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

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) 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 about 900-1200 mg of leucine in the form of a free amino acid; and
    (ii) component (b) comprising about 200-550 mg of metformin and
    (iii) component (c) comprising about 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 administered to the subject is formulated in a unit dose, and wherein the composition comprises:
    (i) component (a) comprising about 900-1200 mg of leucine in the form of a free amino acid; and
    (ii) component (b) comprising about 200-550 mg of metformin and
    (iii) component (c) comprising about 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.