embodiments, a unit dose comprises about, less than about, or more than about 200 mg of one or more leucine metabolites. In some

embodiments, a unit dose (e.g. a unit dose comprising leucine) is administered as two unit doses two times per day. In some embodiments, a unit dose (e.g. a unit dose comprising one or more leucine metabolites, such as HMB) is administered as one unit dose two timer per day.

[00141] The agents described herein (e.g., branched chain amino acid in free amino acid form, metabolites thereof, sirtuin pathway activators, PDE inhibitors) can be administered to a subject orally or by any other methods. Methods of oral administration include administering the composition as a liquid, a solid, or a semi-solid that can be taken in the form of a dietary supplement or a food stuff.

[00142] The agents described herein can be co-administered. The agents can be administered simultaneously, e.g., in a single composition, or can be administered sequentially. The agents can be administered sequentially within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 30, 60, 90, or 120 minutes from each other.

[00143] The agents described herein can be administered periodically. For example, the agents can be administered one, two, three, four times a day, or even more frequent. The subject can be administered every 1, 2, 3, 4, 5, 6 or 7 days. In some embodiments, the agents are administered three times daily. The administration can be concurrent with meal time of a subject. The period of treatment or diet supplementation can be for about 1, 2, 3, 4, 5, 6, 7, 8, or 9 days, 2 weeks, 1-11 months, or 1 year, 2 years, 5 years or even longer. In some embodiments, the subject is administered the agents for six weeks or more. In some embodiments of the invention, the dosages that are administered to a subject can change or remain constant over the period of treatment. For example, the daily dosing amounts can increase or decrease over the period of administration.

[00144] The length of the period of administration and/or the dosing amounts can be determined by a physician, a nutritionist, or any other type of clinician. The physician, nutritionist, or clinician can observe the subject's response to the administered compositions and adjust the dosing based on the subject's performance. For example, dosing for subjects that show reduced effects in energy regulation can be increased to achieve desired results.

Compositions

[00145] Any of the agents described herein can be administered to the subject in one or more compositions. A composition for use in practicing any of the methods of the invention can comprise any combination of the agents described herein. For example, an invention

composition can comprise one, two, three, four, or more than four of the agents described herein.

[00146] Compositions described herein can be compounded into a variety of different dosage forms. For example, compositions can be formulated for oral administration, e.g., as a tablet, chewable tablet, caplets, capsule, soft gelatin capsules, lozenges or solution. Compositions can be formulated as a nasal spray or for injection when in its solution form. In some embodiments, the composition is a liquid composition suitable for oral consumption.

[00147] In some embodiments, the agents are formulated into a composition suitable for oral administration. Compositions of the invention suitable for oral administration can be presented as discrete dosage forms, such as capsules, cachets, or tablets, or liquids or aerosol sprays each containing a predetermined amount of an active ingredient as a powder or in granules, a solution, or a suspension in an aqueous or non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion, including liquid dosage forms (e.g., a suspension or slurry), and oral solid dosage forms (e.g., a tablet or bulk powder). Oral dosage forms may be formulated as tablets, pills, dragees, capsules, emulsions, lipophilic and hydrophilic suspensions, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by an individual or a patient to be treated. Such dosage forms can be prepared by any of the methods of formulation. For example, the active ingredients can be brought into association with a carrier, which constitutes one or more necessary ingredients. Capsules suitable for oral administration include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. Optionally, the inventive composition for oral use can be obtained by mixing a composition a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP).In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation. For example, a tablet can be prepared by compression

or molding, optionally with one or more accessory ingredients. Compressed tablets can be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as powder or granules, optionally mixed with an excipient such as, but not limited to, a binder, a lubricant, an inert diluent, and/or a surface active or dispersing agent. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

[00148] The compositions may be in liquid form. Exemplary liquid forms, which may be formulated for oral administration or for administration by injection, include aqueous solution, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil as well as elixirs and similar pharmaceutical vehicles. Suitable dispersing or suspending agents for aqueous suspensions include synthetic natural gums, such as tragacanth, acacia, alginate, dextran, sodium carboxymethyl cellulose, methylcellulose, polyvinylpyrrolidone or gelatin. A method of the invention may comprise administering to a subject a combination of an injectable composition and a composition for oral administration.

[00149] Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for reconstitution with water or other suitable vehicles before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters or ethyl alcohol); preservatives (e.g., methyl or propyl p-hydroxybenzoates or sorbic acid); and artificial or natural colors and/or sweeteners.

[00150] The preparation of pharmaceutical compositions of this invention can be conducted in accordance with generally accepted procedures for the preparation of pharmaceutical preparations. See, for example, Remington's Pharmaceutical Sciences 18th Edition (1990), E. W. Martin ed., Mack Publishing Co., PA. Depending on the intended use and mode of administration, it may be desirable to process the magnesium-counter ion compound further in the preparation of pharmaceutical compositions. Appropriate processing may include mixing with appropriate non-toxic and non-interfering components, sterilizing, dividing into dose units, and enclosing in a delivery device.

[00151] Embodiments of the invention further encompass anhydrous compositions and dosage forms comprising an active ingredient, since water can facilitate the degradation of

some compounds. For example, water may be added (e.g., 5%) in the arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. Anhydrous compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Compositions and dosage forms of the invention which contain lactose can be made anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected. An anhydrous composition may be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions may be packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastic or the like, unit dose containers, blister packs, and strip packs.

[00152] An agent described herein can be combined in an intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier can take a wide variety of forms depending on the form of preparation desired for administration. In preparing the compositions for an oral dosage form, any of the usual pharmaceutical media can be employed as carriers, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid preparations (such as suspensions, solutions, and elixirs) or aerosols; or carriers such as starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents can be used in the case of oral solid preparations, in some embodiments without employing the use of lactose. For example, suitable carriers include powders, capsules, and tablets, with the solid oral preparations. If desired, tablets can be coated by standard aqueous or nonaqueous techniques.

[00153] Some examples of materials which may serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free

water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

[00154] Binders suitable for use in dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, microcrystalline cellulose, and mixtures thereof.

[00155] Lubricants which can be used to form compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethylaureate, agar, or mixtures thereof. Additional lubricants include, for example, a syloid silica gel, a coagulated aerosol of synthetic silica, or mixtures thereof. A lubricant can optionally be added, in an amount of less than about 1 weight percent of the composition. Lubricants can be also be used in conjunction with tissue barriers which include, but are not limited to, polysaccharides, polyglycans, seprafilm, interceed and hyaluronic acid.

[00156] Disintegrants may be used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Too much of a disintegrant may produce tablets which may disintegrate in the bottle. Too little may be insufficient for disintegration to occur and may thus alter the rate and extent of release of the active ingredient(s) from the dosage form. Thus, a sufficient amount of disintegrant that is neither too little nor too much to detrimentally alter the release of the active ingredient(s) may be used to form the dosage forms of the compounds disclosed herein. The amount of disintegrant used may vary based upon the type of formulation and mode of administration, and may be readily discernible to those of ordinary skill in the art. About 0.5 to about 15 weight percent of disintegrant, or about 1 to about 5 weight percent of disintegrant, may be used in the pharmaceutical composition. Disintegrants that can be used to form compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-

gelatinized starch, other starches, clays, other algins, other celluloses, gums or mixtures thereof.

[00157] Examples of suitable fillers for use in the compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof.

[00158] When aqueous suspensions and/or elixirs are desired for oral administration, the active ingredient therein may be combined with various sweetening or flavoring agents, coloring matter or dyes and, if so desired, emulsifying and/or suspending agents, together with such diluents as water, ethanol, propylene glycol, glycerin and various combinations thereof.

[00159] The tablets can be uncoated or coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed. Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin or olive oil.

[00160] Compositions may include a solubilizer to ensure good solubilization and/or dissolution of the compound of the present invention and to minimize precipitation of the compound of the present invention. This can be useful for compositions for non-oral use, e.g., compositions for injection. A solubilizer may also be added to increase the solubility of the hydrophilic drug and/or other components, such as surfactants, or to maintain the composition as a stable or homogeneous solution or dispersion.

[00161] The composition can further include one or more pharmaceutically acceptable additives and excipients. Such additives and excipients include, without limitation, detackifiers, anti-foaming agents, buffering agents, polymers, antioxidants, preservatives, chelating agents, viscomodulators, tonicifiers, flavorants, colorants, odorants, opacifiers, suspending agents, binders, fillers, plasticizers, lubricants, and mixtures thereof. A non-exhaustive list of examples of excipients includes monoglycerides, magnesium stearate, modified food starch, gelatin, microcrystalline cellulose, glycerin, stearic acid, silica, yellow beeswax, lecithin, hydroxypropylcellulose, croscarmellose sodium, and crospovidone.

[00162] The compositions described herein can also be formulated as extended-release, sustained-release or time-release such that one or more components are released over time. Delayed release can be achieved by formulating the one or more components in a matrix of a variety of materials or by microencapsulation. The compositions can be formulated to release one or more components over a time period of 4, 6, 8, 12, 16, 20, or 24 hours. The release of the one or more components can be at a constant or changing rate.

[00163] Using the controlled release dosage forms provided herein, the one or more cofactors can be released in its dosage form at a slower rate than observed for an immediate release formulation of the same quantity of components. In some embodiments, the rate of change in the biological sample measured as the change in concentration over a defined time period from administration to maximum concentration for an controlled release formulation is less than about 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10% of the rate of the immediate release formulation. Furthermore, in some embodiments, the rate of change in concentration over time is less than about 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10% of the rate for the immediate release formulation.

[00164] In some embodiments, the rate of change of concentration over time is reduced by increasing the time to maximum concentration in a relatively proportional manner. For example, a two-fold increase in the time to maximum concentration may reduce the rate of change in concentration by approximately a factor of 2. As a result, the one or more cofactors may be provided so that it reaches its maximum concentration at a rate that is significantly reduced over an immediate release dosage form. The compositions of the present invention may be formulated to provide a shift in maximum concentration by 24 hours, 16 hours, 8 hours, 4 hours, 2 hours, or at least 1 hour. The associated reduction in rate of change in concentration may be by a factor of about 0.05, 0.10, 0.25, 0.5 or at least 0.8. In certain embodiments, this is accomplished by releasing less than about 30%, 50%, 75%, 90%, or 95% of the one or more cofactors into the circulation within one hour of such administration.

[00165] Optionally, the controlled release formulations exhibit plasma concentration curves having initial (e.g., from 2 hours after administration to 4 hours after administration) slopes less than 75%, 50%, 40%, 30%, 20% or 10% of those for an immediate release formulation of the same dosage of the same cofactor.

[00166] In some embodiments, the rate of release of the cofactor as measured in dissolution studies is less than about 80%, 70%, 60% 50%, 40%, 30%, 20%, or 10% of the rate for an immediate release formulation of the same cofactor over the first 1, 2, 4, 6, 8, 10, or 12 hours.

[00167] The controlled release formulations provided herein can adopt a variety of formats. In some embodiments, the formulation is in an oral dosage form, including liquid dosage forms (e.g., a suspension or slurry), and oral solid dosage forms (e.g., a tablet or bulk powder), such as, but not limited to those, those described herein.

[00168] The controlled release tablet of a formulation disclosed herein can be of a matrix, reservoir or osmotic system. Although any of the three systems is suitable, the latter two systems can have more optimal capacity for encapsulating a relatively large mass, such as for the inclusion of a large amount of a single cofactor, or for inclusion of a plurality of cofactors, depending on the genetic makeup of the individual. In some embodiments, the slow-release tablet is based on a reservoir system, wherein the core containing the one or more cofactors is encapsulated by a porous membrane coating which, upon hydration, permits the one or more cofactors to diffuse through. Because the combined mass of the effective ingredients is generally in gram quantity, an efficient delivery system can provide optimal results.

[00169] Thus, tablets or pills can also be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate. In some embodiments, a formulation comprising a plurality of cofactors may have different cofactors released at different rates or at different times. For example, there can be additional layers of cofactors interspersed with enteric layers.

[00170] Methods of making sustained release tablets are known in the art, e.g., see U.S. Patent Publications 2006/051416 and 2007/0065512, or other references disclosed herein. Methods such as described in U.S. Patent Nos. 4,606,909, 4,769,027, 4,897,268, and 5,395,626 can be used to prepare sustained release formulations of the one or more cofactors determined by the genetic makeup of an individual. In some embodiments, the formulation is prepared using OROS® technology, such as described in U.S. Patent Nos. 6,919,373, 6,923,800, 6,929,803, and 6,939,556. Other methods, such as described in U.S. Patent Nos.

6,797,283, 6,764,697, and 6,635,268, can also be used to prepare the formulations disclosed herein.

[00171] In some embodiments, the compositions can be formulated in a food composition. For example, the compositions can be a beverage or other liquids, solid food, semi-solid food, with or without a food carrier. For example, the compositions can include a black tea supplemented with any of the compositions described herein. The composition can be a dairy product supplemented any of the compositions described herein. In some embodiments, the compositions can be formulated in a food composition. For example, the compositions can comprise a beverage, solid food, semi-solid food, or a food carrier.

[00172] In some embodiments, liquid food carriers, such as in the form of beverages, such as supplemented juices, coffees, teas, sodas, flavored waters, and the like can be used. For example, the beverage can comprise the formulation as well as a liquid component, such as various deodorant or natural carbohydrates present in conventional beverages. Examples of natural carbohydrates include, but are not limited to, monosaccharides such as, glucose and fructose; disaccharides such as maltose and sucrose; conventional sugars, such as dextrin and cyclodextrin; and sugar alcohols, such as xylitol and erythritol. Natural deodorant such as taumatin, stevia extract, levaudioside A, glycyrrhizin, and synthetic deodorant such as saccharin and aspartame may also be used. Agents such as flavoring agents, coloring agents, and others can also be used. For example, pectic acid and the salt thereof, alginic acid and the salt thereof, organic acid, protective colloidal adhesive, pH controlling agent, stabilizer, a preservative, glycerin, alcohol, or carbonizing agents can also be used. Fruit and vegetables can also be used in preparing foods or beverages comprising the formulations discussed herein.

[00173] Alternatively, the compositions can be a snack bar supplemented with any of the compositions described herein. For example, the snack bar can be a chocolate bar, a granola bar, or a trail mix bar. In yet another embodiment, the present dietary supplement or food compositions are formulated to have suitable and desirable taste, texture, and viscosity for consumption. Any suitable food carrier can be used in the present food compositions. Food carriers of the present invention include practically any food product. Examples of such food carriers include, but are not limited to food bars (granola bars, protein bars, candy bars, etc.), cereal products (oatmeal, breakfast cereals, granola, etc.), bakery products (bread, donuts, crackers, bagels, pastries, cakes, etc.), beverages (milk-based beverage, sports drinks, fruit juices, alcoholic beverages, bottled waters), pastas, grains (rice, corn, oats, rye, wheat, flour,

etc.), egg products, snacks (candy, chips, gum, chocolate, etc.), meats, fruits, and vegetables. In an embodiment, food carriers employed herein can mask the undesirable taste (e.g., bitterness). Where desired, the food composition presented herein exhibit more desirable textures and aromas than that of any of the components described herein. For example, liquid food carriers may be used according to the invention to obtain the present food compositions in the form of beverages, such as supplemented juices, coffees, teas, and the like. In other embodiments, solid food carriers may be used according to the invention to obtain the present food compositions in the form of meal replacements, such as supplemented snack bars, pasta, breads, and the like. In yet other embodiments, semi-solid food carriers may be used according to the invention to obtain the present food compositions in the form of gums, chewy candies or snacks, and the like.

[00174] The dosing of the combination compositions can be administered about, less than about, or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times a daily. A subject can receive dosing for a period of about, less than about, or greater than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more days, weeks or months. A unit dose can be a fraction of the daily dose, such as the daily dose divided by the number of unit doses to be administered per day. A unit dose can be a fraction of the daily dose that is the daily dose divided by the number of unit doses to be administered per day and further divided by the number of unit doses (e.g. tablets) per administration. The number of unit doses per administration may be about, less than about, or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. The number of doses per day may be about, less than about, or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. The number of unit doses per day may be determined by dividing the daily dose by the unit dose, and may be about, less than about, or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, 20, or more unit doses per day. For example, a unit dose can be about 1/2, 1/3, 1/4, 1/5, 1/6, 1/7, 1/8, 1/9, 1/10. A unit dose can be about one-third of the daily amount and administered to the subject three times daily. A unit dose can be about one-half of the daily amount and administered to the subject twice daily. A unit dose can be about one-fourth of the daily amount with two unit doses administered to the subject twice daily. In some embodiments, a unit dose comprises about, less than about, or more than about 50 mg resveratrol. In some embodiments, a unit dose comprises about, less than about, or more than about 550 mg leucine. In some embodiments, a unit dose comprises about, less than about, or more than about 200 mg of one or more leucine metabolites. In some embodiments, a unit dose (e.g. a unit dose comprising leucine) is administered as two unit doses two times per

day. In some embodiments, a unit dose (e.g. a unit dose comprising one or more leucine metabolites, such as HMB) is administered as one unit dose two timer per day. Compositions disclosed herein can further comprise a flavorant and can be a solid, liquid, gel or emulsion.

Exemplary compositions:

[00175] A composition comprising a unit dosage form can be formulated to provide a dosage of a branched chain amino acid in free amino acid form, or a metabolite thereof. The dosage of the branched chain amino acid in free amino acid form or metabolite thereof can be a therapeutic dose or sub-therapeutic dose. For example, a unit dosage composition can comprise about 10-3000 mg (e.g., 50, 100, 200, 250, 300, 400, 500, 750, 1000, 1125, 1500, or 3000 mg) of leucine in free amino acid form, 5-500 mg of HMB (e.g., 5, 10, 20, 50, 100, 200, 300, 400, or 500 mg of HMB), about 20 to about 300 mg of KIC (e.g., 20, 50, 100, 200, 300 mg of KIC), or any combination thereof. In some embodiments, a weight % of the branched amino acid in free amino acid form or metabolite thereof (e.g., leucine, HMB, or KIC) in the composition, excluding excipients (e.g., fillers), is 50-95%.

[00176] Any of the above unit dosage compositions can further comprise an additional agent. The additional agent can be a sirtuin pathway activator and/or PDE inhibitor. Exemplary sirtuin pathway activators and PDE inhibitors are described herein. In some embodiments, a weight % of the additional agent in the composition, excluding excipients (e.g., fillers) is 5-50%. In particular embodiments, the additional agent is metformin. The metformin can be present in the unit dosage composition in a therapeutic or sub-therapeutic dose. For example, the unit dosage composition can further comprise about 2.5 to about 500 mg of metformin (e.g., about 2.5, 5, 10, 20, 25, 50, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, or 500 mg of metformin). In some embodiments, the additional agent is a PDE inhibitor (e.g., a PDE5 inhibitor). In some embodiments, the additional agent is icariin. The icariin can be present in the unit dosage composition in a therapeutic or sub-therapeutic dose. For example, the unit dosage composition can further comprise about 1 to about 2000 mg of icariin (e.g., about 1, 10, 25, 50, 100, 500, 600, 700, 800, 900, 1000, 1500, or 2000 mg of icariin). In some embodiments, the additional agent is sildenafil. The sildenafil can be present in the unit dosage composition in a therapeutic or sub-therapeutic dose. For example, the unit dosage composition can further comprise about 1 to about 2000 mg of sildenafil (e.g., about 1, 10, 25, 50, 100, 500, 600, 700, 800, 900, 1000, 1500, or 2000 mg of sildenafil).

[00177] The unit dosage can comprise about 250 to about 2000 mg of leucine. The unit dosage can comprise about 500 to about 1500 mg of leucine. The unit dosage can comprise

about 900 to about 1300 mg of leucine. The unit dosage can further comprise metformin. The weight ratio of metformin relative to leucine can be 0.01-0.6. The weight ratio of metformin relative to leucine can be 0.1-0.5. The weight ratio of metformin relative to leucine can be 0.15-0.3. The unit dosage can optionally further comprise resveratrol. An exemplary unit dosage composition comprises about 125 mg metformin, 1.125 g leucine and, optionally, 50 mg resveratrol. A unit dosage can comprise about 50 mg metformin, 1.125 g leucine and optionally 50 mg resveratrol. A unit dosage can comprise about 250 mg metformin, 1.125 g leucine and optionally 50 mg resveratrol. A unit dosage can comprise about 500 mg metformin, 1.125 g leucine and optionally 50 mg resveratrol. An exemplary formulation of a unit dosage comprising leucine and metformin is shown in Table 1:

Table 1: Formulation 1

Component	Mass (mg)	Wt ratio (relative to Leucine)
Leucine	1110	1
Metformin	125	0.1126

[00178] Another exemplary formulation of a unit dosage comprising leucine, metformin, and resveratrol is shown in Table 2:

Table 2: Formulation 2

Component	Mass (mg)	Wt ratio (relative to Leucine)
Leucine	1110	1
Metformin	125	0.1126
Resveratrol	50	0.045

[00179] In some embodiments, a unit dosage comprises leucine and icariin. The unit dosage can comprise about 250 to about 2000 mg of leucine. The unit dosage can comprise about 500 to about 1500 mg of leucine. The unit dosage can comprise about 900 to about 1300 mg of leucine. The weight ratio of icariin relative to leucine can be 0.01-0.1. The weight ratio of icariin relative to leucine can be 0.02-0.06. The weight ratio of icariin relative to leucine can be 0.03-0.05. An exemplary formulation of a unit dosage comprising leucine and icariin is shown in Table 3.

Table 3: Formulation 3

Component	Mass (mg)	Wt ratio (relative to Leucine)
Leucine	1110	1
Icariin	50	0.045

[00180] In some embodiments, a unit dosage comprises leucine, icariin, and resveratrol. The unit dosage can comprise about 250 to about 2000 mg of leucine. The unit dosage can comprise about 500 to about 1500 mg of leucine. The unit dosage can comprise about 900 to about 1300 mg of leucine. The weight ratio of icariin relative to leucine can be 0.01-0.1. The weight ratio of icariin relative to leucine can be 0.02-0.06. The weight ratio of icariin relative to leucine can be 0.03-0.05. The weight ratio of resveratrol relative to leucine can be 0.01-0.1. The weight ratio of resveratrol relative to leucine can be 0.02-0.06. The weight ratio of resveratrol relative to leucine can be 0.03-0.05. An exemplary formulation of a unit dosage comprising leucine, icariin, and resveratrol is shown in Table 4.

Table 4: Formulation 4

Component	Mass (mg)	Wt ratio (relative to Leucine)
Leucine	1110	1
Icariin	50	0.045
Resveratrol	50	0.045

[00181] In some embodiments, a unit dosage comprises leucine, icariin, and metformin. The unit dosage can comprise about 250 to about 2000 mg of leucine. The unit dosage can comprise about 500 to about 1500 mg of leucine. The unit dosage can comprise about 900 to about 1300 mg of leucine. The weight ratio of icariin relative to leucine can be 0.01-0.1. The weight ratio of icariin relative to leucine can be 0.02-0.06. The weight ratio of icariin relative to leucine can be 0.03-0.05. The weight ratio of metformin relative to leucine can be 0.01-0.6. The weight ratio of metformin relative to leucine can be 0.1-0.5. The weight ratio of metformin relative to leucine can be 0.15-0.3. The weight ratio of metformin relative to leucine can be 0.22-0.23. An exemplary formulation of a unit dosage comprising leucine, icariin, and metformin is shown in Table 5.

Table 5: Formulation 5

Component	Mass (mg)	Wt ratio (relative to Leucine)
Leucine	1110	1
Icariin	50	.045
metformin	250	.225

[00182] In some embodiments, a unit dosage comprises leucine, sildenafil, and optionally, resveratrol. The unit dosage can comprise about 250 to about 2000 mg of leucine. The unit dosage can comprise about 500 to about 1500 mg of leucine. The unit dosage can comprise 900-1300 mg of leucine. The weight ratio of resveratrol relative to leucine can be 0.00001-0.05. The weight ratio of resveratrol relative to leucine can be 0.0001-0.03. The weight ratio of resveratrol relative to leucine can be 0.001-0.02. The weight ratio of sildenafil relative to leucine can be 0.00001-0.05. The weight ratio of sildenafil relative to leucine can be 0.0001-0.03. The weight ratio of sildenafil relative to leucine can be 0.001-0.02. The weight ratio of sildenafil relative to leucine can be 0.005-0.015. Two exemplary formulations of a unit dosage comprising leucine and sildenafil, and another unit dosage comprising leucine, resveratrol, and sildenafil are shown in Table 6.

Table 6:

Formulation 6A

Component	Mass (mg)	Wt ratio (relative to Leucine)
Leucine	1110	1
Sildenafil	10	0.009

Formulation 6B

Component	Mass (mg)	Wt ratio (relative to Leucine)
Leucine	1110	1
Resveratrol	10	0.009
Sildenafil	10	0.009

[00183] The unit dosage can comprise about 5-1000 mg of HMB. The unit dosage can comprise about 100-500 mg of HMB. The unit dosage can comprise 150-400 mg of HMB. The unit dosage can comprise 200-300 mg of HMB. The unit dosage can further comprise

metformin. The weight ratio of metformin relative to HMB can be 0.01-1. The weight ratio of metformin relative to HMB can be 0.5-0.5. The weight ratio of metformin relative to HMB can be 0.1-0.3. The unit dosage can optionally further comprise resveratrol. An exemplary unit dosage composition comprises about 125 mg metformin, 250 mg HMB and, optionally, 50 mg resveratrol. A unit dosage can comprise about 50 mg metformin, 1250 mg HMB, and optionally 50 mg resveratrol. A unit dosage can comprise about 250 mg metformin, 250 mg HMB and optionally 50 mg resveratrol. A unit dosage can comprise about 500 mg metformin, 500 mg HMB and optionally 50 mg resveratrol. An exemplary formulation of a unit dosage comprising HMB and metformin is shown in Table 7:

Table 7: Formulation 7

Component	Mass (mg)	Wt ratio (relative to HMB)
HMB	250	1
Metformin	125	0.5

[00184] Another exemplary formulation of a unit dosage comprising HMB, metformin, and resveratrol is shown in Table 8:

Table 8: Formulation 8

Component	Mass (mg)	Wt ratio (relative to HMB)
HMB	250	1
Metformin	125	0.5
Resveratrol	50	0.2

[00185] In some embodiments, a unit dosage comprises HMB and icariin. The unit dosage can comprise about 5-1000 mg of HMB. The unit dosage can comprise about 100-500 mg of HMB. The unit dosage can comprise 150-400 mg of HMB. The unit dosage can comprise 200-300 mg of HMB. The weight ratio of icariin relative to HMB can be 0.05-0.5. The weight ratio of icariin relative to HMB can be 0.07-0.4. The weight ratio of icariin relative to HMB can be 0.1-0.3. An exemplary formulation of a unit dosage comprising HMB and icariin is shown in Table 9:

Table 9: Formulation 9

Component	Mass (mg)	Wt ratio (relative to HMB)
HMB	250	1
Icariin	50	0.2

[00186] In some embodiments, a unit dosage comprises HMB, icariin, and resveratrol. The unit dosage can comprise about 5-1000 mg of HMB. The unit dosage can comprise about 100-500 mg of HMB. The unit dosage can comprise about 150-400 mg of HMB. The unit dosage can comprise 200-300 mg of HMB. The weight ratio of icariin relative to HMB can be 0.05-0.5. The weight ratio of icariin relative to HMB can be 0.07-0.4. The weight ratio of icariin relative to HMB can be 0.1-0.3. The weight ratio of resveratrol relative to HMB can be 0.05-0.5. The weight ratio of resveratrol relative to HMB can be 0.07-0.4. The weight ratio of resveratrol relative to HMB can be 0.1-0.3. An exemplary formulation of a unit dosage comprising HMB, icariin, and resveratrol is shown in Table 10.

Table 10: Formulation 10

Component	Mass (mg)	Wt ratio (relative to HMB)
HMB	250	1
Icariin	50	0.2
Resveratrol	50	0.2

[00187] In some embodiments, a unit dosage comprises HMB, icariin, and metformin. The unit dosage can comprise about 5 to about 1000 mg of HMB. The unit dosage can comprise about 100 to about 500 mg of HMB. The unit dosage can comprise about 150 to about 400 mg of HMB. The unit dosage can comprise about 200 to about 300 mg of HMB. The weight ratio of icariin relative to HMB can be 0.05-0.5. The weight ratio of icariin relative to HMB can be 0.07-0.4. The weight ratio of icariin relative to HMB can be 0.1-0.3. The weight ratio of metformin relative to HMB can be 0.2-4. The weight ratio of metformin relative to HMB can be 0.5-2. The weight ratio of metformin relative to HMB can be 0.75-1.25. An exemplary formulation of a unit dosage comprising HMB, icariin, and metformin is shown in Table 11.

Table 11: Formulation 11

Component	Mass (mg)	Wt ratio (relative to HMB)
HMB	250	1
Icariin	50	0.2
Metformin	250	1

[00188] In some embodiments, a unit dosage comprises HMB and sildenafil. The unit dosage can comprise about 5-1000 mg of HMB. The unit dosage can comprise about 100-500 mg of HMB. The unit dosage can comprise about 150-400 mg of HMB. The unit dosage can comprise 200-300 mg of HMB. The weight ratio of sildenafil relative to HMB can be 0.01-0.1. The weight ratio of sildenafil relative to HMB can be 0.02-0.08. The weight ratio of sildenafil relative to HMB can be 0.03-0.05. An exemplary formulation of a unit dosage comprising HMB and sildenafil is shown in Table 12.

Table 12: Formulation 12

Component	Mass (mg)	Wt ratio (relative to HMB)
HMB	250	1
Sildenafil	10	0.04

[00189] In some embodiments, a unit dosage comprises HMB, sildenafil, and resveratrol. The unit dosage can comprise about 5-1000 mg of HMB. The unit dosage can comprise about 100-500 mg of HMB. The unit dosage can comprise about 150-400 mg of HMB. The unit dosage can comprise 200-300 mg of HMB. The weight ratio of sildenafil relative to HMB can be 0.01-0.1. The weight ratio of sildenafil relative to HMB can be 0.02-0.08. The weight ratio of sildenafil relative to HMB can be 0.03-0.05. The weight ratio of resveratrol relative to HMB can be 0.01-0.1. The weight ratio of resveratrol relative to HMB can be 0.02-0.08. The weight ratio of resveratrol relative to HMB can be 0.03-0.05. An exemplary formulation of a unit dosage comprising HMB and sildenafil is shown in Table 13.

Table 13: Formulation 13

Component	Mass (mg)	Wt ratio (relative to HMB)
HMB	250	1
Sildenafil	10	0.04
Resveratrol	10	0.04

[00190] The unit dosage can comprise about 20-1000 mg of KIC. The unit dosage can comprise about 100-500 mg of KIC. The unit dosage can comprise 150-400 mg of KIC. The unit dosage can comprise 200-300 mg of KIC. The unit dosage can further comprise metformin. The weight ratio of metformin relative to KIC can be 0.01-1. The weight ratio of metformin relative to KIC can be 0.5-0.5. The weight ratio of metformin relative to KIC can be 0.1-0.3. The unit dosage can optionally further comprise resveratrol. An exemplary unit dosage composition comprises about 50 mg metformin, 250 mg KIC and, optionally, 50 mg resveratrol. A unit dosage can comprise about 100 mg metformin, 1250 mg KIC, and optionally 50 mg resveratrol. A unit dosage can comprise about 250 mg metformin, 250 mg KIC and optionally 50 mg resveratrol. A unit dosage can comprise about 500 mg metformin, 500 mg KIC and optionally 50 mg resveratrol. An exemplary formulation of a unit dosage comprising KIC and metformin is shown in Table 14:

Table 14: Formulation 14

Component	Mass (mg)	Wt ratio (relative to KIC)
KIC	250	1
Metformin	50	0.2

[00191] Another exemplary formulation of a unit dosage comprising KIC, metformin, and resveratrol is shown in Table 15:

Table 15: Formulation 15

Component	Mass (mg)	Wt ratio (relative to KIC)
KIC	250	1
Metformin	50	0.2
Resveratrol	50	0.2

[00192] In some embodiments, a unit dosage comprises KIC and icariin. The unit dosage can comprise about 20-1000 mg of KIC. The unit dosage can comprise about 100-500 mg of KIC. The unit dosage can comprise 150-400 mg of KIC. The unit dosage can comprise 200-300 mg of KIC. The weight ratio of icariin relative to KIC can be 0.05-0.5. The weight ratio of icariin relative to KIC can be 0.07-0.4. The weight ratio of icariin relative to KIC can be 0.1-0.3. An exemplary formulation of a unit dosage comprising KIC and icariin is shown in Table 16.

Table 16: Formulation 16

Component	Mass (mg)	Wt ratio (relative to KIC)
KIC	250	1
Icariin	50	0.2

[00193] In some embodiments, a unit dosage comprises KIC, icariin, and resveratrol. The unit dosage can comprise about 20-1000 mg of KIC. The unit dosage can comprise about 100-500 mg of KIC. The unit dosage can comprise about 150-400 mg of KIC. The unit dosage can comprise 200-300 mg of KIC. The weight ratio of icariin relative to KIC can be 0.05-0.5. The weight ratio of icariin relative to KIC can be 0.07-0.4. The weight ratio of icariin relative to KIC can be 0.1-0.3. The weight ratio of resveratrol relative to KIC can be 0.05-0.5. The weight ratio of resveratrol relative to KIC can be 0.07-0.4. The weight ratio of resveratrol relative to KIC can be 0.1-0.3. An exemplary formulation of a unit dosage comprising KIC, icariin, and resveratrol is shown in Table 17.

Table 17: Formulation 17

Component	Mass (mg)	Wt ratio (relative to KIC)
KIC	250	1
Icariin	50	0.2
Resveratrol	50	0.2

[00194] In some embodiments, a unit dosage comprises KIC, icariin, and metformin. The unit dosage can comprise about 50 to about 1000 mg of KIC. The unit dosage can comprise about 100 to about 500 mg of KIC. The unit dosage can comprise about 150 to about 400 mg of KIC. The unit dosage can comprise about 200 to about 300 mg of KIC. The weight ratio of icariin relative to KIC can be 0.05-0.5. The weight ratio of icariin relative to KIC can be 0.07-0.4. The weight ratio of icariin relative to KIC can be 0.1-0.3. The weight ratio of metformin relative to KIC can be 0.2-4. The weight ratio of metformin relative to KIC can

be 0.5-2. The weight ratio of metformin relative to KIC can be 0.75-1.25. An exemplary formulation of a unit dosage comprising KIC, icariin, and metformin is shown in Table 18.

Table 18: Formulation 18

Component	Mass (mg)	Wt ratio (relative to KIC)
KIC	250	1
Icariin	50	0.2
Metformin	250	1

[00195] In some embodiments, a unit dosage comprises KIC and sildenafil. The unit dosage can comprise about 20-1000 mg of KIC. The unit dosage can comprise about 100-500 mg of KIC. The unit dosage can comprise about 150-400 mg of KIC. The unit dosage can comprise 200-300 mg of KIC. The weight ratio of sildenafil relative to KIC can be 0.01-0.1. The weight ratio of sildenafil relative to KIC can be 0.02-0.08. The weight ratio of sildenafil relative to KIC can be 0.03-0.05. An exemplary formulation of a unit dosage comprising KIC and sildenafil is shown in Table 19.

Table 19: Formulation 19

Component	Mass (mg)	Wt ratio (relative to KIC)
KIC	250	1
Sildenafil	10	0.04

[00196] In some embodiments, a unit dosage comprises KIC, sildenafil, and resveratrol. The unit dosage can comprise about 20-1000 mg of KIC. The unit dosage can comprise about 100-500 mg of KIC. The unit dosage can comprise about 150-400 mg of KIC. The unit dosage can comprise 200-300 mg of KIC. The weight ratio of sildenafil relative to KIC can be 0.01-0.1. The weight ratio of sildenafil relative to KIC can be 0.02-0.08. The weight ratio of sildenafil relative to KIC can be 0.03-0.05. The weight ratio of resveratrol relative to KIC can be 0.01-0.1. The weight ratio of resveratrol relative to KIC can be 0.02-0.08. The weight ratio of resveratrol relative to KIC can be 0.03-0.05. An exemplary formulation of a unit dosage comprising KIC and sildenafil is shown in Table 20.

Table 20: Formulation 20

Component	Mass (mg)	Wt ratio (relative to KIC)
KIC	250	1
Sildenafil	10	0.04
Resveratrol	10	0.04

[00197] In some embodiments, a unit dosage comprises leucine, metformin, and sildenafil. The unit dosage can comprise about 250 to about 2000 mg of leucine. The unit dosage can comprise about 500 to about 1500 mg of leucine. The unit dosage can comprise 900-1300 mg of leucine. The weight ratio of metformin relative to leucine can be 0.15-0.3. The weight ratio of metformin relative to leucine can be 0.22-0.23. The weight ratio of sildenafil relative to leucine can be 0.00001-0.05. The weight ratio of sildenafil relative to leucine can be 0.0001-0.03. The weight ratio of sildenafil relative to leucine can be 0.001-0.02. The weight ratio of sildenafil relative to leucine can be 0.005-0.015. An exemplary formulation of a unit dosage comprising leucine, metformin and sildenafil is shown in Table 21.

Table 21: Formulation 21

Component	Mass (mg)	Wt ratio (relative to Leucine)
Leucine	1110	1
Metformin	250	0.225
Sildenafil	10	0.009

[00198] In some embodiments, the molar ratio of (a) branched chain amino acids and/or metabolites thereof to (b) a selective PDE inhibitor about or greater than about 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 120, or 150. In other embodiments, the molar ratio of one or more branched chain amino acids and/or metabolites thereof to a selective PDE inhibitor contained in the subject compositions is about or greater than about 20, 30, 40, 50, 60, 70, 80, 90, 95, 90, 95, 100, 105, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 250, 300, 350, 400, or 500. In some embodiments, the molar ratio of component (a) to (b) in said composition is greater than about 20, 40, 60, 80, 100, 120, or 150. In some embodiments, the molar ratio of component (a) to (b) in said composition is greater than about 80, 100, 120, or 150. In some embodiments, the molar ratio of component (a) to (b) in said composition is greater than about 80, 100, 120, or 150. In some embodiments, the molar ratio of component (a) to (b) in said composition is greater than about 200, 250, or 300. In

some embodiments, the molar ratio of component (a) to (b) in said composition is greater than about 40, 150, 250, or 500.

[00199] In some embodiments, the dosing of leucine, any metabolites of leucine, the PDE inhibitor (such as a PDE 5 inhibitor like sildenafil) can be designed to achieve a specified physiological concentration or circulating level of leucine, metabolites of leucine and/or a PDE 5 inhibitor. The physiological concentration can be a circulating level as measured in the blood stream of a subject. The subject can be a human or an animal. A selected dosing can be altered based on the characteristics of the subject, such as weight, rate of energy metabolism, genetics, ethnicity, height, or any other characteristic. The amount of leucine in a unit dose can be such that the circulating level of leucine in a subject is about or greater than about 0.25 mM, 0.5 mM, 0.75 mM, or 1 mM. A dosing of about 1,125 mg leucine (e.g., free leucine), can achieve a circulating level of leucine in a subject that is about 0.5 mM. A dosing of about 300 mg leucine (e.g., free leucine), can achieve a circulating level of leucine in a subject that is about 0.25 mM. The dosing of about sildenafil can achieve a circulating concentration of about or less than about 0.1, 0.5, 1, 2, 5, or 10 nM. In some embodiments, the target or achieved circulating concentration of sildenafil is less than about 1 nM. A unit dose of about 20 mg of sildenafil can achieve a circulating concentration of about 100 nM of sildenafil. A unit dose of about 0.2 mg of sildenafil can achieve a circulating concentration of about 1 nM of sildenafil. The dosing of about icariin can achieve a circulating concentration of about or less than about 0.1, 0.5, 1, 2, 5, or 10 nM. In some embodiments, the target or achieved circulating concentration of icariin is less than about 1 nM. A unit dose of about 20 mg of icariin can achieve a circulating concentration of about 100 nM of icariin. A unit dose of about 0.1 mg of icariin can achieve a circulating concentration of about 1 nM of icariin.

[00200] In some embodiments, the molar or mass ratios are circulating molar or mass ratios achieved after administration one or more compositions to a subject. The compositions can be a combination composition described herein. The molar ratio of a combination composition in a dosing form can be adjusted to achieve a desired circulating molar ratio. The molar ratio can be adjusted to account for the bioavailability, the uptake, and the metabolic processing of the one or more components of a combination composition. For example, if the bioavailability of a component is low, then the molar amount of a that component can be increased relative to other components in the combination composition. In some embodiments, the circulating molar or mass ratio is achieved within about 0.1, 0.5, 0.75, 1, 3, 5, or 10, 12, 24, or 48 hours after administration. The circulating molar or mass ratio can be

maintained for a time period of about or greater than about 0.1, 1, 2, 5, 10, 12, 18, 24, 36, 48, 72, or 96 hours.

[00201] In some embodiments, the circulating molar ratio of leucine to sildenafil is about or greater than about 100,000, 250,000, 500,000, 750,000 or more. In some embodiments, the circulating molar ratio of HMB to sildenafil is about or greater than about 1,000, 2,500, 5,000, 7,500 or more. In some embodiments, the circulating molar ratio of resveratrol to sildenafil is about or greater than about 50, 100, 200, 400, 800 or more.

[00202] The compositions can be administered to a subject such that the subject is administered a selected total daily dose of the composition. The total daily dose can be determined by the sum of doses administered over a 24 hour period. The total daily dose of the composition can include about 0.05, 0.1, 0.5, 1, 2, 5, 10, 20, 40, 60, 80, or 100 mg of sildenafil. The total daily dose of the composition can include about 1, 10, 20, 50, 100, 150, 300, 400, 500, 750, 1000, 1500, or 2000 mg of icariin. The total daily dose of the composition can include about 1, 10, 20, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 900, or 1000 mg of metformin. The total daily dose of the composition can include at least about 250, 500, 750, 1000, 1125, 2000, 2250 mg, 2550 mg or more of a branched chain amino acid or metabolite thereof. The branched chain amino acid can be leucine, HMB, or any other branched chain amino acid described herein.

[00203] In some embodiments, a selected dose of a composition can be administered to a subject such that the subject achieves a desired circulating level of the composition. The desired circulating level of the leucine can be at least about 0.25, 0.5, 0.75, 1 mM or more of leucine. The desired circulating level of the sildenafil can be about 0.1, 0.5, 1, 2, 5, 10 nM or more of sildenafil. The desired circulating level of the icariin can be about 0.1, 0.5, 1, 2, 5, 10 nM or more of icariin. The desired circulating level of the metformin can be about 1, 2, 4, 5 μM or more of metformin. The desired circulating level of the rosiglitazone can be about 1, 10, 25, 50, 100, 400 nM or more of rosiglitazone. The desired circulating level of the pioglitazone can be about 0.25, 0.50, 1.0, 2.0 μM or more of pioglitazone. The selected dose can be chosen based on the characteristics of the subject, such as weight, height, ethnicity, or genetics.

EXAMPLES

[00204] Example 1: Effects of chronic high fat diet on liver mass and hepatic lipid accumulation.

[00205] Chronic high fat diet was used in C57Bl/6 mice as an experimental model of hepatic steatosis. Male C57/BL6 mice were either maintained on standard low-fat diet (control; LFD; 10% energy from fat) or placed on a high fat (HFD; 60% energy from fat, Research Diets #D12492, Research Diets, Inc, New Brunswick, NJ) at six weeks of age to induce obesity and fatty infiltration of the liver. Following six weeks on the high fat diet, there was a significant, substantial increase in liver mass ($p<0.0001$, FIG. 2). This increase in liver mass was accompanied by a marked increase in hepatic lipid infiltration, as shown histologically in FIG. 3. Animals fed with LFD have normal cellular structure in liver cells. Black dots denote cell nuclei. Typically, cell nuclei can be illustrated by staining with hematoxylin, methylene blue, nile blue, or neutral/Toluylene red. By contrast, animals fed with HFD have increased liver mass and increased hepatic lipid infiltration are shown in the HFD panel. Numerous lipid vesicles are found in liver cells in the HFD panel, arrows point to exemplary lipid vesicles. Liver cells in the HFD panel also have disrupted cellular structure, as shown by the distribution of nuclei. These results indicate that chronic HFD induces hepatic steatosis in mice. Animals were then randomized to treatments for an additional six weeks, with 10 animals per group, as specified in the following examples. Data for all examples were analyzed via one-way analysis of variance; when ANOVA showed significant differences, means were separated via Tukey's multiple comparison test (Graphpad Prism, Version 6.0).

[00206] Example 2: effect of various combinations of metformin, resveratrol and leucine on liver mass and hepatic steatosis.

[00207] In this study, the effects of metformin alone (therapeutic dose), subtherapeutic metformin alone, subtherapeutic leucine, and various combinations of leucine, metformin, and resveratrol on liver weight and hepatic lipid accumulation were assessed. The 1.5 g/kg diet of metformin corresponds to a therapeutically effective dose of metformin in humans. The remaining metformin doses (0.05 – 0.5 g/kg) represent sub-therapeutic doses that exert no independent effects. Likewise, the 24 g/kg dose of leucine represents a sub-therapeutic dose that was predicted to exert no significant independent effects on liver mass or hepatic steatosis. Following induction of hepatic steatosis as described in Example 1, animals were treated according to the following treatment groups as depicted in Table 21:

Table 21: Example 2 Treatment Groups

Low fat diet (LFD) control
High fat diet (HFD) control
HFD + therapeutic metformin alone (1.5 g /kg diet; calculated human equivalent dose, 1,500 mg/day)
HFD + leucine alone (24 g leucine/kg diet)
HFD + leucine (24 g/kg diet) + resveratrol (12.5 mg/kg diet)
HFD + Leucine (24 g/kg diet) + resveratrol (12.5 mg/kg diet) + metformin (0.05 g/kg diet; calculated human equivalent dose, 50 mg/day) HFD + Leucine (24 g/kg diet) + resveratrol (12.5 mg/kg diet) + metformin (0.15 g/kg diet; calculated human equivalent dose, 125 mg/day)
HFD + Leucine (24 g/kg diet) + resveratrol (12.5 mg/kg diet) + metformin (0.25 g/kg diet; calculated human equivalent dose, 250 mg/day)

[00208] FIGS. 4 and 5 depict results of the above study. High fat diet increased liver weight by approximately 70% (FIG. 4). Administration of therapeutic dosages of metformin partially reverse the effect of high fat diet on liver weight back to control levels ($p<0.02$ vs. HFD and LFD, FIG. 4). Administration of sub-therapeutic leucine alone had no significant effect on liver weight (FIG. 4). Likewise, administration of sub-therapeutic leucine + sub-therapeutic resveratrol had no significant effect on liver weight (FIG. 4). Surprisingly, combining subtherapeutic leucine, subtherapeutic resveratrol, and sub-therapeutic metformin (0.15 g/kg) significantly reduced the effect of high fat diet on liver weight ($p<0.02$ vs. HFD and LFD, FIG. 4). Furthermore, combining sub-therapeutic leucine, sub-therapeutic resveratrol and 0.25 g/kg metformin effected a complete reversal of the effects of high fat diet on liver weight back to control levels ($p<0.0002$ vs. HFD; n.s. vs. LFD, FIG. 4).

[00209] Histological analysis reveals that animals fed with low fat diet (LFD) have normal cellular structure and show no detectable lipid vesicles. By contrast, animals fed with high fat diet (HFD) induced significant hepatic steatosis (FIG. 5). Administration of therapeutic dosages of metformin effected a partial reversal of the effects of HFD on hepatic steatosis (FIG. 5). The cells appear to have reduced hepatic accumulation and reduced lipid vesicles. Administration of leucine alone nor leucine + resveratrol did not appear to significantly reduce hepatic steatosis, as shown by the appearance lipid vesicles and lipid accumulation (FIG. 5). However, combination therapy with sub-therapeutic dosages of leucine, resveratrol, and metformin appeared to reverse hepatic steatosis, as shown by decreased hepatic lipid

accumulation and decreased lipid vesicles (FIG. 5). Cells also appear to show comparable cellular structure to cells in the LFD panel.

[00210] Example 3: effect of various combinations of metformin and leucine on liver mass and hepatic steatosis.

[00211] In this study, the effects of metformin alone (therapeutic dose) vs. combination therapy with sub-therapeutic amounts of metformin and leucine on liver weight and hepatic lipid accumulation were assessed. Following induction of hepatic steatosis as described in Example 1, animals were treated for six weeks according to the following treatment groups as depicted in Table 22:

Table 22: Example 3 Treatment Groups

Treatment Group
Low fat diet (LFD) control
High fat diet (HFD) control
HFD + therapeutic metformin alone (1.5 g metformin/kg diet; calculated human equivalent dose, 1,500 mg/day)
HFD + Leucine (24 g/kg diet; calculated human equivalent dose, exclusive of diet, 2-3 g/day) +metformin (0.15 g/kg diet metformin; calculated human equivalent dose, 125 mg/day of metformin)
HFD + Leucine (24 g/kg diet; calculated human equivalent dose, exclusive of diet, 2-3 g/day) +metformin (0.25 g/kg diet; calculated human equivalent dose, 250 mg/day)
HFD + Leucine (24 g/kg diet; calculated human equivalent dose, exclusive of diet, 2-3 g/day) + metformin (0.50 g/kg diet; calculated human equivalent dose, 500 mg/day)

[00212] FIGS. 6-8 depict the primary results of the above study. High fat diet increased liver weight by approximately 30% (FIG. 6) and liver triglyceride by approximately five-fold (FIG. 7). Administration of therapeutic dosages of metformin reversed the effect of high fat diet on liver weight back to control levels ($p<0.02$ vs. HFD, n.s. compared to LFD, FIG. 6). Surprisingly, combining sub-therapeutic dosages of leucine with sub-therapeutic dosages of metformin (0.25 and 0.5 g/kg) also reversed the effect of high fat diet on liver weight back to control levels ($p<0.02$ vs. HFD, n.s. compared to LFD, FIG. 6). Administration of therapeutic dosages of metformin partially reversed the effect of high fat diet on liver triglycerides (FIG. 7), but the sub-therapeutic dosage of metformin (0.5 g/kg) combined with leucine was significantly more effective and fully reversed the effects of the high fat diet on liver triglycerides (FIG 7).

[00213] Histological analysis reveals that control animals fed with low fat diet (LFD) have no detectable lipid accumulation in liver cells. By contrast, animals fed with high fat diet (HFD) induced significant hepatic steatosis (FIG. 8). Administration of therapeutic dosages of leucine partially reversed hepatic steatosis, as shown by reduced lipid accumulation and lipid vesicles in liver cells. Administration of therapeutic dosages of metformin partially reversed hepatic steatosis, as shown by reduced lipid accumulation and lipid vesicles in liver cells (FIG.8). Combining subtherapeutic dosage of leucine with metformin (0.5 g/kg) also reversed hepatic steatosis (FIG. 8). The number of lipid vesicles and lipid accumulation in liver cells appeared to be greatly reduced in animals treated with leucine and metformin combination.The cellular structure also appeared to be comparable to the cells in the LFD panel.

[00214] The high fat diet markedly increased liver expression of lipogenic genes, including acetyl-CoA carboxykase (ACC), strearoyl-CoA desaturase 1 (SCD1) and fatty acid synthase (FAS)(FIG 9), while adding leucine with low-dos metformin resulted in significant dose-dependent reductions in lipogenic gene expression (FIG 9). Notably, the combination of metformin (0.5 g/kg) and leucine exerted a significantly greater effect on reducing ACC than did the therapeutic dose of metformin (FIG 9).

[00215] The expression of hepatic inflammatory genes interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α and monocyte chemotactic protein (MCP)-1 were significantly increased by the high fat diet and reduced by the leucine-metformin combinations, and the low-dose combinations exerted significantly greater effects on IL1β and MCP-1 than did the therapeutic dose of metformin (FIG 10).

[00216] Example 4: effect of various combinations of metformin and HMB on liver mass and hepatic steatosis.

[00217] In this study, the effects of metformin alone (therapeutic dose) vs. combination therapy with sub-therapeutic amounts of metformin and HMB on liver weight and hepatic lipid accumulation were assessed. Following induction of hepatic steatosis as described in Example 1, animals were treated for six weeks according to the following treatment groups as depicted in Table 23:

Table 23: Example 4 treatment groups

Low fat diet (LFD) control
High fat diet (HFD) control
HFD + therapeutic metformin alone (1.5 g metformin/kg diet; calculated human equivalent dose, 1,500 mg/day)
HFD + HMB (CaHMB, 2 g/kg diet; calculated human equivalent dose, ~500 mg/day) + metformin (0.15 g/kg diet)
HMB + metformin (0.25 g/kg diet)
HMB + metformin (0.50 g/kg diet)

[00218] FIGS. 11 and 12 depict results of the above study. High fat diet increased liver weight by approximately 25% (FIG. 11). Administration of therapeutic dosages of metformin reversed the effect of high fat diet on liver weight back to control levels ($p<0.03$,FIG. 8). Surprisingly, combining sub-therapeutic dosages of HMB with sub-therapeutic dosages of metformin (0.25 and 0.5 g/kg) also reversed the effect of high fat diet on liver weight back to control levels $p<0.03$, FIG. 11).

[00219] Histological analysis revealed that control animals fed with low fat diet (LFD) have normal cellular structure and no detectable lipid accumulation. By contrast, animals fed with high fat diet (HFD) induced significant hepatic steatosis, as shown by lipid accumulation and the appearance of numerous lipid vesicles in liver cells (FIG. 12). Administration of therapeutic dosages of metformin partially reversed hepatic steatosis, as shown by reduced lipid accumulation and reduced lipid vesicles in liver cells (FIG. 12). Combining subtherapeutic dosage of HMB with metformin (0.25 g/kg) also reversed hepatic steatosis (FIG. 12). The liver cells appear to have comparable cellular structure with liver cells in the LFD panel.

[00220] Example 5: effect of various combinations of a PDE5-specific inhibitor and leucine on liver mass and hepatic steatosis.

[00221] In this study, the effects of leucine alone vs. combination therapy with leucine and the PDE5-specific inhibitor icariin on liver weight and hepatic lipid accumulation were assessed. Previous published studies of icariin have demonstrated no effect of 25 mg icariin/kg diet; corresponding to 4 mg/kg body weight/day) on liver mass or hepatic lipid accumulation. Following induction of hepatic steatosis as described in Example 1, animals were treated for six weeks according to the following treatment groups as depicted in Table 24:

Table 24: Example 5 treatment groups

Low fat diet (LFD) control
High fat diet (HFD) control
HFD + leucine alone (24 g/kg diet; calculated human equivalent dose, exclusive of diet, 2-3 g/day)
HFD + leucine (24 g/kg diet; calculated human equivalent dose, exclusive of diet, 2-3 g/day) + icariin (25 mg/kg diet; calculated human equivalent dose, ~10 mg)

[00222] FIGS. 10 and 11 depict results of the above study. High fat diet increased liver weight by approximately 75% (FIG. 13). Administration of leucine alone had no effect on liver weight, FIG. 10). Surprisingly, combining sub-therapeutic dosages of leucine with sub-therapeutic dosages of icariin (25 mg/kg) significantly reduced the effect of high fat diet on liver weight ($p<0.01$ vs. HFD, FIG. 13).

[00223] Histological analysis reveals that control animals fed with low fat diet (LFD) have normal cellular structure and no detectable lipid accumulation. By contrast, animals fed with high fat diet (HFD) induced significant hepatic steatosis, as shown by the appearance of lipid vesicles and lipid accumulation in liver cells (FIG. 14). Combining subtherapeutic dosages of leucine with icariin appeared to reverse hepatic steatosis (FIG. 14). The liver cells show comparable cellular structure with liver cells in the LFD panel.

[00224] Combining subtherapeutic dosages of leucine with icariin resulted in marked stimulation of liver fatty acid oxidation gene expression (FIG 15).

[00225] Likewise, combining subtherapeutic dosages of leucine with icariin resulted in marked suppression of hepatic inflammatory genes interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α and monocyte chemotactic protein (MCP)-1 (FIG 16). Moreover, using circulating C-reactive protein (CRP) as an integrated marker of hepatic inflammatory stress, the leucine-PDE5 inhibitor combination reversed the HFD-induced elevation of CRP, while leucine alone exerted no independent effect (FIG 17).

[00226] Example 6: prophylactic effect of various combinations of leucine, metformin, and resveratrol on hepatic steatosis.

[00227] In this study, the prophylactice effects of metformin alone, and various combinations of metformin, leucine, and resveratrol on liver mass and hepatic steatosis are assessed. C57Bl/6 mice are assigned to the following experimental groups as depicted in Table 25:

Table 25: Example 6 experimental groups

Treatment Group
Low fat diet (LFD) alone
High fat diet (HFD) alone
HFD + therapeutic metformin alone (1.5 g metformin/kg diet; calculated human equivalent dose, 1,500 mg/day)
HFD + Leucine (24 g/kg diet; calculated human equivalent dose, exclusive of diet, 2-3 g/day) +metformin (0.15-0.50 g/kg diet metformin; calculated human equivalent dose, 125-500 mg/day of metformin)
HFD + Leucine (as above) + metformin (as above) + resveratrol (12.5 mg/kg diet)

[00228] Drug treatments and high fat diet feeding (as described in Example 1) are initiated on the same day and are carried out for six weeks. The effects of the treatments and diet on liver mass and hepatic lipid accumulation are assessed as described in the preceding examples. High fat diet alone results in increased liver mass and hepatic lipid accumulation. Administration of therapeutic dosages of metformin alone, concurrent with high fat feeding, attenuates the HFD-induced increase in liver mass and hepatic lipid accumulation. Co-administration of leucine and sub-therapeutic doses of metformin, with or without resveratrol prevents the HFD-induced increase in liver mass and hepatic lipid accumulation.

[00229] Example 7: prophylactic effect of various combinations of HMB, metformin, and resveratrol on hepatic steatosis.

[00230] In this study, the prophylactice effects of metformin alone, and various combinations of metformin, HMB, and resveratrol on liver mass and hepatic steatosis are assessed. C57Bl/6 mice are assigned to the following experimental groups as depicted in Table 26:

Table 26: Example 7 experimental groups

Treatment Group
Low fat diet (LFD) alone
High fat diet (HFD) alone
HFD + therapeutic metformin alone (1.5 g metformin/kg diet; calculated human equivalent dose, 1,500 mg/day)
HFD + HMB (CaHMB, 2 g/kg diet; calculated human equivalent dose, ~500 mg/day)) + metformin (0.15-0.50 g/kg diet metformin; calculated human equivalent dose, 125-500 mg/day of metformin)
HFD + HMB (as above) + metformin (as above) + resveratrol (12.5 mg/kg diet)

[00231] Drug treatments and high fat diet feeding (as described in Example 1) are initiated on the same day and are carried out for six weeks. The effects of the treatments and diet on

liver mass and hepatic lipid accumulation are assessed as described in the preceding examples. High fat diet alone results in increased liver mass and hepatic lipid accumulation. Administration of therapeutic dosages of metformin alone, concurrent with high fat feeding, attenuates the HFD-induced increase in liver mass and hepatic lipid accumulation. Co-administration of HMB and sub-therapeutic doses of metformin, with or without resveratrol, prevents the HFD-induced increase in liver mass and hepatic lipid accumulation.

[00232] Example 8: prophylactic effect of various combinations of leucine and a PDE5-specific inhibitor on hepatic steatosis.

[00233] In this study, the prophylactic effects of sub-therapeutic leucine alone, and co-administration of leucine and the PDE5-specific inhibitor icariin on liver mass and hepatic steatosis are assessed. C57Bl/6 mice are assigned to the following experimental groups as depicted in Table 27:

Table 27: Example 8 experimental groups

Treatment Group
Low fat diet (LFD) alone
High fat diet (HFD) alone
HFD + sub-therapeutic leucine alone (24 g/kg diet; calculated human equivalent dose, exclusive of diet, 2-3 g/day)
HFD + leucine (as above) + icariin (25 mg/kg diet; calculated human equivalent dose, 25 mg/day of icariin)

[00234] Drug treatments and high fat diet feeding (as described in Example 1) are initiated on the same day and are carried out for six weeks. The effects of the treatments and diet on liver mass and hepatic lipid accumulation are assessed as described in the preceding examples. High fat diet alone results in increased liver mass and hepatic lipid accumulation. Administration of therapeutic dosages of sub-therapeutic leucine alone has no significant effect on HFD-induced increase in liver mass and hepatic lipid accumulation. Co-administration of leucine and sub-therapeutic doses of icariin prevents the HFD-induced increase in liver mass and hepatic lipid accumulation.

[00235] Example 9. Interactive effects of leucine, metformin and sildenafil in hepatocytes.

[00236] In this study, the *in vitro* effects of sub-therapeutic doses of leucine (0.5 mM), metformin, and PDE5 inhibition with sildenafil (0.1 – 10 nM) on hepatocyte sirtuin signaling, fat oxidation and triglyceride content were evaluated in HepG2 cells.

[00237] Although leucine-metformin and leucine-sildenafil stimulated Sirt1 and AMPK activation, the three-way combination stimulated both Sirt1 and AMPK to a significantly

greater degree than either of the two way combinations (leucine-metformin, leucine-sildenafil).

[00238] Moreover, although both leucine-metformin and leucine sildenafil stimulated hepatocyte fat oxidation and inhibited palmitate-induced hepatocyte triglyceride accumulation, the three-way combination of leucine, metformin and sildenafil was twice as effective as either of the two-way combinations (FIG 18).

[00239] Example 10. Interactive effects of leucine-metformin-sildenafil versus leucine-metformin and leucine-sildenafil in a mouse model of non-alcoholic steatohepatitis (NASH).

[00240] In this study, the effects of subtherapeutic doses of leucine, metformin and sildenafil were compared as two-way and three-way combinations.

[00241] C57Bl/6 mice were assigned to the following experimental groups, as depicted in table 28.

Table 28: Example 10 experimental groups

Treatment Group
Low fat diet (LFD) alone
High fat-atherogenic diet (HF/ATH) alone
HF/ATH + + Leucine (24 g/kg diet; calculated human equivalent dose, exclusive of diet, 2-3 g/day) +metformin (0.25 g/kg diet metformin; calculated human equivalent dose, 250 mg/day of metformin)
HF/ATH + sildenafil (25 mg/kg diet; calculated human equivalent dose, 1 mg/day)
HF/ATH + Leucine (24 g/kg diet; calculated human equivalent dose, exclusive of diet, 2-3 g/day) + sildenafil (25 mg/kg diet; calculated human equivalent dose, 1 mg/day)
HFD + sildenafil (25 mg/kg diet; calculated human equivalent dose, 1 mg/day) + metformin (0.25 g/kg diet metformin; calculated human equivalent dose, 250 mg/day of metformin)
HFD + leucine (24 g/kg diet; calculated human equivalent dose, exclusive of diet, 2-3 g/day) + sildenafil (25 mg/kg diet; calculated human equivalent dose, 1 mg/day) + metformin (0.25 g/kg diet metformin; calculated human equivalent dose, 250 mg/day of metformin)

[00242] Drug treatments and high fat diet feeding (as described in Example 1) are initiated on the same day and are carried out for six weeks. The effects of the treatments and diet on liver mass and hepatic lipid accumulation are assessed as described in the preceding examples.

[00243] The HF/ATH diet resulted in significant hepatocellular injury, as evidenced by a ~six-fold increase in alanine aminotransferase (ALT; Figure 19). Treatment efficacy of the leucine-metformin-sildenafil combination was evident within two weeks of treatment; although both leucine-metformin and leucine-sildenafil reduced ALT levels, the triple combination exerted a significantly greater effect, resulting in a ~50% decrease in ALT at

two weeks (Figure 19), and a decrease of >60% at 6 weeks (Figure 20). The HF/ATH diet also resulted in a significant 29% increase in body weight-normalized liver mass ($p<0.0001$; Figure 21). Both leucine-metformin and leucine-sildenafil combinations resulted in non-significant reductions in liver mass. However, the triple combination of leucine, metformin and sildenafil resulted in a 12% decrease in liver mass ($p<0.003$), reflecting a reduction in hepatic lipid accumulation.

[00244] Example 11. Interactive effects of leucine-metformin-sildenafil versus leucine-metformin and leucine-sildenafil in human of non-alcoholic steatohepatitis (NASH).

[00245] In this study, the effects of subtherapeutic doses of leucine, metformin and sildenafil are compared as two-way and three-way combinations.

[00246] Human with non-alcoholic steatohepatitis is assigned to the following experimental groups, as depicted in Table 29. The duration of such trial includes about 12 months.

Table 29: Example 11 experimental groups

Treatment Group
Placebo
Metformin (250 mg, administered twice a day)
Leucine (1.1 g, administered twice a day) + metformin (250 mg, administered twice a day)
Sildenafil (1 mg, administered twice a day)
Leucine (1.1 g, administered twice a day) + sildenafil (1 mg, administered twice a day)
Sildenafil (1 mg, administered twice a day) + metformin (250 mg administered twice a day)
Leucine (1.1 g, administered twice a day) + sildenafil (1 mg, administered twice a day) + metformin (250 mg, administered twice a day)

[00247] The effects of the treatments and diet on hepatic lipid accumulation are assessed using a liver biopsy, a blood test, a FibroTest, a SteatoTest, a gene expression profiling of the genes provided in Example 3 and Example 4, or combinations thereof. A liver biopsy is performed and improvement in NAFLD Activity Score (NAS) is evaluated. An improvement of resolution of NASH is also evaluated.

[00248] The placebo group is expected to maintain significant hepatocellular injury. In response to the treatments, it is expected to improve NAFLD Activity Score by at least two points and to improve resolution of NASH in fibrosis in both leucine and metformin double combination, with significant further improvement resulting from the leucine, metformin and sildenafil triple combination.

[00249] It should be understood from the foregoing that, while particular implementations have been illustrated and described, various modifications can be made thereto and are contemplated herein. It is also not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the preferable embodiments herein are not meant to be construed in a limiting sense. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. Various modifications in form and detail of the embodiments of the invention will be apparent to a person skilled in the art. It is therefore contemplated that the invention shall also cover any such modifications, variations and equivalents.

CLAIMS

What is claimed is:

1. Use of a composition comprising:
    a) an amount of leucine in the form of a free amino acid and/or metabolite thereof, wherein the metabolite is β-hydroxy β-methylbutyrate (HMB) or keto-isocaproic acid (KIC); and
    b) an amount of metformin, and
    c) an amount of sildenafil,
    in the manufacture of a medicament for reducing non-alcoholic steatohepatitis (NASH).
2. The use of claim 1, wherein the non-alcoholic steatohepatitis (NASH) in the subject is evidenced by an accumulation of hepatic fat, fibrosis and/or inflammation detectable by one or more methods selected from the group consisting of ultrasonography, computed tomography (CT), magnetic resonance imaging, magnetic resonance spectroscopy (MRS), magnetic resonance elastography (MRE), transient elastography (TE), hepatic steatosis, inflammation and/or fibrosis detected by biopsy, and detection of a biomarker indicative of liver damage.
3. The use of claim 1, wherein a subject has been diagnosed with the non-alcoholic steatosis (NASH), or exhibits a non-alcoholic fatty liver disease (NAFLD) selected from the group consisting of non-alcoholic fatty liver (NAFL), NASH-related cirrhosis and hepatic steatosis.
4. The use of claim 1, wherein the amount of component (a) to be administered to the subject is 0.25-3 g/day of leucine in the form of a free amino acid, and/or 0.2-3 g/day of the metabolite thereof.
5. The use of claim 1, wherein component (a) is leucine, the molar ratio of the leucine to sildenafil in the composition is at least about 20.
6. The use of claim 1, wherein component (c) comprises 0.05-100 mg/day of sildenafil.
7. The use of claim 1, wherein a sub-therapeutic amount of metformin is to be administered to the subject.
8. The use of claim 7, wherein the amount of metformin to be administered to the subject is 10-1000 mg/day.

9. The use of claim 1, wherein the composition is to be administered to the subject twice a day and wherein the composition reduces hepatic steatosis in the subject by at least 5% within 6 weeks.
10. The use of claim 1, wherein the composition is substantially free of one or more free amino acids selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
11. The use of claim 1, wherein the component (a) comprises leucine existing in form of a free amino acid.
12. The use of claim 1, wherein the composition to be administered to the subject is formulated in a unit dose, and wherein the composition comprises:
    (i) component (a) comprising 900-1200 mg of leucine in the form of a free amino acid; and
    (ii) component (b) comprising 200-550 mg of metformin and
    (iii) component (c) comprising 0.1-10 mg of sildenafil.
13. The use of claim 12, wherein the unit dose is to be administered twice a day.
14. The use of claim 12, wherein the components of the unit dose are to be co-administered to the subject simultaneously as a single composition or sequentially.
15. The use of claim 1, wherein the composition to be administered reduces non-alcoholic steatohepatitis (NASH) in the subject to a greater extent than component (a) alone.
16. The use of claim 15, wherein the biomarker indicative of liver damage is elevated serum alanine transferase, and/or aspartate transferase.
17. Use of a composition comprising
    a. an amount of leucine in the form of a free amino acid and/or metabolite thereof, wherein the metabolite is β-hydroxy β-methylbutyrate (HMB) or keto-isocaproic acid (KIC); and
    b. an amount of metformin,
    c. an amount of sildenafil,

in the manufacture of a medicament for preventing non-alcoholic steatohepatitis (NASH) in a subject exhibiting a propensity to NASH, wherein said composition is to be administered prior to manifestation of a hepatic steatosis symptom.

18. The use of claim 17, wherein the composition to be administered to the subject is formulated in a unit dose, and wherein the composition comprises:
    (i) component (a) comprising 900-1200 mg of leucine in the form of a free amino acid; and
    (ii) component (b) comprising 200-550 mg of metformin and
    (iii) component (c) comprising 0.1-10 mg of sildenafil.
19. The use of claim 18, wherein the components of the unit dose are to be co-administered to the subject simultaneously as a single composition or sequentially, and the unit dose is to be administered twice a day.