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; and

b) an amount of a sirtuin pathway activator

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; and

b. an amount of one or more sirtuin pathway activators,

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; and
    b) an amount of a sirtuin pathway activator,

    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 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 the subject ~~a~~ 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 leucine metabolite thereof is β-hydroxy β-methylbutyrate (HMB) or keto-isocaproic acid (KIC).
5. 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.
6. The use of claim 1, wherein component (a) is leucine and component (b) is a selective PDE inhibitor, the molar ratio of the leucine to the PDE 5 specific inhibitor in the composition is at least about 20.
7. The use of claim 1, wherein component (b) comprises a PDE-5 specific inhibitor selected from the group consisting of icariin, sildenafil, tadalafil, vardenafil, avanafil, lodenafil, mirodenafil and udenafil.
8. The use of claim 7, wherein component (b) comprises a PDE5 specific inhibitor selected from the group consisting of 1-2000 mg/day of icariin, 0.05-100 mg/day of sildenafil, 0.01-

20 mg/day of tadalafil, 0.01-20 mg/day of vardenafil, 1-200 mg/day of avanafil, 1-200 mg/day lodenafil, 1-100 mg/day of mirodenafil, 1-200 mg/day of udenafil, and 1-2000 mg/day of zaprinast.

9. The use of claim 1, wherein component (b) comprises a PDE5 specific inhibitor and a biguanide.
10. The use of claim 9, wherein the biguanide is metformin.
11. The use of claim 10, wherein a sub-therapeutic amount of metformin is to be administered to the subject.
12. The use of claim 11, wherein the amount of metformin to be administered to the subject is 10-1000 mg/day.
13. The use of claim 1, wherein component (b) comprises a thiazolidinedione.
14. The use of claim 13, wherein the thiazolidinedione comprises rosiglitazone or pioglitazone.
15. The use of claim 13, wherein the amount of thiazolidinedione to be administered to the subject is 0.1-4 mg of rosiglitazone or 0.1-15 mg of pioglitazone.
16. The use of claim 1, comprising administering the composition to the subject twice daily and the liver mass of the subject is decreased by 25% within 6 weeks.
17. 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.
18. The use of claim 1, wherein component (b) comprises sildenafil.
19. The use of claim 1, wherein the component (a) comprises leucine existing in form of a free amino acid and the component (b) comprises metformin.
20. The use of claim 1, wherein component (b) comprises at least two sirtuin pathway activators.
21. The use of claim 9, wherein the PDE5 specific inhibitor comprises sildenafil.
22. The use of claim 9, wherein component (a) comprises leucine in the form of a free amino acid and (b) comprises sildenafil and metformin.
23. The use of claim 22, 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 about 0.1-10 mg of sildenafil.

24. The use of claim 23, wherein the unit dose is administered twice a day.

25. The use of claim 23, wherein the components of the unit dose are co-administered to the subject simultaneously as a single composition or sequentially.

26. The use of claim 1, wherein the administering of (a) and (b) reduces non-alcoholic steatohepatitis (NASH) to a greater extent than administration of (a) alone, thereby reducing non-alcoholic steatohepatitis (NASH) in the subject.

27. The use of claim 26, wherein the biomarker indicative of liver damage is elevated serum alanine transferase, and/or aspartate transferase.

28. Use of a composition comprising

a. an amount of leucine in the form of a free amino acid and/or metabolite thereof; and

b. an amount of one or more sirtuin pathway activators,

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.

29. The use of claim 28, 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 about 0.1-10 mg of sildenafil.

30. The use of claim 29, 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.

31. The use of claim 1, wherein component (b) comprises one or more sirtuin pathway activators selected from the group consisting of an AMPK activator, a PGC-1α activator, a PDE5 specific inhibitor, a biguanide and a thiazolidinedione.

CLAIMS

What is claimed is:

1. 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 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.
2. 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.
3. 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.
4. 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.

5. 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;
    b) an amount of metformin; and
    c) an amount of sildenafil;

    wherein 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.
6. 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;
    b) an amount of metformin; and
    c) an amount of sildenafil;

    wherein 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.
7. The method of claim 1, 2, 3, 4 or 5, further comprising administering to the subject (c) a second sirtuin pathway activator.
8. The method of claim 7, wherein the sirtuin pathway activator comprises a biguanide and the second sirtuin pathway activator comprises a sirtuin activator.
9. The method of claim 7, wherein the sirtuin pathway activator comprises a sirtuin activator and the second sirtuin pathway activator comprises a thiazolidinedione.
10. The method of claim 7, wherein the sirtuin pathway activator comprises a sirtuin activator and the second sirtuin pathway activator comprises a PDE5-specific inhibitor.
11. The method of claim 7, wherein the sirtuin pathway activator comprises a biguanide and the second sirtuin pathway activator comprises a thiazolidinedione.
12. The method of claim 7, wherein the sirtuin pathway activator comprises a biguanide and the second sirtuin pathway activator comprises a PDE5-specific inhibitor.
13. The method of claim 7, wherein the sirtuin pathway activator comprises a thiazolidinedione and the second sirtuin pathway activator comprises a PDE5-specific inhibitor.

14. The method of claim 3, 4, or 6, wherein the subject is administered periodically a composition comprising components (a), (b), and (c) over a course of at least 2 months.
15. The method of any of claim 1, 2 or 5, wherein the subject has been diagnosed with said hepatic steatosis.
16. The method of any of the preceding claims, wherein 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.
17. The method of any of the preceding claims, wherein 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.
18. The method of any of claim 1, 2 or 5, wherein the subject exhibits a non-alcoholic fatty liver disease (NAFLD).
19. The method of claim 18, wherein the NAFLD is selected from the group consisting of non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH) and NASH-related cirrhosis.
20. The method of claim 1, 2 or 5, wherein the subject exhibits one or more symptoms of hepatic steatosis selected from the group consisting of weakness, fatigue, unexplained weight loss, ache and jaundice.
21. The method of any of the preceding claims, wherein the leucine metabolite thereof is β-hydroxy β-methylbutyrate (HMB) or keto-isocaproic acid (KIC).
22. The method of any of preceding claims, wherein the amount of leucine administered to the subject is about 0.25-3 g/day.
23. The method of any of the preceding claims, wherein the amount of leucine administered to the subject is about 0.25-3 g.
24. The method of any of the preceding claims, wherein the amount of leucine metabolite administered to the subject is about 0.2-3 g/day.
25. The method of any of the preceding claims, wherein the amount of leucine metabolite administered to the subject is about 0.2-3 g.

26. The method of any of the preceding claims, wherein a wt% of the leucine in the composition, excluding fillers, is 50-95 wt%.
27. The method of any of the preceding claims, wherein a wt% of the leucine metabolite in the composition, excluding fillers, is 50-95 wt%.
28. The method of any of the preceding claims, wherein a wt% of the sirtuin pathway activator in the composition, excluding fillers, is 5-50 wt%.
29. The method of any of the preceding claims, wherein the molar ratio of leucine to sirtuin pathway activator is at least about 20.
30. The method of claim 1, 3 or 6, wherein component (a) is hydroxymethylbutyrate and component (b) is metformin.
31. The method of claim 1, 3 or 6, wherein component (a) is leucine existing in form of a free amino acid and component (b) is metformin.
32. The method of claim 1, 3 or 6, wherein the sirtuin pathway activator is a sirtuin activator.
33. The method of claim 32, wherein the sirtuin activator is resveratrol.
34. The method of claim 32, wherein 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.
35. The method of claim 33, wherein the amount of resveratrol administered to the subject is 0.5-100 mg/day.
36. The method of claim 34, wherein 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.
37. The method of claim 2, 4 or 6, wherein the PDE-5 specific inhibitor is selected from the group consisting of icariin, sildenafil, tadalafil, vardenafil, avanafil, lodenafil, mirodenafil and udenafil.
38. The method of claim 37, wherein the amount of icariin administered to the subject is 1-2000 mg/day.
39. The method of claim 37, wherein the amount of sildenafil administered to the subject is 0.05-100 mg/day.
40. The method of claim 37, wherein the amount of tadalafil administered to the subject is 0.01-20 mg/day.

41. The method of claim 37, wherein the amount of vardenafil administered to the subject is 0.01-20 mg/day.
42. The method of claim 37, wherein the amount of avanafil administered to the subject is 1-200 mg/day.
43. The method of claim 37, wherein the amount of lodenafil administered to the subject is 1-200 mg/day.
44. The method of claim 37, wherein the amount of mirodenafil administered to the subject is 1-100 mg/day.
45. The method of claim 37, wherein the amount of udenafil administered to the subject is 1-200 mg/day.
46. The method of claim 37, wherein the amount of zaprinast administered to the subject is 1-2000 mg/day.
47. The method of claim 1, 3 or 6, wherein the sirtuin pathway activator is an AMPK activator.
48. The method of claim 47, wherein the AMPK activator is a biguanide.
49. The method of claim 48, wherein the biguanide is metformin.
50. The method of claim 49, wherein the amount of metformin is a therapeutic amount of metformin.
51. The method of claim 50, wherein the therapeutic amount of metformin is about 1 g to about 2.55 g/day.
52. The method of claim 50, wherein the therapeutic amount of metformin is about 0.5 g to about 1.25 g.
53. The method of claim 49, wherein the amount of metformin is a sub-therapeutic amount of metformin.
54. The method of claim 53, wherein the sub-therapeutic amount of metformin is about 20-1000 mg metformin/day.
55. The method of claim 54, wherein the sub-therapeutic amount of metformin is about 10-500 mg metformin.
56. The method of claim 1, 3 or 6, wherein the sirtuin pathway activator is a PGC-1α activator
57. The method of claim 56, wherein the PGC-1α activator is a thiazolidinedione.
58. The method of claim 57, wherein the thiazolidinedione is selected from the group consisting of rosiglitazone and pioglitazone.

59. The method of claim 58, wherein the amount of rosiglitazone administered is 0.1 – 4 mg.
60. The method of claim 58, wherein the amount of pioglitazone administered is 0.1 – 45 mg.
61. The method of claim 1, 3 or 6, wherein the amount of sirtuin pathway activator is a sub-therapeutic amount.
62. The method of claim 1, 2 or 5, wherein the reduction in hepatic steatosis is characterized by a reduction in hepatic liver vacuole number or size or density.
63. The method of any of the preceding claims, wherein the amounts of (a) and (b) are co-administered.
64. The method of claim 63, wherein the amounts of (a) and (b) are administered simultaneously as a single composition.
65. The method of claims 63, wherein the amounts of (a) and (b) are administered sequentially.
66. The method of claim 65, wherein all of the amounts are administered sequentially within 15 minutes, 60 minutes or 2 hours.
67. The method of claim 65, wherein all components of the composition are administered 15 minutes, 60 minutes or 2 hours.
68. The method of any of the preceding claims, comprising administering (a) and (b) 1, 2, 3, 4, 5 or more times per day.
69. The method of any of the preceding claims, comprising administering (a) and (b) 3 times daily and the liver mass of the subject is decreased by 25% within 6 weeks.
70. The method of any of the preceding claims, 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.
71. 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;

    wherein 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).

72. The composition of claim 71, wherein 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.

73. The composition of claim 71, wherein the composition is administered twice a day.