need to accurately and reliably measure BHB levels in a subject. This can be through invasive, minimally invasive, or non-invasive mechanisms. The present disclosure solves this need by describing novel enzymes for aiding in the measurement of BHB. Useful systems, devices, and associated methods are further provided herein.

BRIEF SUMMARY

[0006] The instant disclosure relates to enzymes with oxidase activity. In an example, enzymes described herein are engineered and/or modified relative to native enzymes. In examples described herein enzymes are capable of oxidizing beta-hydroxybutyrate (BHB) to produce 3-oxobutanoate. In some examples, beta-hydroxybutyrate (BHB) is (R)-beta-hydroxybutyrate. In some examples, beta-hydroxybutyrate (BHB) is (S)-beta-hydroxybutyrate. In some examples, beta-hydroxybutyrate (BHB) is a mixture of (R)-beta-hydroxybutyrate and (S)-beta-hydroxybutyrate.

[0007] In some examples, the enzyme, for example an engineered enzyme, includes (e.g., comprises, consists essentially of or consists of) an amino acid sequence derived from an oxidase of the EC 1.1.3.6 family. In some examples, the oxidase from the EC 1.1.3.6 family is a cholesterol oxidase.

[0008] In some examples, the enzyme is non-naturally occurring and/or engineered and includes (e.g., comprises, consists essentially of or consists of) one or more amino acid substitutions, deletions, or truncations relative to a native enzyme.

[0009] In an example, the enzyme described herein covers modified Oxidase 8, derived from *Scytonema sp.* In another example, the enzyme is a modified version of SEQ ID NO: 8 which includes one or more amino acid substitutions, deletions, or truncations. In an example, SEQ ID NO: 8 is optimized to yield improved BHB detection activity.

[0010] In some examples, the enzyme includes (e.g., comprises, consists essentially of or consists of) an amino acid sequence encompassing one or more mutations corresponding to N137G, Y235Q, and/or A455Y of SEQ ID NO: 8. In other examples, enzymes for use herein include SEQ ID NO: 1 – 43. In some examples, enzymes for use herein include (e.g., comprise, consist essentially of or consist of) any of SEQ ID NO: 24 or SEQ ID NO: 40 – 43, fragments, or modified proteins therein.

[0011] In some examples, the enzyme includes (e.g., comprises, consists essentially of or consists of) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any of SEQ ID NO: 1 – 43. In some examples, the enzyme includes (e.g., comprises, consists essentially of or consists of) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any of SEQ ID NO: 24 or SEQ ID NO: 40 – 43, fragments, or modified proteins therein.

[0012] In additional examples, the enzyme includes (e.g., comprises, consists essentially of or consists of) an amino acid sequence of any of SEQ ID NO: 1 – 43, wherein the amino acid sequence includes one, two, three, four, five, six, seven, eight, nine, ten, fifteen or more amino acid substitutions. In some examples, the enzyme includes an amino acid sequence of any of SEQ ID NO: 24 or 40 – 43, wherein the amino acid sequence includes (e.g., comprises, consists essentially of or consists of) one, two, three, four, five, six, seven, eight, nine, ten, fifteen or more amino acid substitutions. In examples, the amino acid substitution(s) are conservative substitutions. In examples the substitutions are made only to the portions of the protein which are responsible for binding and/or propagating activity.

[0013] In an example, the disclosure provides for a protein including (e.g., comprising, consisting essentially of or consisting of) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any of SEQ ID NO: 1 – 43. In some examples, the disclosure provides for an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any of SEQ ID NO: 24 or SEQ ID NO: 40 – 43, fragments, or modified proteins therein.

[0014] In additional examples, the disclosure provides for a protein including (e.g., comprising, consisting essentially of or consisting of) an amino acid sequence of any of SEQ ID NO: 1 – 43, wherein the amino acid sequence includes (e.g., comprises, consists essentially of or consists of) one, two, three, four, five, six, seven, eight, nine, ten, or fifteen or more amino acid substitutions. In some examples, the disclosure provides for a protein with an amino acid sequence of any of SEQ ID NO: 24 or 40 – 43, wherein the amino acid sequence includes one, two, three, four, five, six, seven, eight, nine, ten, or fifteen or more amino acid

substitutions. In examples, the amino acid substitution(s) are conservative substitutions.

[0015] In additional examples, engineered or modified enzymes described herein exhibit increased BHB activity relative to the corresponding wild-type enzyme. As used herein, wild-type, wildtype, wild type, and WT are all synonymous and refer to an enzyme found naturally (i.e., in its wild or state in nature). As used herein, mutant, mut, MUT are all synonymous and refer to a modified enzyme and/or a mutated enzyme, such as a modified and/or mutant enzyme created by enzyme design and/or enzyme engineering. Directed evolution, rational design, and semirational design are the major in silico assisted approaches used widely in enzyme engineering. In an example, the BHB activity is measured by peroxide strips, acetoacetic strips, and/or liquid chromatography–mass spectrometry (LCMS). In an example, the peroxide strips are Bartovation strips and the acetoactic strips are Bayer Ketostix® strips. In an examples, the BHB activity for the modified or engineered enzyme is about one (1), two (2), three (3), four (4), five (5), six (6), seven (7), eight (8), nine (9), ten (10), twenty (20), thirty (30), fourty (40), fifty (50), sixty (60), seventy (70), eighty (80), ninety (90), one hundred (100), two hundred (200), three hundred (300), four hundred (400), five hundred (500), six hundred (600), seven hundred (700), eight hundred (800), nine hundred (900), one thousand (1,000), ten thousand (10,000), one hundred thousand (100,000) or more times greater than the corresponding non-modified wild-type when measured by LCMS.

[0016] In another example, SEQ ID NO: 24 or 40 – 43 and derivatives thereof exhibit an increased BHB activity relative to SEQ ID NO: 8. In an example, the BHB activity is measured by peroxide strips, acetoacetic strips, or liquid chromatography–mass spectrometry (LCMS). In an examples, the peroxide strips are Bartovation strips and the acetoactic strips are Bayer Ketostix® strips. In an examples, the BHB activity for SEQ ID NO: 24 or 40 – 43 and their derivatives is about one (1), two (2), three (3), four (4), five (5), six (6), seven (7), eight (8), nine (9), ten (10), twenty (20), thirty (30), fourty (40), fifty (50), sixty (60), seventy (70), eighty (80), ninety (90), one hundred (100), two hundred (200), three hundred (300), four hundred (400), five hundred (500), six hundred (600), seven hundred (700), eight hundred (800), nine hundred (900), one thousand (1,000), ten thousand (10,000), one hundred thousand (100,000) or more times greater than SEQ ID NO: 8 when measured by LCMS.

[0017] In another example, SEQ ID NO: 24 exhibits increased BHB activity relative to SEQ ID NO: 8. In an example, the BHB activity is measured by peroxide strips, acetoacetic strips, or liquid chromatography–mass spectrometry (LCMS). In an examples, the peroxide strips are Bartovation strips and the acetoactic strips are Bayer Ketostix® strips. In an examples, the BHB activity for SEQ ID NO: 24 is about one (1), two (2), three (3), four (4), five (5), six (6), seven (7), eight (8), nine (9), ten (10), twenty (20), thirty (30), fourty (40), fifty (50), sixty (60), seventy (70), eighty (80), ninety (90), one hundred (100), two hundred (200), three hundred (300), four hundred (400), five hundred (500), six hundred (600), seven hundred (700), eight hundred (800), nine hundred (900), one thousand (1,000), ten thousand (10,000), one hundred thousand (100,000) or more times greater than SEQ ID NO: 8 when measured by LCMS.

[0018] In examples, enzymes described herein are non-naturally occurring, engineered, isolated, and/or purified.

[0019] The disclosure further provides for sensors capable of detecting and/or measuring BHB concentration, including utilizing an enzyme described herein, for non-wearable sensing systems. An example format of a non-wearable sensing system is a test strip, which detects and or measures BHB concentration. The test strip may be single or multiple use.

[0020] In some examples, the test strip includes: a substrate layer; and one or more sensing reagents dispensed upon at least a portion of the substrate layer, wherein at least one of the sensing reagents includes the enzyme.

[0021] In some examples, the at least one of the sensing reagents further includes a co-factor, a mediator, and/or another adjuvant or excipient. In some examples, the co-factor includes flavin adenine dinucleotide (FAD). In some examples, the mediator includes one or more of ferricyanide, phenazine, phenothiazine, thionine, methylene green/blue, tetrathiafulvalene, quinone derivatives, ferrocene, organometallic osmium complexes, and/or organometallic ruthenium complexes.

[0022] In some examples, the material of the substrate layer includes one or more of a composite material, a fibrous material, a woven textile, a non-woven textile, a polymer, an adhesive, a film, a gel, PTFE, and /or silicone.

[0023] The sensor, for example, a non-wearable sensor, can be used to detect or measure BHB in any body fluid. The fluid can be obtained through non-invasive,

minimally invasive, or invasive means, such as by finger stick, blood draw, spinal tap, as examples of invasive means, or sweat or saliva collection, as examples of non-invasive or minimally invasive means. Body fluid refers to all body fluid including but not limited to whole blood, serum, plasma, a blood fraction other than serum or plasma, lymph, cerebrospinal fluid (CSF), interstitial fluid (ISF), intracellular fluid, transcellular fluid, saliva, tears, sweat, vaginal discharge, milk, mucus, chyme, pus, bile, semen, urine, amniotic fluid, synovial fluid, peritoneal fluid, pericardial fluid, peritoneum, glandular secretions, exudate, contents of cysts, and/or ascites.

[0024] The disclosure further provides for sensors capable of detecting and/or measuring BHB concentration, including utilizing an enzyme described herein, for continuous, continual, or on-demand detection or measurement. Continuous is meant to be uninterrupted; unbroken; not intermittent or occasional; so persistently repeated at short intervals as to constitute virtually an unbroken series. Continual means repeated regularly and frequently in a steady suggestion. On demand means the detection or measurement can readily be obtained when the sensor or display of sensor data is actuated by the user.

[0025] In some examples, the BHB sensor capable of sensing, detecting, and/or measuring BHB concentration includes: a sensor, comprising a sensing electrode; a sensing reagent, wherein the sensing reagent composition includes an enzyme described herein; and a reference electrode. In some examples, the sensing reagent is on or adjacent to the sensing electrode. In some examples, the sensing reagent is dispensed to be in contact with the sensing electrode at the time of use.

[0026] In some examples, wherein the sensing reagent composition further includes a co-factor and/or a mediator.

[0027] In some examples, the BHB sensor is configured as a wearable sensor to measure a BHB concentration of a subject and output a data stream in a continuous, continual, or on-demand fashion. In some examples, some or all of the sensors are to be located within body tissue (in-the-body sensors) to measure fluid in that tissue. Body tissue can include any tissue, including, but not limited to epidermal, dermal, subcutaneous, muscular, intraperitoneal space. Example examples of in-the-body sensors include, but are not limited to, a continuous BHB sensor in a needle-based sensor, a microneedle-based sensor, or an implantable sensor. In other examples, the BHB sensor is configured to measure

BHB in body fluid that has been sampled from the body. The sample can be obtained through non-invasive, minimally invasive, or invasive means. Body fluid refers to all body fluid including but not limited to whole blood, serum, plasma, a blood fraction other than serum or plasma, lymph, cerebrospinal fluid (CSF), interstitial fluid (ISF), intracellular fluid, transcellular fluid, saliva, tears, sweat, vaginal discharge, milk, mucus, chyme, pus, bile, semen, urine, amniotic fluid, synovial fluid, peritoneal fluid, pericardial fluid, peritoneum, glandular secretions, exudate, contents of cysts, and/or ascites. The body fluids can either be from direct contact within body tissue internal to the body or from body fluids that are sampled either non-invasively or invasively from the body and sensed “on-body”.

[0028] The disclosure further relates to a system for detecting and measuring BHB concentration by utilizing an enzyme described herein.

[0029] In some examples, the system for detecting and measuring BHB concentration includes: a BHB sensor, wherein the BHB sensor is configured to continuously, continually, or on-demand measure a BHB concentration of a subject and output a data stream; a sensor can use any method of BHB-measurement, including enzymatic, chemical, physical, electrochemical, spectrophotometric, polarimetric, calorimetric, iontophoretic, radiometric, immunochemical, and the like; and a device connected to the BHB sensor, wherein the device comprises: a processor configured to process the data stream from the BHB sensor; and an interface configured to display and/or communicate measured BHB concentration values.

[0030] The disclosure further relates to a method of detecting and measuring BHB concentration by utilizing an enzyme described herein.

[0031] In some examples, the method of detecting and measuring BHB concentration includes: obtaining a body fluid from a subject; subjecting the body fluid to a BHB sensor; determining a single point BHB concentration in the body fluid; and displaying and/or communicating the single point BHB concentration on an interface.

[0032] In other examples, the enzymes, devices, or systems described herein are used in methods of improving health and wellness in a subject in need thereof. In other examples, the enzymes, devices, or systems described herein are used in methods of weight loss. In other examples, enzymes, devices, or systems described herein are used for improving mental and/or metabolic health. In yet other

examples, enzymes, devices, or systems described herein are used in methods of controlling intake or monitoring of carbohydrates. In other examples, enzymes, devices, or systems described herein are used in methods of monitoring ketone body levels for improvement of cognitive function, to treat various neurological disorders, such as epilepsy, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), traumatic brain injury, Alzheimer's disease, and dementias, to treat mental health, mental illness, psychiatric problems, and psychiatric disorders such as depression, bi-polar disorders, schizophrenia, to improve cardiac metabolism, improve immunotherapeutic, chemotherapeutic and radiotherapeutic response to cancer treatments, modulate inflammatory pathways and immune function, to treat obesity and diabetes, for cholesterol quantification and monitoring, and to monitor and detect alcoholic or diabetic ketoacidosis.

[0033] In other examples, enzymes, devices, or systems described herein are used in conjunction with other weight loss or wellness methods or therapies. In other examples, enzymes, devices, or systems described herein are used in conjunction with food or supplements which contain a low carbohydrate amount or sugar alcohols in the formulation.

[0034] In some examples, the food item or supplement comprises a ketone supplement or additive. In another example, the food item or supplement comprises an exogenous ketone and/or ketogenic supplement. In some examples, the exogenous ketone and/or ketogenic supplement comprises ketone bodies and/or precursors of ketone bodies. In some examples, the ketone bodies and/or precursors of ketone bodies comprise one or more of acetone, acetoacetic acid, beta-hydroxybutyrate (BHB), beta-ketopentanoate, beta-hydroxypentanoate, 1,3-butanediol, and medium chain triglycerides (MCT) containing fatty acids with hydrocarbon side chains in the length of 6-12 carbons. In some examples, the ketone bodies and/or precursors of ketone bodies are in the form of salts and/or esters. In some examples, the MCT comprises one or more of the following: caproic acid (C6), caprylic acid (C8), capric acid (C10), and lauric acid (C12).

[0035] In examples, devices, systems, or methods described herein are prescribed to a subject in need thereof by a medical professional. In other examples, the patient the patient in need thereof is obese or afflicted with an underlying medical condition such as heart disease.

CLAIMS

1. An engineered enzyme with oxidase activity comprising an amino acid sequence at least 85% identical to SEQ ID NO: 8 and comprising at least three amino acid substitutions relative to SEQ ID NO: 8, wherein said engineered enzyme is engineered to exhibit improved beta-hydroxybutyrate (BHB) activity as compared to an enzyme comprising SEQ ID NO: 8.

2. The engineered enzyme of claim 1, wherein the amino acid sequence of the engineered enzyme comprises one or more amino acid substitutions corresponding to N137G, Y235Q, and/or A455Y of SEQ ID NO: 8.

3. The engineered enzyme of any one of claims 1 – 2, wherein the amino acid sequence of the engineered enzyme comprises an N-terminal truncation of at most 3 amino acids, at most 5 amino acids, at most 10 amino acids, at most 15 amino acids, at most 20 amino acids, at most 25 amino acids, at most 26 amino acids, at most 27 amino acids, at most 28 amino acids, at most 29 amino acids, at most 30 amino acids, at most 31 amino acids, at most 32 amino acids, or at most 35 amino acids in one or more of SEQ ID NO: 8.

4. The engineered enzyme of any one of claims 1 – 2, wherein the amino acid sequence of the engineered enzyme comprises an N-terminal truncation corresponding to amino acid positions 2-32 of SEQ ID NO: 8.

5. The engineered enzyme of any one of claims 1 – 4 comprising an amino acid sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, or at least 99.8% identical to SEQ ID NO: 8.

6. The engineered enzyme of any one of claims 1 – 4, wherein said amino acid sequence comprises at most three, at most four, at most five, at most six, at

most seven, at most eight, at most nine, or at most ten amino acid substitutions.

7. The engineered enzyme of claim 6, wherein the amino acid substitutions are made only to the portions of the protein which are responsible for binding and/or propagating BHB activity.

8. The engineered enzyme of any one of claims 6 or 7 wherein the amino acid substitution(s) are conservative substitutions.

9. The engineered enzyme of anyone of claims 1 – 7, wherein the engineered enzyme exhibits increased BHB activity as measured by peroxide strips, acetoacetic strips, or liquid chromatography–mass spectrometry (LCMS).

10. The engineered enzyme of claim 9, wherein the engineered enzyme exhibits increased BHB activity as measured by LCMS.

11. The engineered enzyme of claim 10, wherein the engineered enzyme exhibits about one, about two, about three, about four, about five, about ten, or about twenty times greater BHB activity than the enzyme comprising SEQ ID NO: 8 when measured by LCMS.

12. The engineered enzyme of claim 10, wherein the engineered enzyme comprises a sequence at least 90%, at least 95%, or at least 98% identitcal to SEQ ID NO: 24 and wherein SEQ ID NO: 24 exhibits increased BHB activity as measured by liquid chromatography–mass spectrometry (LCMS) as compared to SEQ ID NO: 8.

13. The engineered enzyme of claim 1, wherein the beta-hydroxybutyrate is (R)-beta-hydroxybutyrate.

14. The engineered enzyme of claim 1, wherein the beta-hydroxybutyrate is (S)-beta-hydroxybutyrate.

15. The engineered enzyme of claim 1, wherein the beta-hydroxybutyrate is a mixture of (R)-beta-hydroxybutyrate and (S)-beta-hydroxybutyrate.

16. A non-wearable BHB sensor capable of detecting and/or measuring BHB concentration comprising utilizing the enzyme of any one of claims 1 – 15.

17. The non-wearable BHB sensor of claim 16, wherein the non-wearable BHB sensor is a test strip.

18. The test strip of claim 17, wherein said test strip comprises:

a substrate layer; and

one or more sensing reagents dispensed upon at least a portion of the substrate layer, wherein at least one of the sensing reagents comprise an engineered enzyme of any one of claims 1 – 15.

19. The test strip of claim 17 or 18, wherein the at least one sensing reagents comprises a co-factor, a mediator, an adjuvant, a carrier, and/or an excipient.

20. The test strip of claim 19, wherein the co-factor comprises flavin adenine dinucleotide (FAD).

21. The test strip of any one of claims 19 – 20, wherein the mediator comprises one or more of ferricyanide, phenazine, phenothiazine, thionine, methylene green/blue, tetrathiafulvalene, quinone derivatives, ferrocene, organometallic osmium complexes, and/or organometallic ruthenium complexes.

22. The test strip of any one of claims 18 – 21, wherein a material of the substrate layer comprises one or more of a composite material, a fibrous material, a woven textile, a non-woven textile, a polymer, an adhesive, a film, a gel, PTFE, and /or silicone.

23. The non-wearable BHB sensor of claim 16, wherein the non-wearable sensor is a fingertip sweat sensor.

24. The non-wearable BHB sensor of claim 16, wherein the non-wearable sensor can be used to detect or measure BHB in any body fluid.

25. The non-wearable BHB sensor of claim 24, wherein the body fluid can be obtained through non-invasive, minimally invasive, or invasive means, optionally via finger stick, blood draw, spinal tap, sweat and/or saliva collection.

26. The non-wearable BHB sensor of claim 25, wherein the body fluid comprises one or more of whole blood, serum, plasma, a blood fraction other than serum or plasma, lymph, cerebrospinal fluid (CSF), interstitial fluid (ISF), intracellular fluid, transcellular fluid, saliva, tears, sweat, vaginal discharge, milk, mucus, chyme, pus, bile, semen, urine, amniotic fluid, synovial fluid, peritoneal fluid, pericardial fluid, peritoneum, glandular secretions, exudate, contents of cysts, and/or ascites.

27. A wearable BHB sensor capable of detecting, measuring, and/or monitoring BHB concentration, comprising:

a sensor, comprising a sensing electrode;

a sensing reagent, wherein the sensing reagent composition comprises the engineered enzyme of any one of claims 1 – 15; and

a reference electrode.

28. The wearable BHB sensor of claim 27, wherein the sensing reagent is on or adjacent to the sensing electrode.

29. The wearable BHB sensor of claim 27, wherein the sensing reagent is dispensed to be in contact with the sensing electrode at the time of use.

30. The wearable BHB sensor of claim 27, wherein the wearable BHB sensor is a continuous sensor, a continual sensor, or an on-demand sensor.

31. The wearable BHB sensor of any one of claims 27 – 30, wherein the sensing reagent composition further comprises a co-factor, a mediator, an adjuvant, a carrier, and/or an excipient.

32. The wearable BHB sensor of any one of claims 27 – 31, wherein the wearable BHB sensor is configured to continuously measure a BHB concentration of a subject and output a data stream.

33. The wearable BHB sensor of any one of claims 27 – 32, wherein the wearable BHB sensor is a needle-based sensor, a micro-needle-based sensor, a reverse iontophoretic sensor, a sweat-based sensor, or an implantable sensor.

34. The wearable BHB sensor of claim 33, wherein the wearable BHB sensor is located in body tissues.

35. The wearable BHB sensor of any of claims 27 – 34, wherein the wearable BHB sensor is configured to measure a BHB concentration in a body fluid.

36. The wearable BHB sensor of claim 35, wherein the body fluid comprises one or more of whole blood, serum, plasma, a blood fraction other than serum or plasma, lymph, cerebrospinal fluid (CSF), interstitial fluid (ISF), intracellular fluid, transcellular fluid, saliva, tears, sweat, vaginal discharge, milk, mucus, chyme, pus, bile, semen, urine, amniotic fluid, synovial fluid, peritoneal fluid, pericardial fluid, peritoneum, glandular secretions, exudate, contents of cysts, and/or ascites.

37. The wearable BHB sensor of any of claims 27 – 36, wherein the wearable BHB sensor is an in-body wearable BHB sensor or an on-body wearable BHB sensor.

38. A system for detecting, measuring, and/or monitoring BHB concentration, comprising:

the wearable BHB sensor of any one of claims 27 – 37, wherein the wearable BHB sensor is configured to continuously, continually, or on-demand measure a BHB concentration of a subject and output a data stream; and

a device connected to the wearable BHB sensor, wherein the device comprises:

a processor configured to process the data stream from the wearable BHB sensor; and

an interface configured to display and/or communicate measured BHB concentration values.

39. A method of detecting, measuring, and/or monitoring BHB concentration, comprising:

a body fluid to be obtained from a subject;

subjecting the body fluid to the wearable BHB sensor of any one of claims 27 – 37;

determining a single point BHB concentration in the body fluid; and

displaying and/or communicating the single point BHB concentration on an interface.

40. A method of improving health and/or wellness in a subject in need thereof comprising using a non-wearable BHB sensor, device, or apparatus of any one of claims 16 – 26.

41. A method of improving health and/or wellness in a subject in need thereof comprising using a test kit, wherein the test kit utilizes the enzyme of any one of claims 1 – 15.

42. A method of aiding weight loss in a subject in need thereof comprising using a non-wearable BHB sensor, device, or apparatus of any one of claims 16 – 26.

43. The method of any one of claims 40-42 used in conjunction with a food item or supplement comprising at least one sugar alcohol or low carbohydrate composition.

44. The method of anyone of claims 40 – 43, wherein the food item or supplement contain less than about 5 grams of carbohydrates, less than about 8 grams of carbohydrates, or less than about 10 grams of carbohydrates per unit.

45. The method of any one of claims 43 and 44 used in conjunction with a food item or supplement, wherein the food item or supplement comprises exogenous ketone supplement and/or ketogenic ingredients.

46. The method of claim 45, wherein the exogenous ketone supplement comprises ketone bodies and/or precursors of ketone bodies.

47. The method of claim 46, wherein the ketone bodies and/or precursors of ketone bodies comprise one or more of acetone, acetoacetic acid, beta-hydroxybutyrate (BHB), beta-ketopentanoate, beta-hydroxypentanoate, 1,3-butanediol, and medium chain triglycerides (MCT) containing fatty acids with hydrocarbon side chains in the length of 6-12 carbons.

48. The method of claim 47, wherein the ketone bodies and/or precursors of ketone bodies are in the form of salts and/or esters.

49. The method of claim 47, wherein the MCT comprises one or more of the following: caproic acid (C6), caprylic acid (C8), capric acid (C10), and lauric acid (C12).