Indirect calorimetry measure energy expenditure or increase in metabolic rate with BAT activation. Blood exosomal miRNA detects specific miRNA patterns or signatures associated with BAT. All methods above aim to provide a safer and more cost-effective modality with good reliability and precision that can one day replace PET-CT as the gold standard. PET-CT uses positron emission and X-rays absorption to measure glucose uptake by active BAT. Having said that, the above methods can be highly costly, inconvenient and/or exposes the subject to radioactive tracers.

[0004] In view of the above, there is a need to provide alternative means for detecting brown adipose tissues (BAT) activation and/or browning of white adipose tissues (WAT) to BAT, that overcomes, or at least ameliorates, one or more of the disadvantages described in the currently available methods.

SUMMARY

[0005] According to an example, there is provided a method for detecting brown adipose tissues (BAT) activation and/or browning of white adipose tissues (WAT) to BAT in a subject, wherein the method comprises:

a. obtaining a first blood sample from the subject;
b. determining a baseline level of methylene tetrahydrofolate dehydrogenase 1-like (MTHFD1L) protein in the first blood sample;
c. exposing the subject to a stimulation;
d. obtaining a second blood sample from the subject after the stimulation; and
e. measuring a post-stimulation level of MTHFD1L protein in the second blood sample;

e1. if the post-stimulation level of MTHFD1L protein is **higher** than the baseline level of MTHFD1L protein, determining **presence** of BAT activation and/or browning of WAT to BAT in the subject, or

e2. if the post-stimulation level of MTHFD1L protein is **the same or lower** than the baseline level of MTHFD1L protein, determining **absence** of BAT activation and/or browning of WAT to BAT in the subject .

[0006] According to one aspect, there is provided a method for detecting brown adipose tissues (BAT) activation and/or browning of white adipose tissues (WAT) to BAT in a subject, wherein the method comprises:

a. determining a baseline level of methylene tetrahydrofolate dehydrogenase 1-like (MTHFD1L) protein in a first blood sample obtained from the subject;

b. measuring a post-stimulation level of MTHFD1L protein in a second blood sample obtained from the subject after the subject has been exposed to a stimulation; and

b1. if the post-stimulation level of MTHFD1L protein is **higher** than the baseline level of MTHFD1L protein, determining **presence** of BAT activation and/or browning of WAT to BAT in the subject, or

b2. if the post-stimulation level of MTHFD1L protein is **the same or lower** than the baseline level of MTHFD1L protein, determining **absence** of BAT activation and/or browning of WAT to BAT in the subject .

[0007] According to one aspect, there is provided a qualitative BAT activity test kit for detecting brown adipose tissues (BAT) activation and/or browning of white adipose tissues (WAT) to BAT in a subject according to the method as disclosed herein, wherein the qualitative BAT activity test kit comprising:

- a test strip A made from porous material allowing for capillary flow of a buffer,
  - wherein the test strip A comprises a sample receiving region A and an active reagents region A,
  - wherein the active reagents region A is located downstream of the sample receiving region A,
  - wherein the active reagents region A comprises one or more active reagents capable of a visually detectable change in the presence of MTHFD1L protein, and
  - wherein the sample receiving region A is for application of a blood sample obtained from a subject **prior to** a stimulation,

and

- a test strip B made from porous material allowing for capillary flow of a buffer,
  - wherein the test strip B comprises a sample receiving region B and an active reagents region B,
  - wherein the active reagents region B is located downstream of the sample receiving area B,
  - wherein the active reagents region B comprises one or more active reagents capable of a visually detectable change in the presence of MTHFD1L protein,

and

- wherein the sample receiving region B is for application of a blood sample obtained from a subject **after** a stimulation,

wherein when the blood sample obtained from a subject **prior to** a stimulation and/or the blood sample obtained from a subject **after** a stimulation comprises MTHFD1L protein, the buffer is capable of dissolving said MTHFD1L protein,

wherein if the buffer comprises dissolved MTHFD1L protein, the dissolved MTHFD1L protein catalyzes a chemical reaction on the one or more active reagents capable of a visually detectable change in the presence of MTHFD1L protein thereby resulting in a visually detectable change in the active reagent region A and/or the active reagent region B which indicates the presence of MTHFD1L protein in the blood sample.

[0008] According to one aspect, there is provided an enzymatic chemistry based quantitative BAT activity test kit for detecting brown adipose tissues (BAT) activation and/or browning of white adipose tissues (WAT) to BAT in a subject according to the method as disclosed herein, wherein the enzymatic chemistry based quantitative BAT activity test kit comprises:

a. a sample collection stick, wherein the sample collection stick comprises one or more active reagents capable of undergoing an enzymatic reaction in the presence of MTHFD1L protein,
b. a detector system capable of detecting a product formed by the enzymatic reaction, and
c. a monitoring device capable of determining the level of MTHFD1L in the blood sample based on the amount of product formed by the enzymatic reaction.

[0009] According to one aspect, there is provided an antibody based quantitative BAT activity test kit for detecting brown adipose tissues (BAT) activation and/or browning of white adipose tissues (WAT) to BAT in a subject according to the method as disclosed herein, wherein the quantitative BAT activity test kit comprises:

a. a sample collection stick, wherein the sample collection stick comprises a capture antibody capable of binding to MTHFD1L protein and a detection antibody capable of binding to a capture antibody-MTHFD1L protein complex, wherein the detection antibody is connected to a tag, wherein the tag is a fluorescent tag capable of emitting

fluorescence in the presence of MTHFD1L protein or a tag capable of undergoing a color change in the presence of MTHFD1L protein,

wherein when the tag is a tag capable of undergoing a color change in the presence of MTHFD1L protein, the sample collection stick further comprises a substrate capable of reacting with the tag,

b. a detector system capable of detecting the fluorescence or the color change formed by the tag, and

c. a monitoring device capable of determining the level of MTHFD1L in the blood sample based on the amount of fluorescence formed by the tag or based on the intensity of color change formed by the tag.

[0010] According to one aspect, there is provided a method for detecting or monitoring a metabolic disorder or metabolic syndrome that affect BAT activity in a subject using the method as disclosed herein, the qualitative BAT activity test kit as disclosed herein, the enzymatic chemistry based quantitative BAT activity test kit as disclosed herein, and/or the antibody based quantitative BAT activity test kit as disclosed herein.

[0011] According to one aspect, there is provided method of discovering a drug, a nutraceutical, and/or a functional food that activate BAT and/or induce browning of WAT to BAT in a subject using the method as disclosed herein, wherein the stimulation is the drug, the nutraceutical, and/or the functional food; and

wherein if the post-stimulation level of MTHFD1L protein is **higher** than the baseline level of MTHFD1L protein, determine that the drug, the nutraceutical, and/or the functional food activates BAT and/or induces browning of WAT to BAT in a subject.

DEFINITION OF TERMS

[0012] The following words and terms used herein shall have the meaning indicated:

[0013] The term “brown adipose tissue” or “BAT” refers to a form or a type of adipose tissue that is found in almost all mammals important for thermoregulation. “Brown adipose tissue” or “BAT” is thermogenic and consumes substantial amounts of glucose and fatty acids as fuel for thermogenesis and energy expenditure. Therefore, it may play a role in combating obesity and/or metabolic disease.

CLAIMS

1. A method for detecting brown adipose tissues (BAT) activation and/or browning of white adipose tissues (WAT) to BAT in a subject, wherein the method comprises:

a. determining a baseline level of methylene tetrahydrofolate dehydrogenase 1-like (MTHFD1L) protein in a first blood sample obtained from the subject;

b. measuring a post-stimulation level of MTHFD1L protein in a second blood sample obtained from the subject after the subject has been exposed to a stimulation; and

b1. if the post-stimulation level of MTHFD1L protein is higher than the baseline level of MTHFD1L protein, determining presence of BAT activation and/or browning of WAT to BAT in the subject, or

b2. if the post-stimulation level of MTHFD1L protein is the same or lower than the baseline level of MTHFD1L protein, determining absence of BAT activation and/or browning of WAT to BAT in the subject.

2. The method of claim 1, wherein the blood sample is plasma, serum, or whole blood.

3. The method of claim 1 or 2, wherein the subject is a mammal.

4. The method of claim 3, wherein the mammal is a human or a rat.

5. The method any one of claims 1 to 4, wherein the stimulation is a stimulation or a combination of stimulations that results in BAT activation and/or browning of WAT to BAT.

6. The method of claim 5, wherein the stimulation that results in BAT activation and/or browning of WAT to BAT is capsinoid stimulation, cold stimulation, and/or hyperthyroid treatment.

7. A qualitative BAT activity test kit for detecting brown adipose tissues (BAT) activation and/or browning of white adipose tissues (WAT) to BAT in a subject according to the method of any one of claims 1 to 6, wherein the qualitative BAT activity test kit comprising:

- a test strip A made from porous material allowing for capillary flow of a buffer,

- wherein the test strip A comprises a sample receiving region A and an active reagents region A,
- wherein the active reagents region A is located downstream of the sample receiving region A,
- wherein the active reagents region A comprises one or more active reagents capable of a visually detectable change in the presence of MTHFD1L protein, and
- wherein the sample receiving region A is for application of a blood sample obtained from a subject prior to a stimulation,

and

- a test strip B made from porous material allowing for capillary flow of a buffer,
  - wherein the test strip B comprises a sample receiving region B and an active reagents region B,
  - wherein the active reagents region B is located downstream of the sample receiving area B,
  - wherein the active reagents region B comprises one or more active reagents capable of a visually detectable change in the presence of MTHFD1L protein,

  and
  - wherein the sample receiving region B is for application of a blood sample obtained from a subject after a stimulation,

wherein when the blood sample obtained from a subject prior to a stimulation and/or the blood sample obtained from a subject after a stimulation comprises MTHFD1L protein, the buffer is capable of dissolving said MTHFD1L protein,

wherein if the buffer comprises dissolved MTHFD1L protein, the dissolved MTHFD1L protein catalyzes a chemical reaction on the one or more active reagents capable of a visually detectable change in the presence of MTHFD1L protein thereby resulting in a visually detectable change in the active reagent region A and/or the active reagent region B which indicates the presence of MTHFD1L protein in the blood sample.

8. The qualitative BAT activity test kit of claim 7, wherein the visually detectable change is a color change.

9. The qualitative BAT activity test kit of claim 7 or 8, wherein the active reagents capable of a visually detectable change in the presence of MTHFD1L protein are tetrahydrofolate (THF), adenosine triphosphate (ATP), formate, formyltetrahydrofolate dehydrogenase (FDH), and ultrasensitive pH colorimetric indicator.

10. The qualitative BAT activity test kit of any one of claims 7 to 9, wherein when the active reagents capable of a visually detectable change in the presence of MTHFD1L protein are tetrahydrofolate (THF), adenosine triphosphate (ATP), formate, formyltetrahydrofolate dehydrogenase (FDH), and ultrasensitive pH colorimetric indicator, the visually detectable change is a change from a blue band to a red band.

11. An enzymatic chemistry based quantitative BAT activity test kit for detecting brown adipose tissues (BAT) activation and/or browning of white adipose tissues (WAT) to BAT in a subject according to the method of any one of claims 1 to 6, wherein the enzymatic chemistry based quantitative BAT activity test kit comprises:

a. a sample collection stick, wherein the sample collection stick comprises one or more active reagents capable of undergoing an enzymatic reaction in the presence of MTHFD1L protein,
b. a detector system capable of detecting a product formed by the enzymatic reaction, and
c. a monitoring device capable of determining the level of MTHFD1L in the blood sample based on the amount of product formed by the enzymatic reaction.

12. The enzymatic chemistry based quantitative BAT activity test kit of claim 11, wherein the active reagents capable of undergoing enzymatic reaction in the presence of MTHFD1L protein are tetrahydrofolate (THF), adenosine triphosphate (ATP), and formate.

13. The enzymatic chemistry based quantitative BAT activity test kit of claim 11 or 12, wherein the detector system capable of detecting a product formed by the enzymatic reaction is a detector capable of detecting formyl-THF, a colorimetric detector system capable of detecting CO2, or a sensitive microchip of semi-conductor device capable of detecting a change of electrical current and voltage generated due to electron transfers of a redox reaction.

14. An antibody based quantitative BAT activity test kit for detecting brown adipose tissues (BAT) activation and/or browning of white adipose tissues (WAT) to BAT in a subject according to the method of any one of claims 1 to 6, wherein the quantitative BAT activity test kit comprises:

a. a sample collection stick, wherein the sample collection stick comprises a capture antibody capable of binding to MTHFD1L protein and a detection antibody capable of binding to a capture antibody-MTHFD1L protein complex, wherein the detection antibody is connected to a tag, wherein the tag is a fluorescent tag capable of emitting fluorescence in the presence of MTHFD1L protein or a tag capable of undergoing a color change in the presence of MTHFD1L protein,
wherein when the tag is a tag capable of undergoing a color change in the presence of MTHFD1L protein, the sample collection stick further comprises a substrate capable of reacting with the tag,
b. a detector system capable of detecting the fluorescence or the color change formed by the tag, and
c. a monitoring device capable of determining the level of MTHFD1L in the blood sample based on the amount of fluorescence formed by the tag or based on the intensity of color change formed by the tag.

15. The antibody based quantitative BAT activity test kit of claim 14, wherein the tag capable of undergoing a color change in the presence of MTHFD1L protein is a horseradish peroxidase or an alkaline phosphatase.

16. The antibody based quantitative BAT activity test kit of claim 14 or 15, wherein the substrate capable of reacting with the tag is tetramethylbenzidine (TMB).

17. A method for detecting or monitoring a metabolic disorder or metabolic syndrome that affect BAT activity in a subject using the method of any one of claims 1 to 6, the qualitative BAT activity test kit of any one of claims 7 to 10, the enzymatic chemistry based quantitative BAT activity test kit of any one of claims 11 to 13, and/or the antibody based quantitative BAT activity test kit of any one of claims 14 to 16.

18. The method of claim 17 wherein the metabolic disorder or metabolic syndrome that affect BAT activity is selected from the group consisting of thyroid dysfunction, obesity, diabetes, and pheochromocytoma.

19. A method of discovering a drug, a nutraceutical, and/or a functional food that activate BAT and/or induce browning of WAT to BAT in a subject using the method of any one of claims 1 to 6, wherein the stimulation is the drug, the nutraceutical, and/or the functional food; and

wherein if the post-stimulation level of MTHFD1L protein is higher than the baseline level of MTHFD1L protein, determine that the drug, the nutraceutical, and/or the functional food activates BAT and/or induces browning of WAT to BAT in a subject.