

**METHODS AND COMPOSITIONS FOR MAINTAINING SKELETAL MUSCLE MOBILITY
AND/OR MASS, AND/OR FOR RECOVERY OF SKELETAL MUSCLE STRENGTH**

[0001] The present invention claims priority to United States Provisional Patent Application No. 63/517,178, filed on August 2, 2023, the entirety of which is incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The present invention relates to methods for maintaining skeletal muscle mobility and/or mass, and/or for recovery of skeletal muscle strength in an individual in need thereof. The present invention also relates to nutritional compositions for maintaining skeletal muscle mobility and/or mass, and/or for recovery of skeletal muscle strength in an individual in need thereof which employ β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol.

BACKGROUND OF THE INVENTION

[0003] Aging is generally associated with numerous changes that may, directly or indirectly, affect health and/or life span. One of the major problems in the aging population is sarcopenia: a progressive loss in skeletal muscle mass, muscle strength, and functionality. Generally, aging is associated with a chronic state of slightly increased plasma levels of pro-inflammatory mediators, often referred to as a low-grade inflammation. It has been demonstrated that inflammatory cytokines activate skeletal muscle breakdown while repressing muscle protein synthesis, leading to net loss of muscle mass and strength in individuals, and subsequently problems in walking, and, in turn, disability.

[0004] The term "inflammaging" has been used to refer to the chronic low-grade inflammation typical of aging. Inflammaging is implicated in the pathogenesis of several diseases, such as type-2 diabetes, osteoporosis, Alzheimer's disease, rheumatoid arthritis, and coronary heart disease. Inflammaging can also result in individuals being susceptible to conditions such as sepsis and COPD. In addition, high-grade inflammation has also been

associated with lower muscle mass and lower muscle strength. From a physiological perspective, inflammation, particularly in older populations, has been linked to dysregulation of protein metabolism as it has been demonstrated to induce a hypercatabolic state which leads to reduced muscle mass. Inflammation does not only negatively affect muscle mass, but also muscle strength and function, including mobility.

[0005] Dietary polyphenols have shown potent antioxidant and anti-inflammatory properties. In particular, hydroxytyrosol, which is present in olive fruit, leaves and oil, has shown anti-inflammatory, antioxidant, cardioprotective, and anti-atherogenic properties. In particular, hydroxytyrosol has been shown to inhibit the development of inflammatory cascade following lipopolysaccharide (LPS) administration.

[0006] HMB, which is a metabolite of the amino acid leucine, is known to regulate protein synthesis and degradation processes. In addition, it has been shown that HMB administration attenuated sepsis-induced diaphragm weakness in an animal model of endotoxin-induced sepsis, preserving muscle force generation.

[0007] While there have been previous interventions to combat sarcopenia, age-related muscle wasting, and improving muscle strength and functions, over catabolic conditions and diseases, most treatment options have focused on resistance training and/or protein supplementation to overcome the anabolic inflexibility from which the elderly or individuals in a pro-inflammatory state suffer. However, considering the pro-inflammatory state that certain individuals, particularly elderly populations face, promoting skeletal muscle strength and function remains a challenge.

[0008] In view of the above, there is an urgent need to develop new and accessible technologies that stimulate skeletal muscle mass and/or muscle strength recovery.

[0009] Accordingly, methods and compositions that stimulate skeletal muscle mass and/or muscle strength recovery are desired.

SUMMARY OF THE INVENTION

[0010] It is therefore an object of the invention to provide a convenient means for maintaining skeletal muscle mobility and/or mass, and/or for recovery of skeletal muscle strength in an individual in need thereof.

[0011] In one embodiment, the invention is directed to a method for maintaining skeletal muscle mobility and/or mass, and/or for recovery of skeletal muscle strength in an individual in need thereof, comprising administering β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol to the individual.

[0012] In another embodiment, the invention is directed to a nutritional composition for maintaining skeletal muscle mobility and/or mass, and/or for recovery of skeletal muscle strength in an individual in need thereof comprising HMB, at least one olive polyphenol, and at least one of: at least one source of protein, at least one source of fat, at least one source of carbohydrate.

[0013] The methods for maintaining skeletal muscle mobility and/or mass, and/or for recovery of skeletal muscle strength, as well as the nutritional compositions according to the present invention, are advantageous in that they, for example, maintain skeletal muscle mobility and/or mass, and/or stimulate recovery of skeletal muscle strength in a convenient manner. These and additional objects and advantages of the invention will be more fully apparent in view of the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The drawings are illustrative of certain embodiments of the invention and are exemplary in nature and are not intended to limit the invention defined by the claims, wherein:

[0015] **FIG. 1** illustrates a flow diagram for studying body weight and grip strength in relation to nutritional intervention in an animal model as described in Example 1.

[0016] **FIG. 2** illustrates loss of grip strength after administration of LPS in the animal model as described in Example 1.

[0017] **FIG. 3** illustrates grip strength recovery after administration of LPS in the animal model as described in Example 1.

[0018] **FIG. 4** illustrates muscle performance (mobility) after administration of LPS in an animal model as described in Example 1.

[0019] **FIG. 5** illustrates protein degradation on cells treated with dexamethasone as described in Example 2.

[0020] **FIG. 6** illustrates protein synthesis on cells treated with dexamethasone as described in Example 3.

[0021] **FIG. 7** illustrates a flow diagram for studying the *in vitro* effects of HMB, Hydroxytyrosol (OHT), and a combination thereof on muscle cells subjected to an inflammatory (LPS) as described in Example 4.

[0022] **FIG. 8** illustrates the *in vitro* effects of HMB, OHT, and a combination thereof on Interleukin 6 (IL-6) expression from muscle cells subjected to an inflammatory (LPS) as described in Example 4.

[0023] **FIG. 9** illustrates the *in vitro* effects of HMB, OHT, and a combination thereof on Atrogin-1 expression from muscle cells subjected to an inflammatory (LPS) as described in Example 4.

[0024] **FIG. 10** illustrates the *in vitro* effects of HMB, OHT, and a combination thereof on Phospho-P70 S6 Kinase (P-p70S6K) expression from muscle cells subjected to an inflammatory (LPS) as described in Example 4.

[0025] **FIG. 11** illustrates the *in vitro* effects of HMB, OHT, and a combination thereof on the glucose uptake of L6 muscle cells subjected to glyoxal, as described in Example 5.

[0026] **FIG. 12** illustrates a flow diagram for studying the *in vitro* effects of HMB, Hydroxytyrosol (OHT), and a combination thereof on the regeneration of muscle cells from aged rats as described in Example 6.

[0027] **FIG. 13** illustrates the *in vitro* effects of HMB, OHT, and a combination thereof on MyoD, a marker of the cell proliferation stage of muscle cell regeneration as described in Example 6.

[0028] **FIG. 14** illustrates the *in vitro* effects of HMB, OHT, and a combination thereof on Myogenin (MyoG), a marker of the cell differentiation stage of muscle cell regeneration as described in Example 6.

[0029] **FIG. 15** illustrates the *in vitro* effects of HMB, OHT, and a combination thereof on Myf6, a marker of the cell fusion and maturation stage of muscle cell regeneration as described in Example 6.

DETAILED DESCRIPTION

[0030] Specific embodiments of the invention are described herein. The invention can, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided to illustrate more specific features of certain embodiments of the invention to those skilled in the art.

[0031] The terminology as set forth herein is for description of the embodiments only and should not be construed as limiting the disclosure as a whole. All references to singular characteristics or limitations of the present disclosure shall include the corresponding plural characteristic or limitation, and vice versa, unless otherwise specified or clearly implied to the contrary by the context in which the reference is made. Unless otherwise specified, “a,” “an,” “the,” and “at least one” are used interchangeably. Furthermore, as used in the description and the appended claims, the singular forms “a,” “an,” and “the” are inclusive of their plural forms, unless the context clearly indicates otherwise.

[0032] To the extent that the term “includes” or “including” is used in the description or the claims, it is intended to be inclusive of additional elements or steps, in a manner similar to the term “comprising” as that term is interpreted when employed as a transitional word in a claim. Furthermore, to the extent that the term “or” is employed (e.g., A or B), it is intended to mean “A or B or both.” When the “only A or B but not both” is intended, then the term “only A or B but not both” is employed. Thus, use of the term “or” herein is the inclusive, and not the exclusive use. When the term “and” as well as “or” are used together, as in “A and/or B” this indicates A or B as well as A and B.

[0033] All ranges and parameters, including but not limited to percentages, parts, and ratios disclosed herein are understood to encompass any and all sub-ranges subsumed therein, and every number between the endpoints. For example, a stated range of “1 to 10” should be considered to include any and all sub-ranges beginning with a minimum value of 1 or more and ending with a maximum value of 10 or less (e.g., 1 to 6.1, or 2.3 to 9.4), and to each integer (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10) contained within the range.

[0034] Any combination of method or process steps as used herein can be performed in any order, unless otherwise specified or clearly implied to the contrary by the context in which the referenced combination is made.

[0035] All percentages are percentages by weight unless otherwise indicated.

[0036] The term “HMB” as used herein, unless otherwise specified, refers to β -hydroxy- β -methylbutyrate (also referred to as beta-hydroxyl-3-methyl butyric acid, beta-hydroxy isovaleric acid) and sources thereof. All weights, percentages, and concentrations as used herein to characterize HMB are based on the weight of HMB, regardless of source, unless otherwise specified.

[0037] The term “calcium HMB” as used herein, unless otherwise specified, refers to the calcium salt of HMB, which is most typically in a monohydrate form. All weights, percentages,

and concentrations as used herein to characterize calcium HMB are based on the weight of calcium HMB monohydrate, unless otherwise specified.

[0038] The methods and nutritional compositions of the present invention employ HMB, which is a naturally occurring amino acid metabolite that is known for use in a variety of nutritional products and supplements. HMB is a metabolite of the essential amino acid leucine and has been shown to modulate protein turnover and inhibit proteolysis. While HMB is commonly used in nutritional products to help build or maintain healthy muscle in selected individuals, the present inventors have surprisingly discovered that HMB, in combination with an olive polyphenol, more specifically, hydroxytyrosol, synergistically maintain skeletal muscle mobility and/or mass and also stimulate recovery of skeletal muscle strength in individuals in need thereof.

[0039] Any source of HMB is suitable for use in the methods and nutritional compositions of the invention. Examples include HMB as the free acid, a salt, including an anhydrous salt or a hydrate salt, an ester, a lactone, or other product forms that otherwise provide a bioavailable form of HMB. In specific embodiments of the methods and compositions of the invention, the source of HMB is selected from the group consisting of alkali metal HMB, alkaline earth metal HMB, HMB free acid, HMB lactone and combinations of two or more thereof, or the HMB is selected from the group consisting of sodium HMB, potassium HMB, magnesium HMB, chromium HMB, calcium HMB and combinations of two or more thereof, or the HMB is calcium HMB monohydrate.

[0040] The term "olive polyphenol" as used herein, unless otherwise specified, refers to a polyphenol that is derived from olives, such as from olive fruit, leaves or oil.

[0041] The term "hydroxytyrosol" as used herein, unless otherwise specified, refers to the organic compound with the formula $(\text{HO})_2\text{C}_6\text{H}_3\text{CH}_2\text{CH}_2\text{OH}$. Hydroxytyrosol is an olive polyphenol known to exert bioactive properties including antioxidant and anti-inflammatory properties.

[0042] Any source of hydroxytyrosol is suitable for use in the methods and nutritional compositions of the invention. Examples include hydroxytyrosol as an extract from olive fruit or olive leaves, hydroxytyrosol found in olive oil, or purified hydroxytyrosol.

[0043] The terms “nutritional product” and “nutritional composition” as used herein, unless otherwise specified, refer to nutritional liquids and nutritional powders, the latter of which may be reconstituted to form a nutritional liquid, and are suitable for oral consumption by a human.

[0044] The term “nutritional powder” as used herein, unless otherwise specified, refers to nutritional powders that are generally flowable particulates and that, in certain embodiments, are reconstitutable with an aqueous liquid, and which are suitable for oral administration to a human.

[0045] The term “nutritional liquid” as used herein, unless otherwise specified, refers to nutritional products made in ready-to-drink liquid form and to nutritional liquids made by reconstituting nutritional powders described herein prior to use.

[0046] In specific embodiments of the invention, the methods and compositions are provided to an individual, where the individual may be a human adult or pediatric subject.

[0047] In one embodiment, the subject is an adult at or above the age of about 40 years old, at or above the age of about 50 years, or about 40-80 years old, or about 40-70 years old, or about 50-80 years old.

[0048] In one embodiment, the invention is directed to a method for maintaining skeletal muscle mobility and/or mass, and/or a method for recovery of skeletal muscle strength in an individual in need thereof.

[0049] In a specific embodiment of the invention, the individual has or is at risk of losing skeletal muscle mass, mobility, or strength.

[0050] In specific embodiments of the invention, reduced muscle mass or risk thereof is associated with sarcopenia, hospitalization, immobilization, acquired immunodeficiency syndrome (AIDS), diabetes, cancer, chronic obstructive pulmonary disease (COPD), burn injury,

nonalcoholic fatty liver disease (NAFLD), long term steroid use, including but not limited to glucocorticoid use, inflammatory bowel disease (IBD), Crohn's disease, malnutrition, and/or an acute or chronic condition with a hypercatabolic state. Specifically, a hypercatabolic state may occur in conditions where muscle catabolism is favored over muscle anabolism.

[0051] In specific further specific embodiments of the invention, reduced muscle strength or risk thereof is associated with inflammation, oxidative stress, immobilization, sepsis, infection, surgery, cancer, COPD, obesity, diabetes, cardiovascular disease, dementia, aging, sarcopenia, malnutrition, and/or an acute or chronic condition with a systemic pro-inflammatory state.

[0052] In further specific embodiments of the invention, the inventive methods comprise preventing decline in muscle mobility or mass loss during a systemic pro-inflammatory state or catabolic state.

[0053] The methods and nutritional compositions according to specific embodiments of the invention can be used to restore muscle homeostasis during catabolic periods, preserve muscle during catabolic periods or conditions, enhance muscle protein synthesis in conditions where protein synthesis is decreased (such as in muscle atrophy), enhance muscle anabolism during a catabolic state, decrease muscle protein breakdown during a catabolic state, enhance faster recovery of muscle strength following a catabolic pro-inflammatory period, and/or reduce the recovery time for regaining muscle strength following a catabolic pro-inflammatory period. Catabolic pro-inflammatory periods may include periods following surgery, hospitalization, immobilization, treatment with steroids, post-infections, etc.

[0054] The methods of the invention comprise administering HMB and an olive polyphenol, or specifically, hydroxytyrosol, to an individual in need thereof. In additional embodiments of the invention, nutritional compositions are provided. According to one embodiment, the nutritional composition comprises HMB and an olive polyphenol, or specifically, hydroxytyrosol.

[0055] In specific embodiments of the methods of the invention, the HMB and olive polyphenol, or specifically, hydroxytyrosol are administered to the subject orally.

[0056] In accordance with the methods and nutritional compositions of the present invention, to maintain skeletal muscle mobility and/or mass, or to recover skeletal muscle strength, the HMB and olive polyphenol, or specifically, hydroxytyrosol, may be administered to the individual in need thereof for a time period of at least 2 days, or at least 3 days, or at least 5 days, or at least 6 days, or at least 1 week, or at least 2 weeks, or at least 3 weeks, or at least 4 weeks, or at least 5 weeks, or at least 6 weeks, or at least 7 weeks, or at least 8 weeks, or at least 9 weeks, or at least 10 weeks, or at least 11 weeks, or at least 12 weeks, or at least 14 weeks, or at least 16 weeks, or at least 18 weeks, or at least 24 weeks or longer. In specific embodiments of the methods and nutritional compositions of the invention, the HMB and olive polyphenol, or specifically, hydroxytyrosol, are administered to an individual once or multiple times daily or weekly. In specific embodiments, the HMB and olive polyphenol, or specifically, hydroxytyrosol, are administered to the subject from about 1 to about 6 times per day or per week, or from about 1 to about 5 times per day or per week, or from about 1 to about 4 times per day or per week, or from about 1 to about 3 times per day or per week. In other specific embodiments, the HMB and olive polyphenol, or specifically, hydroxytyrosol, are administered once or twice daily for a period of at least 2 days, at least 3 days, at least 4 days, at least 5 days or at least 6 days, or at least one week, at least two weeks, at least three weeks, at least four weeks, at least five weeks, or at least six weeks.

[0057] In specific embodiments of the methods of the invention, the desired amounts of HMB and olive polyphenol, or specifically, hydroxytyrosol may be administered in more than one serving or, more specifically, in two servings. Each serving is desirably administered as a single, undivided dose, although the serving may also be divided into two or more partial or divided servings to be taken at two or more times during the day. The methods and nutritional compositions of the present invention include continuous day after day administration, as well as periodic or limited administration, although continuous day after day administration is generally desirable.

[0058] In specific embodiments of the methods of the invention, the individual is administered from about 0.01 gram to about 10 grams per day of HMB. In further specific embodiments of the methods of the invention, the individual is administered from about 0.1 gram to about 10 grams, or from about 0.1 gram to about 6 grams, or from about 0.5 gram to about 3 grams, or from about 0.5 gram to about 1.5 grams, or from about 1 gram to about 5 grams, or from about 2 grams to about 9 grams, or from about 2 grams to about 8 grams, or from about 2 grams to about 7 grams, or from about 2 grams to about 6 grams, or from about 2 grams to about 5 grams, or from about 2 grams to about 4 grams, or from about 2 grams to about 3 grams per day of HMB.

[0059] In specific embodiments of the methods of the invention, the individual is administered from about 0.5 mg to about 50 mg per day of olive polyphenols, or specifically, hydroxytyrosol. In further specific embodiments of the methods and nutritional compositions of the invention, the subject is administered from about 1 mg to about 20 mg, or from about 5 mg to about 15 mg per day of olive polyphenols, or specifically, hydroxytyrosol.

[0060] In specific embodiments of the methods of the invention, the individual is administered about 0.01 gram to about 10 grams, or about 0.1 gram to about 10 grams, or about 0.1 gram to about 6 grams per day of HMB, and about 0.5 mg to about 50 mg, or about 1 mg to about 20 mg, or about 5 mg to about 15 mg per day of olive polyphenol, or specifically, hydroxytyrosol.

[0061] In further specific embodiments, the subject is administered about 2 grams to about 6 grams per day of HMB and about 5 mg to about 20 mg per day of olive polyphenol, or specifically, hydroxytyrosol.

[0062] In further specific embodiments, the subject is administered about 0.5 grams to about 2 grams per day of HMB and about 1 mg to about 15 mg per day of olive polyphenol, or specifically, hydroxytyrosol.

[0063] In specific embodiments of the invention, the nutritional composition comprises from about 0.01 to about 10 wt %, from about 0.1 to about 10 wt %, from about 1 to about 10 wt %, from about 1 to about 6 wt %, from about 3 to about 7 wt %, from about 0.1 to about 6 wt %, from about 0.1 to about 5 wt %, from about 0.1 to about 3 wt %, from about 0.1 to about 2 wt %, or from about 0.1 to about 1.5 wt % of HMB, based on the weight of the nutritional composition. In additional specific embodiments, the nutritional composition comprises from about 1 to about 10 wt %, or from about 3 to about 7 wt % of HMB, based on the weight of the nutritional composition.

[0064] In further specific embodiments of the invention, the nutritional composition comprises from about 0.001 to about 1 wt %, from about 0.005 to about 0.5 wt %, from about 0.005 to about 0.25 wt %, from about 0.008 to about 0.1 wt %, from about 0.01 to about 0.02 wt %, or from about 0.008 to about 0.025 wt % of olive polyphenol, or specifically, hydroxytyrosol, based on the weight of the nutritional composition. In additional specific embodiments, the nutritional composition comprises from about 0.005 to about 0.03 wt %, or from about 0.008 to about 0.025 wt % of olive polyphenol, or specifically, hydroxytyrosol, based on the weight of the nutritional composition.

[0065] In further specific embodiments of the compositions of the invention, the nutritional composition comprises from about 1 to about 10 wt %, or from about 3 to about 7 wt %, of HMB; and from about 0.001 to about 1 wt %, or from about 0.005 to about 0.3 wt % of olive polyphenol, or specifically, hydroxytyrosol, all based on the weight of the nutritional composition.

[0066] In other specific embodiments of the invention, in addition to protein, carbohydrate and fat contents provided by the HMB, olive polyphenol, or specifically, hydroxytyrosol, or other components mentioned above, the nutritional composition further comprise additional sources of protein, carbohydrate, and/or fat. A wide variety of sources and types of protein, carbohydrate, and/or fat can be used in embodiments of nutritional compositions described herein.

[0067] In other specific embodiments, an additional source of carbohydrate in the nutritional compositions comprises fiber, maltodextrin, corn maltodextrin, corn syrup, organic corn maltodextrin, corn syrup solids, sucralose, cellulose gel, cellulose gum, gellan gum, carrageenan, fructooligosaccharide, inositol, hydrolyzed starch, glucose polymers, rice-derived carbohydrate, sucrose, glucose, lactose, honey, sugar alcohols, isomaltulose, sucromalt, pullulan, potato starch, galactooligosaccharide, oat fiber, soy fiber, corn fiber, gum arabic, sodium carboxymethylcellulose, methylcellulose, guar gum, locust bean gum, konjac flour, hydroxypropyl methylcellulose, tragacanth gum, karaya gum, gum acacia, chitosan, arabinoglactin, glucomannan, xanthan gum, alginate, pectin, low methoxy pectin, high methoxy pectin, cereal beta-glucan, psyllium, inulin, or combinations of two or more thereof.

[0068] The nutritional composition may comprise additional carbohydrate source(s) in an amount from about 0.5 wt % to about 75 wt % of the nutritional composition. More specifically, the additional carbohydrate source(s) may be present in an amount from about 0.5 wt % to about 70 wt % of the nutritional compositions, including about 1 wt % to about 70 wt %, about 5 wt % to about 70 wt %, about 5 wt % to about 65 wt %, about 5 wt % to about 50 wt %, about 5 wt % to about 40 wt %, about 5 wt % to about 30 wt %, about 5 wt % to about 25 wt %, about 10 wt % to about 65 wt %, about 10 wt % to about 40 wt %, about 20 wt % to about 65 wt %, about 30 wt % to about 65 wt %, about 40 wt % to about 65 wt %, about 40 wt % to about 70 wt %, or about 15 wt % to about 25 wt %, of the nutritional composition.

[0069] In further specific embodiments, an additional source of protein in the nutritional compositions comprises whey protein concentrate, whey protein isolate, whey protein hydrolysate, milk protein concentrate, milk protein isolate, milk protein hydrolysate, organic milk protein concentrate, soy protein concentrate, soy protein isolate, soy protein hydrolysate, pea protein concentrate, pea protein isolate, pea protein hydrolysate, collagen protein, collagen protein isolate, acid casein, sodium caseinate, calcium caseinate, potassium caseinate, casein hydrolysate, nonfat dry milk, condensed skim milk, L-Carnitine, L-Lysine, taurine, lutein, rice

protein concentrate, rice protein isolate, rice protein hydrolysate, fava bean protein concentrate, fava bean protein isolate, fava bean protein hydrolysate, meat protein, potato protein, chickpea protein, canola protein, mung protein, quinoa protein, amaranth protein, chia protein, hemp protein, flax seed protein, earthworm protein, insect protein, one or more amino acids and/or metabolites thereof, or combinations of two or more thereof.

[0070] In specific embodiments, an additional source of protein in the nutritional compositions comprises at least one milk protein and/or at least one soy protein. In a specific embodiment, an additional source of protein in the nutritional compositions comprises milk protein concentrate, calcium caseinate, or a combination thereof. In another specific embodiment, an additional source of protein in the nutritional compositions comprises sodium caseinate, soy protein isolate, calcium caseinate, or combinations of two or more thereof.

[0071] The collagen protein or collagen protein isolate may be any collagen protein that is suitable for use in nutritional compositions, including, inter alia, collagen from bones, skin, and connective tissue of animals, including cattle, fish, horses, pigs, or rabbits. In a specific embodiment of the invention, the nutritional compositions comprise hydrolyzed beef collagen.

[0072] The one or more amino acids, which may be described as free amino acids, can be any amino acid known for use in nutritional products, in addition to the HMB. The amino acids may be naturally occurring or synthetic amino acids. In a specific embodiment, the one or more amino acids and/or metabolites thereof comprise one or more branched chain amino acids or metabolites thereof. Examples of branched chain amino acids include arginine, glutamine, leucine, isoleucine, and valine. In another specific embodiment, the one or more branched chain amino acids or metabolites thereof comprise alpha-hydroxy-isocaproic acid (HICA, also known as leucic acid), keto isocaproate (KIC), and a combination of two or more thereof.

[0073] The nutritional composition may comprise additional protein source(s) in an amount from about 1 wt % to about 75 wt % of the nutritional composition. More specifically, the additional protein source(s) may be present in an amount from about 1 wt % to about 70 wt % of

the nutritional composition, including about 1 wt % to about 30 wt %, about 1 wt % to about 25 wt %, about 1 wt % to about 20 wt %, about 2 wt % to about 20 wt %, about 1 wt % to about 15 wt %, about 1 wt % to about 10 wt %, about 5 wt % to about 10 wt %, about 10 wt % to about 70 wt %, about 10 wt % to about 25 wt %, or about 10 wt % to about 20 wt % of the nutritional composition. Even more specifically, the additional protein source(s) comprise from about 10 wt % to about 70 wt % of the nutritional composition, or from about 1 wt % to about 30 wt % of the nutritional composition.

[0074] The terms “fat” and “oil” as used herein, unless otherwise specified, are used interchangeably to refer to lipid materials derived or processed from plants or animals. These terms also include synthetic lipid materials so long as such synthetic materials are suitable for oral administration to humans.

[0075] In further specific embodiments, an additional source of fat in the nutritional compositions comprise coconut oil, fractionated coconut oil, soy oil, soy lecithin, corn oil, safflower oil, sunflower oil, palm olein, canola oil monoglycerides, lecithin, canola oil, medium chain triglycerides, one or more fatty acids such as linoleic acid, alpha-linolenic acid, high gamma linolenic (GLA) safflower oil, palm oil, palm kernel oil, marine oil, fish oil, algal oil, borage oil, cottonseed oil, fungal oil, interesterified oil, transesterified oil, structured lipid, omega-3 fatty acid, or combinations of two or more thereof. In a specific embodiment, the omega-3 fatty acid is selected from the group consisting of eicosapentaenoic acid, docosahexaenoic acid, arachidonic acid, and alpha-linolenic acid, and combinations of two or more thereof.

[0076] The nutritional composition may comprise additional fat source(s) in an amount from about 0.1 wt % to about 30 wt % of the nutritional composition. More specifically, the additional fat source(s) may be present in an amount from about 1 wt % to about 25 wt % of the nutritional composition, including about 2 wt % to about 20 wt %, about 1 wt % to about 15 wt %, about 1

wt % to about 10 wt %, about 5 wt % to about 10 wt %, about 10 wt % to about 25 wt %, or about 10 wt % to about 20 wt % of the nutritional composition.

[0077] In a specific embodiment of the invention, the nutritional compositions comprise from about 1 to about 30 wt % of protein, from about 0.1 to about 30 wt % fat, and from about 0.5 to about 70 wt % carbohydrate. In another specific embodiment of the invention, the nutritional compositions comprise from about 1 to about 20 wt % protein and from about 10 to about 40 wt % carbohydrate.

[0078] The concentration and relative amounts of the sources of protein, carbohydrate, and fat in the exemplary nutritional compositions can vary considerably depending upon, for example, the specific dietary needs of the intended user.

[0079] In specific embodiments of the methods and compositions of the invention, the nutritional compositions further comprise one or more vitamins. Non-limiting examples of vitamins include vitamin A, vitamin B12, vitamin C, vitamin E, vitamin K, thiamine, riboflavin, pyridoxine, niacin, folic acid, pantothenic acid, biotin, choline, inositol, and/or salts and derivatives thereof, and combinations thereof. Any vitamin E is suitable for use in the methods and compositions of the present invention, including, inter alia, d-alpha-tocopherol, dl-alpha-tocopherol, and gamma-tocopherol, and salts or esters thereof. In a specific embodiment, the vitamin E is dl-alpha-tocopheryl acetate. In specific embodiments of the invention, the nutritional compositions include one or more vitamins selected from the group consisting of vitamin A, vitamin C, vitamin D, vitamin B12, and combinations thereof.

[0080] In additional specific embodiments of the methods and nutritional compositions of the invention, the nutritional compositions further comprise one or more minerals or trace minerals. Non-limiting examples of minerals and trace minerals include calcium, phosphorus, magnesium, zinc, manganese, sodium, potassium, molybdenum, chromium, iron, copper, and/or chloride, and combinations thereof. In a specific embodiment, the nutritional composition includes one or more minerals selected from the group consisting of zinc, calcium (to the extent the HMB source

does not provide sufficient calcium), and combinations thereof. In a specific embodiment of the methods and compositions of the invention, the nutritional compositions further comprise zinc.

[0081] The nutritional composition may also comprise one or more components to modify the physical, chemical, aesthetic, or processing characteristics of the nutritional composition or serve as additional nutritional components. Non-limiting examples of additional components include preservatives, emulsifying agents (e.g., lecithin), buffers, sweeteners including artificial sweeteners (e.g., saccharine, aspartame, acesulfame K, sucralose), colorants, flavorants, acids (e.g., citric acid, ascorbic acid), thickening agents, stabilizers, and so forth.

[0082] In specific embodiments, the nutritional composition has a neutral pH, i.e., a pH of from about 6 to 8 or, more specifically, from about 6 to 7.5. In more specific embodiments, the nutritional composition has a pH of from about 6.5 to 7.2 or, more specifically, from about 6.8 to 7.1.

[0083] The nutritional compositions may be formulated and administered in any known or otherwise suitable oral product form, so long as they include HMB and olive polyphenol, or specifically, hydroxytyrosol. Any solid, liquid, semi-solid, semi-liquid or powder form, including combinations or variations thereof, are suitable for use in the methods described herein, provided that such forms allow for safe and effective oral delivery to the individual of the ingredients as also defined herein. In specific embodiments of the methods and compositions of the invention, the nutritional composition is in the form of a powder. In other specific embodiments, the nutritional composition is in the form of a liquid.

[0084] When the nutritional composition is a powder, for example, in specific embodiments, a serving size is from about 20 g to about 60 g, about 20 g to about 30 g, about 25 g to about 55 g, about 25 g to 35 g, or about 45 g to about 50 g, to be administered as a powder or to be reconstituted in from about 1 ml to about 500 ml of liquid. In a specific embodiment, when the nutritional composition is a powder, a serving size is from about 25 g to about 30 g, or from about 55 g to about 60 g, to be reconstituted in from about 230 ml to about 300 ml of liquid.

[0085] When the nutritional composition is in the form of a liquid, for example, reconstituted from a powder or manufactured as a ready-to-drink product, in specific embodiments, a serving ranges from about 1 ml to about 500 ml, including from about 110 ml to about 500 ml, from about 110 ml to about 417 ml, from about 120 ml to about 500 ml, from about 120 ml to about 417 ml, from about 177 ml to about 417 ml, from about 207 ml to about 296 ml, from about 230 ml to about 245 ml, from about 230 ml to about 300 ml, from about 110 ml to about 237 ml, from about 120 ml to about 245 ml, from about 110 ml to about 150 ml, and from about 120 ml to about 150 ml. In specific embodiments, the serving is about 1 ml, or about 100 ml, or about 225 ml, or about 230 ml, or about 300 ml, or about 500 ml.

[0086] The nutritional compositions may be formed using any techniques known in the art. In one embodiment, a powder product can be formed by dry blending ingredients, or alternatively, providing an aqueous mixture of ingredients and spray drying the aqueous mixture to form a powder. In another embodiment, if the nutritional composition includes one or more oil soluble components, the nutritional compositions may be formed by preparing an aqueous solution, for example, comprising protein and carbohydrate, preparing an oil blend comprising fat and oil-soluble components, and mixing together the aqueous solution and the oil blend to form an emulsified liquid nutritional composition. The HMB and olive polyphenol, or specifically, hydroxytyrosol may be added at any time as desired in the process, for example, to the aqueous solution or to the emulsified blend. The compositions may be spray-dried or otherwise dried, if a powder product is desired. Typically, the nutritional compositions are subjected to a heat treatment which provides sterilization sufficient to maintain microbiological stability of the compositions over a desired shelf-life.

[0087] The following Example demonstrates various embodiments of the invention.

EXAMPLES

[0088] **Example 1:** In Vivo Study on Inflammation

[0089] This example describes an in vivo study that evaluated the stimulatory effect of chronic administration of HMB and hydroxytyrosol on muscle function in an animal model. The experimental sequence of the study is summarized in Fig. 1.

[0090] Specifically, fifty 12-week-old C57BL/6J mice were divided into five equal groups that were fed varying diets according to Table 1 below.

Table 1: Diets

Group	Diet
1	AIN93M (rodent standard diet)
2	AIN93M
3	AIN93M + HMB
4	AIN93M + hydroxytyrosol (olive extract)
5	AIN93M + HMB + hydroxytyrosol (olive extract)

[0091] The mice of Groups 1-5 were fed the indicated diets for an initial period of 45 days to represent chronic administration.

[0092] The mice that received HMB (Groups 3 and 5) were administered 530 mg of HMB per kg of body weight per day.

[0093] The mice that received hydroxytyrosol (Groups 4 and 5) were administered 20 mg of olive extract per kg of body weight per day.

[0094] After the mice were fed the respective diets for 45 days (the nutritional intervention period), body weight and grip strength of the animals were measured. Groups 2-5 were then given a daily lipopolysaccharide (LPS) injection of 1 mg of LPS per kg of body weight for three days after the initial 45 day nutritional intervention period. The healthy Group 1 received saline solution injection. The mobility (spontaneous motor activity) of the animals was continuously monitored during the active period of the day. The animals were left undisturbed during the

three days of LPS administration. After three days of LPS injection, body weight and grip strength of the animals were measured for all groups.

[0095] To monitor the effects of diet additives, specifically HMB and hydroxytyrosol, on mobility, the mobility of all groups was continuously monitored during the three days of LPS injection as a representation of effects caused by inflammation induction by the LPS injections given to animals in Groups 2-5. FIG. 4 shows mobility of all Groups 1-5, expressed in activity units ($\Delta g/s$) for each of the 3 days of LPS injection (also referred to as the acute inflammatory period). As shown in FIG. 4, across all three days of the acute inflammatory period, animals that were fed diets supplemented with either HMB, hydroxytyrosol (olive extract), or both (Groups 3-5), showed a statistically significant higher level of mobility during the acute inflammatory period compared with Group 2, which included LPS injection but did not include either effector additive. These results indicated that HMB and hydroxytyrosol (olive extract) have a role in preserving muscle function during inflammation-induced muscle catabolism (such as that induced by LPS).

[0096] In FIG. 4, the statistical significance of the results are shown by “#”, which represents $p < 0.05$ vs treatment with LPS (Group 2); “2”, which represents $p < 0.05$ vs treatment with LPS and HMB (Group 3); and “3”, which represents $p < 0.05$ vs treatment with LPS and hydroxytyrosol (olive extract) (Group 4).

[0097] After the 3 days of LPS injection, the mice that received the LPS injections (Groups 2-5) underwent a 10-day recovery period where no LPS was administered. At the end of the 10-day recovery period, body weight and grip strength were again measured for all groups.

[0098] The average body weight of the animals in each group before LPS injection, directly after LPS injection, and after the recovery period, is shown in Table 2 below.

Table 2: Effect of LPS on Body Weight

	Body Weight (g)				
	Group 1	Group 2	Group 3	Group 4	Group 5

Before LPS injection	28.19±0.72	29.50±0.61	29.19±0.52	28.86±1.13	29.20±0.86
Directly After LPS injection	28.31±0.49	25.14±0.58*	24.23±0.45*	23.85±1.18*	24.22±0.92*
	<i>*no LPS injection received*</i>				
After Recovery Period	28.53±0.44	28.85±1.19	28.83±0.43	26.86±0.47	28.01±0.77

[0099] As shown in Table 2, administration of LPS significantly lowered body weight in all of Groups 2-5 that received LPS injections, regardless of differences in experimental diet. At the end of the recovery period, all treatment groups (Groups 2-5) regained body weight levels similar to (within about 93-97%) body weight levels before LPS injection. The body weight data of Table 2 is expressed as mean ± standard SEM (standard error of mean). Data was analyzed using one-way analyses of variance (ANOVA) and post-hoc Fisher's test or using two-way analyses of variance (ANOVA). Analysis was corrected for multiple comparisons using *Dunnnet* statistical hypothesis test. In Table 2, “*” represents $p < 0.05$ for control Group 1 vs treatment Groups 2-5 that received LPS.

[0100] The average muscle strength, based on grip strength (in Newtons) of the animals in each group both before and directly after LPS injection, is shown in FIG. 2. Grip strength was analyzed for monitoring muscle strength during the LPS injection period. As expected, the administration of LPS significantly decreased muscle strength in Groups 2-5 that received LPS, compared with “healthy” Group 1 that did not receive LPS. In FIG. 2, “*” represents $p < 0.05$ vs control Group 1.

[0101] The average grip strength of the animals in each group (in Newtons) after the recovery period, is shown in FIG. 3. At the end of the recovery period, the animals that had received LPS injections alone (Group 2), without any HMB or hydroxytyrosol (olive extract) diet additives, showed little to no muscle strength recovery in grip strength during the recovery

period. Muscle strength values for Group 2 essentially remained the same across the LPS injection period and the recovery period, as shown in FIGS. 2-3.

[0102] In FIG. 3, the statistical significance of Group 5 performance is shown by “#”, which represents $p < 0.05$ vs treatment with LPS (Group 2); “2”, which represents $p < 0.05$ vs treatment with LPS and HMB (Group 3); and “3”, which represents $p < 0.05$ vs treatment with LPS and hydroxytyrosol (olive extract) (Group 4). Surprisingly, Group 5, which included a diet supplemented with the combination of HMB and hydroxytyrosol (olive extract) presented the greatest recovery of grip strength, even statistically significantly higher than Groups 3 and 4 (with either HMB or hydroxytyrosol alone). Group 5 achieved an approximately 47% increase in muscle strength recovery, a significant increase compared to the groups that received either HMB or hydroxytyrosol (olive extract) alone. This surprising result indicated that the combination of HMB and hydroxytyrosol (olive extract) provides a synergistic effect in stimulating skeletal muscle strength recovery following a state of inflammation.

[0103] Additionally, Group 5, also presented the highest motor activity mobility during the LPS injection period, even statistically significantly higher than Groups 3 and 4 (with either HMB or hydroxytyrosol alone). This surprising result indicated that the combination of HMB and hydroxytyrosol (olive extract) also provides a synergistic effect in preserving muscle function during an inflammation-induced state.

[0104] In combination, the results discussed in this Example highlight the surprising and unexpected results that the combination of HMB and hydroxytyrosol (olive extract) have in maintaining mobility and improving the recovery of muscle strength and function in a subject that has experienced inflammation-induced muscle dysfunction.

[0105] **Example 2:** In Vitro Muscle Protein Degradation Assay

[0106] This example describes an *in vitro* study that evaluated the stimulatory effect of administration of HMB and hydroxytyrosol on muscle preservation after induced muscle atrophy. In muscle atrophy, altered protein turnover is partially due to an increase in the rate of protein

breakdown. This breakdown is mediated by an induction of the ubiquitin-proteasome system which involves an increase in ubiquitin activity and ubiquitin ligase levels. In this Example, for the analysis of ubiquitin activity, a 417-bp ubiquitin (UbC) promoter fragment flanking the transcription starting point was amplified from rat genomic DNA and used to construct the plasmid pGL3-UbC.

[0107] Rat skeletal muscle myoblast L6.C11 cells (ATCC CRL-1458) were seeded at 80-90% confluence and transfected using LipofectAMINE2000 and a rat genomic DNA mixture (that was used to construct the plasmid pGL3-UbC) with a concentration of 30 µg of DNA mixture per well, in Dulbecco's Modified Eagle's Medium (DMEM) without Fetal Bovine Serum (FBS). The DNA mixture comprised the pGL3-UbC luciferase reporter and the referenced plasmid pRL-TK at a ratio of 95:5. After 24 hours, the cells were differentiated for 5 days. The differentiated L6.C11 cells (myotubes) were incubated with different effector(s) in accordance with the information provide in Table 3 below.

Table 3: Incubation of Myotubes

Group	Incubation Effector(s)
1	Control (none)
2	25 µM HMB
3	5 µM Hydroxytyrosol (OHT)
4	25 µM HMB + 5 µM Hydroxytyrosol (OHT)

[0108] The cells were incubated according to Table 3 for 48 hours. Cells in Groups 2-4 were then treated with their respective effector(s) for 48 hours. Cells in all Groups 1-4 were then treated in the presence or absence of 5 µM of glucocorticoid dexamethasone (DEX) for 24 additional hours. Luciferase activity was determined for all groups using the Dual Luciferase

method (Promega) in a luminometer FLUOstar Omega (BMG LABTECH) and results were standardized for Renilla luciferase activity.

[0109] DEX is known to provoke skeletal muscle atrophy in that DEX increases muscle protein degradation and suppresses muscle protein synthesis. Muscle protein degradation was evaluated in the cells of Groups 1-4, with and without DEX, by analyzing ubiquitin promoter transcriptional activity, which is required for protein catabolism.

[0110] The results are shown in FIG. 5 and are expressed as relative changes in luciferase activity (ubiquitin promoter transcriptional activity (in RLU%)) and are normalized to a value of 100% for the control, Group 1. As shown by the left portion of FIG. 5, none of HMB, hydroxytyrosol, or the combination of HMB and hydroxytyrosol induced ubiquitin promoter transcriptional activity as compared with the control (far left bar). As shown in the right portion of FIG. 5, incubation with 5 μ M of DEX in each of Groups 1-4 significantly induced ubiquitin promoter transcriptional activity, increasing by about 43% in control Group 1 as shown in FIG. 5. In FIG. 5, “**” represents $p < 0.05$ vs control Group 1; and “#” represents $p < 0.05$ vs control Group 1 treated with DEX.

[0111] As shown on the right half of FIG. 5, in the presence of DEX, each of HMB and hydroxytyrosol alone reduced ubiquitin promoter transcriptional activity, by about 14% in Group 2 with HMB alone, and by about 10% in Group 3 with hydroxytyrosol alone, compared with the control, Group 1. However, surprisingly, only the combination of HMB and hydroxytyrosol in Group 4 was able to statistically significantly decrease ubiquitin promoter activity levels, specifically by about 28%, after treatment with DEX, compared to the control Group 1 treated with DEX. As such, the combination of HMB and hydroxytyrosol in Group 4 was able to reduce the DEX-mediated induction of the ubiquitin gene in a statistically significant manner.

[0112] **Example 3: In Vitro Myotube Protein Synthesis Assay**

[0113] This example describes an *in vitro* study that evaluated the stimulatory effect of administration of HMB and hydroxytyrosol on muscle preservation by evaluating muscle protein synthesis.

[0114] Rat skeletal muscle myoblast L6.C11 cells (ATCC CRL-1458) were plated in 48-well culture plates and were differentiated for 5 days. The differentiated L6.C11 cells (myotubes) were divided into Groups 1-4 and incubated for 2 hours in DMEM with 10% FBS. Cells in Groups 2-4 were additionally treated with different effector(s) as described in Table 3 above in Example 2 for the 2-hour incubation period. Cells in all of Groups 1-4 were then treated with 5 μ M of DEX for 1 hour, followed by incubation with 1 μ Ci/ml of L-[ring-3,5-3H]-Tyrosine (Perkin Elmer, Waltham MA) for 1 hour. After this 1 hour of incubation, the reaction was stopped by placing all culture plates on ice. All wells were washed with ice cold phosphate buffered saline (PBS) and cells were lysed in 0.1 ml of 0.1 mM NaOH/0.1% sodium deoxycholate. Cellular proteins were precipitated out of the wells by adding 0.1 ml of cold 20% trichloroacetic acid (TCA; Sigma). This mixture was incubated at 4°C for 15 minutes and then centrifuged at 16,000 G for 10 minutes at 4°C.

[0115] Muscle protein synthesis was evaluated in the cells of Groups 1-4, with and without the DEX incubation, through conventional radioactive metabolic labelling. Each of the resulting pellets was washed once with cold 10% TCA and the precipitated proteins were dissolved in 0.1 ml of 1 M NaOH. An aliquot of 5 μ l of the NaOH-solubilized material was used for total protein quantitation and the remaining dissolved proteins were mixed with ReadySafe scintillation fluid (Beckman Coulter). Disintegrations per minute (dpm) were recorded with a scintillation counter (Beckman Coulter).

[0116] The results are shown in FIG. 6 and are expressed as percentage of protein synthesis and normalized to a value of 100%, corresponding to the untreated control. As shown on the left side of Fig. 6, all of Groups 1-4 without DEX incubation included an initial protein synthesis percentage at or near the normalized value of 100%. This indicated that HMB and

hydroxytyrosol, alone or in combination, do not reduce or increase protein synthesis *per se*. After 5 μ M of DEX was added to each of Groups 1-4, as expected, DEX significantly decreased protein synthesis, as shown on the right half of FIG. 6. Compared with the untreated control (Group 1), incubation of the Group 1 cells with DEX alone significantly reduced protein synthesis, decreasing by about 22% as shown in FIG. 6 for Group 1. However, that substantial decrease in protein synthesis was not seen in the other Groups 2-4 that were incubated with either HMB, hydroxytyrosol, or both, and DEX. In FIG. 6, “*” represents $p < 0.05$ for control Group 1 with treatment with DEX vs the control Group 1 without DEX treatment; and “#” represents $p < 0.05$ for control Group 4 treated with DEX, HMB and hydroxytyrosol, vs Group 1 treatment with DEX only.

[0117] As shown on the right half of FIG. 6, in the presence of DEX, HMB and hydroxytyrosol alone increased protein synthesis by about 28% in Group 2 with HMB alone, and by about 24% in Group 3 with hydroxytyrosol alone, compared with the control, Group 1 treated with DEX. However, surprisingly, only the combination of HMB and hydroxytyrosol in Group 4 was able to promote a statistically significant increase in protein synthesis levels, by about 60%, compared to the value of control Group 1 after treatment with DEX. As such, the combination of HMB and hydroxytyrosol in Group 4 was able to reduce the decrease in protein synthesis induced by DEX in a statistically significant manner and further promote protein synthesis by surpassing the normalized value of the control even before DEX treatment.

[0118] The in vitro models of Examples 2 and 3 clearly demonstrated that DEX was able to mimic the hypercatabolic conditions observed in acute or chronic conditions such as AIDS, diabetes, cancer, COPD, burn injury, NAFLD, sarcopenia, hospitalization/immobilization, long term steroid use, IBD, Crohn's disease, and prolonged malnutrition – conditions where muscle protein degradation is favored over muscle protein synthesis (muscle atrophy). The surprising synergistic effect of the combination of HMB and hydroxytyrosol was able to counteract the

negative effects of DEX on protein turnover, both in terms of mitigating muscle protein degradation and promoting muscle protein synthesis.

[0119] **Example 4:** Effects on muscle cells during inflammation

[0120] This example describes an *in vitro* study that evaluated the effects of administration of HMB, hydroxytyrosol (OHT), and a combination thereof on muscle cells when subjected to inflammation-producing environment. Inflammation, and in particular chronic inflammation, is known to create a catabolic environment in the muscles that increases proteolysis (protein degradation or breakdown) and reduces protein synthesis, resulting over time in muscle atrophy. The effects of HMB and OHT on muscle cells subjected to an inflammation-producing environment were studied by measuring (a) the relative expression of interleukin 6 (IL-6), a pro-inflammatory cytokine, in muscle cells subjected to an inflammation-producing environment, (b) the relative expression of Atrogin-1, a marker of protein degradation, in muscle cells subjected to an inflammation-producing environment, and (c) the relative intensity of P-p70 S6K, a marker of protein synthesis, in muscle cells subjected to an inflammation-producing environment.

[0121] *In vitro* assays were performed using a cell coculture model that include C2C12 murine skeletal muscle cell line (ATCC CRL-1772) and RAW 264.7 mouse macrophage cell line (ATCC TIB-71). These cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM-high glucose, Sigma-Aldrich) supplemented with 10% FBS (v/v), 2 mmol/L glutamine, 100 units/mL penicillin, and 0.1 mg/mL streptomycin. To induce myoblast differentiation, C2C12 cells were cultured in a differentiation medium containing 2% horse serum (v/v). The cells were maintained at 37°C in a 5% CO₂ and 95% humidity environment. For the coculture experiments, C2C12 cells were seeded in 12-well plates at a density of 70x10³ cells/well containing growth medium. After 24 hours, the C2C12 cells were differentiated for 5 days in the presence of 25 µM HMB, 5 µM HMB, and their combination in accordance with the information provide in table 4 below. RAW 264.7 cells were plated at a density of 30x10³ cells on inserts in a companion 12-well plate. The macrophages were allowed to settle and adhere for 24 hours, followed by activation with 0.05

µg/mL LPS for 6 hours. Once C2C12 cells were differentiated and RAW 264.7 cells were activated, coculture experiments were conducted for 24 or 48 hours.

Table 4. Coculture *in vitro* model: experimental groups

Group	Incubation Effector(s)
1	Activated Control
2	25 µM HMB
3	5 µM Hydroxytyrosol
4	25 µM HMB + 5 µM Hydroxytyrosol

[0122] After 48 hours of coculture, C2C12 cells were lysed with RIPA buffer supplemented with phosphatase and protease inhibitors. Cells were collected and sonicated, and the extracts were centrifuged for 10 minutes at 14,000 rpm. Supernatants were collected, and protein concentration was measured using the bicinchoninic acid method. Proteins (20 µg) were separated by SDS-PAGE, transferred onto nitrocellulose membranes, and immunoblotted with specific antibodies: anti-β-Actin, anti-phosphoP70s6K (Thermo Scientific), anti-P70s6K, and anti-Atrogin (Santa Cruz Biotechnology). Immunoblots were developed using an enhanced chemiluminescence detection method with Chemidoc MP (Biorad).

[0123] Total RNA from differentiated C2C12 cells was obtained after 24 hours of coculture using the RNeasy Plus Mini Kit (Qiagen). The quantity and quality of the isolated RNA were determined with QubitFlex (Thermo Scientific). cDNA was synthesized using the iScript™ cDNA Synthesis Kit (Biorad), and subsequently amplified using the SsoAdvanced Universal SYBR Green Supermix reagent (Biorad) and the LightCycler 480 II thermal cycler (Roche), following the manufacturer's recommendations. IL-6 mRNA levels were assessed using specific primers (see table 5). The housekeeping gene ACTIN was used as an internal control, and expression levels were calculated using the ΔΔCT method.

Table 5. IL-6 and beta-actin mice primers for RT-PCR

Gene Name	Forward Primer	Reverse Primer
IL-6	TCCTTCAGAGAGATACAGAAAC	TTCTGTGACTCCAGCTTATC
Actin	GATGTATGAAGGCTTTGGTC	TGTGCACTTTTATTGGTCTC

[0124] A general flowchart for this testing is shown in Figure 7. The results of this testing are shown in Figures 8 to 10.

[0125] As shown in Figure 8, contacting the muscle cells with HMB (25 μ M) resulted in a reduction in IL-6 expression of about 50% compared to the control and contacting the muscle cells with OHT (5 μ M) resulted in a reduction in IL-6 expression of about 40% compared to the control, with both showing efficacy. However, contacting the muscle cells with a combination of HMB (25 μ M) and OHT (5 μ M) resulted in a reduction in IL-6 expression of about 95% compared to the control, which is greater than would have been expected based on the individual results produced by each agent. As such, the combination HMB and OHT has been found to produce a synergistic effect in reducing expression of the inflammatory IL-6 cytokine in muscle cells subjected to an inflammation-producing environment. This represents a surprising ability of HMB and OHT, when administered together, to reduce the effects of an inflammatory environment on muscle cells.

[0126] As shown in Figure 9, contacting the muscle cells with HMB (25 μ M) resulted in a reduction in Atrogin-1 expression of about 17% compared to the control and contacting the muscle cells with OHT (5 μ M) resulted in a reduction in Atrogin-1 expression of about 12% compared to the control. However, contacting the muscle cells with a combination of HMB (25 μ M) and OHT (5 μ M) resulted in a reduction in Atrogin-1 expression of about 52% compared to the control, which is significantly greater than would have been expected based on the

individual results produced by each agent. As such, the combination HMB and OHT has been found to produce a synergistic effect in reducing expression of Atrogin-1, which is a known marker of protein degradation (proteolysis), in muscle cells subjected to an inflammation-producing environment. This represents a surprising ability of HMB and OHT, when administered together, to combat the catabolic effect on muscle cells that is produced by an inflammatory environment.

[0127] As shown in Figure 10, contacting the muscle cells with HMB (25 μ M) resulted in an increase in Phosphor-p70 S6 Kinase (P-p70S6K) expression of about 34% compared to the control and contacting the muscle cells with OHT (5 μ M) resulted in an increase in P-p70S6K expression of about 9% compared to the control. However, contacting the muscle cells with a combination of HMB (25 μ M) and OHT (5 μ M) resulted in an increase in P-p70S6K expression of about 177% compared to the control, which is significantly greater than would have been expected based on the individual results produced by each agent. As such, the combination HMB and OHT has been found to produce a synergistic effect in increasing the expression of P-p70S6K, which is a known marker of protein synthesis, in muscle cells subjected to an inflammation-producing environment. This represents a surprising ability of HMB and OHT, when administered together, to not only combat the catabolic effect on muscle cells that is produced by an inflammatory environment but also to promote protein synthesis in the face of that inflammatory environment.

[0128] Taken together, the results of Example 4 demonstrate that HMB and OHT, when administered together, produce a surprising and synergistic ability to counteract the negative effects of inflammation on muscle cells and thus improve muscle outcomes during periods of inflammation. In doing so, the combination of HMB and OHT to preserve muscle during periods of inflammation, such as occur during aging, sarcopenia, or chronic disease.

[0129] **Example 5:** Effects on the insulin sensitivity/resistance of muscle cells

[0130] This example describes an *in vitro* study that evaluated the effects of administration of HMB, hydroxytyrosol (OHT), and a combination thereof on muscle cells when subjected to Glyoxal, a di-carbonyl compound that promotes the formation of advanced glycation end products (AGEs).

[0131] AGEs are harmful compounds that are formed when protein or lipids combine with sugar in the bloodstream. Given that low glucose uptake, e.g. from insulin resistance, and consequent high blood glucose levels are a major driving factor in AGE generation, AGEs tend to accumulate in diabetic individuals and with aging (as insulin resistance is relatively common in the aging population). AGEs also themselves induce insulin resistance, creating a cycle in which the accumulation of AGEs promotes the formation of more AGEs, furthering the negative effects associated with AGE accumulation. Among those negative effects are inflammation in muscles and skeletal muscle atrophy.

[0132] By treating the cells with Glyoxal, the present example aims to mimic the situation that occurs during aging and/or the development/worsening of diabetes. Moreover, by measuring the effects of HMB and OHT on glucose uptake by muscle cells that have been treated in this manner, this example aims to test the effects of these compounds on the muscles of the aging and/or those with diabetes.

[0133] Rat skeletal muscle myoblast L6.C11 cell line (ATCC CRL-1458) was seeded in 48-well plates and cells were differentiated into myotubes by culturing them for 5 days in differentiation medium (DMEM containing 2% Fetal bovine Serum; FBS (v/v)). Once differentiated, myotubes were incubated in DMEM without FBS for 24 hours. After this time, cells were incubated for 24 hours with different effector(s) in accordance with the information provide in Table 6 below.

Table 6: Incubation of Myotubes

Group	Incubation Effector(s)
1	Control (none)

2	3 mM Glyoxal
3	3 mM Glyoxal + 25 μ M HMB
4	3 mM Glyoxal + 5 μ M Hydroxytyrosol
5	3 mM Glyoxal + 25 μ M HMB + 5 μ M Hydroxytyrosol

[0134] Then, glucose uptake was measured using a Glucose Uptake-Glo Assay (Promega), which is based on the detection of 2-deoxyglucose-6-phosphate (2DG6P) in mammalian cells. The assay was performed according to the manufacturing kit.

[0135] The results of this testing are shown in Figure 11 and are expressed as percentage of glucose uptake and normalized to a value of 100%, corresponding to the untreated control. As shown in Figure 11, contacting the Glyoxal-treated muscle cells with HMB (25 μ M) resulted in an increase in glucose uptake of 8.1% compared to the control (here, the Glyoxal-treated muscle cells) and contacting the Glyoxal-treated muscle cells with OHT (5 μ M) resulted in an increase in glucose uptake of 0.3% compared to the control (here, the Glyoxal-treated muscle cells). However, contacting the Glyoxal treated muscle cells with a combination of HMB (25 μ M) and OHT (5 μ M) resulted in an increase in glucose uptake of 29.1% compared to the control (here, the Glyoxal-treated muscle cells), which is significantly greater than would have been expected based on the individual results produced by each agent. As such, the combination HMB and OHT has been found to produce a synergistic effect in increasing the glucose uptake of muscle cells that have been treated to simulate the AGE production cycle that occurs in aging individuals and/or individuals with diabetes. This represents a surprising ability of HMB and OHT, when administered together, to promote glucose uptake in muscle cells, and in particular in muscle cells that are subjected to conditions associated with aging and/or diabetes, and thus improving muscle health in aging and/or diabetic individuals.

[0136] The results of Example 5 thus demonstrate that the combination of HMB and OHT has a synergistic and surprising ability to promote glucose uptake in muscle cells, and in particular in muscle cells that are subjected to conditions associated with aging and/or diabetes, and thus improving muscle health in aging and/or diabetic individuals. By controlling blood

sugar levels, e.g. by improving muscle cell insulin sensitivity and/or reducing muscle cell insulin resistance, these compounds can improve muscle health in aging and/or diabetic individuals by slowing the cycle of AGE formation and thus reducing/preventing muscle inflammation and skeletal muscle atrophy. These results also demonstrate that the combination of HMB and OHT has a synergistic and surprising ability to help control blood sugar levels in diabetic patients, improve muscle health in diabetic, aging, and sarcopenic subjects, and slow or prevent complications associated with the progression of diabetes.

[0137] **Example 6:** Effects on muscle regeneration

[0138] This example describes an *in vitro* study that evaluated the effects of administration of HMB, hydroxytyrosol (OHT), and a combination thereof on satellite muscle cells harvested from aged (greater than 6 months old) rats. Satellite cells are the cells that are responsible for the regeneration of muscle. Once activated (which naturally occurs when muscles are damaged), the satellite cells undergo a multi-stage process by which they are converted into new muscle cells. These stages are, in order, proliferation, differentiation, fusion, and maturation. An illustration of this process is shown in Figure 12. Different myogenic regulatory factors are associated with, and act as markers for, each stage of this process. Thus, by measuring the effects of HMB, OHT, and the combination thereof on different myogenic regulatory factors, one can measure the effects of each on the various stages of the muscle regeneration process. Specifically, MyoD is a known marker of muscle cell proliferation, MyoG (i.e., Myogenin) is a known marker of muscle cell differentiation, and Myf6 is known marker of muscle cell fusion and maturation.

[0139] Primary culture preparations were obtained by seeding the satellite cells obtained from one gram of gastrocnemius muscle in gelatin coated plates. First, each muscle was carefully processed to discard fat and connective tissues and washed several times in sterile phosphate buffered saline (PBS) (pH 7.4). The muscles were then minced with scissors and digested for 2 h in 40 ml of 0.17% protease from *Streptomyces griseus* Type XIV, ≥ 3.5 units/mg

solid (#P5147, Merck) diluted in Ham's F-12 medium plus 10% fetal calf serum (FCS) at 37°C with orbital agitation. Then, the digested muscles were centrifuged 150 g for 5 min to discard debris and non-digested muscles and the supernatant was centrifuged (350 g for 20 min). The satellite cells in the pellet were re-suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS and 10% horse serum (HS). Cells were re-suspended in 9 mL of the grown medium and distributed in 3 flasks (60 000 cells/25 cm² flask). To facilitate the adhesion of muscular cells to the plastic surface, and to avoid attachment of fibroblasts, flasks were pre-coated with gelatin (0.5% in water). The cells were grown in DMEM plus 10% FCS and 10% HS supplemented with penicillin (0.1 mg/ml) and streptomycin (100 U/ml) at 37°C in a humidified atmosphere of 5% CO₂. When the cells reach an 80-90 % confluence, they were frozen.

[0140] To analyze the effects of the different ingredients on the expression of the different transcriptional factors involved in the muscle regeneration process, the satellite cells were thawed and grown in DMEM medium supplemented with 10% FBS and 10% calf serum. When they reach a confluence of 80-90%, they were incubated with 25 µM HMB, 5 µM OHT, or the mixture of both HMB (25 µM) and OHT (5 µM) for 24 hours.

[0141] To study the protein expression of different proteins involved in the satellite cell differentiation process, cells were flash frozen and lysed with RIPA buffer supplemented with phosphatase and protease inhibitors, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM EGTA, 20 nM okadaic acid, 10 µg/ml aprotinin, 10 µg/ml leupeptin and 10 µg/ml pepstatin. The protein concentration was measured using the bicinchoninic acid method. Proteins (20 µg) were separated by sodium dodecyl sulfate-polyacrylamide (SDS-PAGE), transferred onto nitrocellulose membranes, and immunoblotted with specific antibodies. To determine the expression of these proteins, the expression of GAPDH is used as load control. Immunoblots were developed using fluorescent secondary

antibodies and detected using a Chemidoc Imager. Quantification was performed by densitometry with the Image Lab Program (Bio-Rad) or the NIH Image Software.

[0142] The results of this testing are shown in Figures 13-15.

[0143] As shown in Figure 13, contacting the satellite cells with HMB (25 μ M) resulted in a decrease in MyoD expression of about 5% compared to the control and contacting the muscle cells with OHT (5 μ M) resulted in an increase in MyoD expression of about 11% compared to the control. However, contacting the muscle cells with a combination of HMB (25 μ M) and OHT (5 μ M) resulted in an increase in MyoD expression of about 39% compared to the control, which is much greater than would have been expected based on the individual results produced by each agent. As such, the combination HMB and OHT has been found to produce a synergistic effect on MyoD expression in activated muscle satellite cells. This represents a surprising ability of HMB and OHT, when administered together, to promote the proliferation stage of muscle cell regeneration.

[0144] As shown in Figure 14, contacting the satellite cells with HMB (25 μ M) resulted in an increase in MyoG expression of about 8% compared to the control and contacting the muscle cells with OHT (5 μ M) resulted in a decrease in MyoG expression of about 2% compared to the control. However, contacting the muscle cells with a combination of HMB (25 μ M) and OHT (5 μ M) resulted in an increase in MyoG expression of about 65% compared to the control, which is much greater than would have been expected based on the individual results produced by each agent. As such, the combination HMB and OHT has been found to produce a synergistic effect on MyoG expression in activated muscle satellite cells. This represents a surprising ability of HMB and OHT, when administered together, to promote the differentiation stage of muscle cell regeneration.

[0145] As shown in Figure 15, contacting the satellite cells with HMB (25 μ M) resulted in an increase in Myf6 expression of about 7% compared to the control and contacting the muscle cells with OHT (5 μ M) resulted in a decrease in Myf6 expression of about 3% compared to the

control. However, contacting the muscle cells with a combination of HMB (25 μ M) and OHT (5 μ M) resulted in an increase in Myf6 expression of about 48% compared to the control, which is much greater than would have been expected based on the individual results produced by each agent. As such, the combination HMB and OHT has been found to produce a synergistic effect on Myf6 expression in activated muscle satellite cells. This represents a surprising ability of HMB and OHT, when administered together, to promote the fusion and maturation stage of muscle cell regeneration.

[0146] Taken together, the results of Example 4 demonstrate that HMB and OHT, when administered together, produce a surprising and synergistic ability to promote muscle regeneration, i.e. to promote the production of new, functional skeletal muscle. As a person ages, the capacity of his or her body to regenerate muscle cells decreases. Accordingly, the ability to promote muscle regeneration may be particularly beneficial to the aging adult and/or elderly population. The ability to promote muscle regeneration may also be particularly beneficial for preserving and rebuilding muscle during recovery from immobilization and/or injury. Finally, the ability to promote muscle regeneration may also be particularly beneficial for those recovering from muscle damage, e.g. due to extended physical activity, such as athletes.

[0147] In summary, the methods of the present application provide a convenient means in the form of the combination of HMB and hydroxytyrosol for a host of skeletal muscle improvements, specifically, maintaining skeletal muscle mobility and/or mass by mitigating muscle protein degradation, promoting muscle protein synthesis, improving the recovery of skeletal muscle strength, and/or improving recovery of skeletal muscle strength, especially where an individual is in need thereof, such as when an individual is in a pro-inflammatory state or is suffering from muscle atrophy or similar catabolic condition.

[0148] The specific embodiments and examples described herein are exemplary only and are not limiting to the invention defined in the claims. Additionally, while the present invention has been illustrated by the description of embodiments thereof, and while the embodiments

have been described in considerable detail, such descriptions are not intended to restrict or in any way limit the scope of the appended claims to such detail. Additional advantages and modifications will readily appear to those skilled in the art. Therefore, the invention, in its broader aspects, is not limited to the specific details, the representative compositions and processes, or illustrative examples shown and described. Accordingly, departures may be made from such details without departing from the spirit or scope of the general inventive concept.

Claims

What is claimed is:

1. A method for maintaining skeletal muscle mobility and/or mass, and/or for recovery of skeletal muscle strength in an individual in need thereof, comprising administering β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol to the individual.
2. The method of claim 1, wherein the at least one olive polyphenol comprises hydroxytyrosol.
3. The method of any one of claims 1 or 2, wherein the individual has or is at risk of losing muscle mass.
4. The method of claim 3, wherein the reduced muscle mass or risk thereof is associated with aging, sarcopenia, hospitalization, immobilization, acquired immunodeficiency syndrome (AIDS), diabetes, cancer, chronic obstructive pulmonary disease (COPD), burn injury, nonalcoholic fatty liver disease (NAFLD), long term steroid use, inflammatory bowel disease (IBD), Crohn's disease, malnutrition, and/or an acute or chronic condition with a hypercatabolic state.
5. The method of any one of claims 1-4, wherein the individual is being treated with a glucocorticoid.
6. The method of any one of claims 1 or 2, wherein the individual has or is at risk of muscle catabolism.

7. The method of any one of claims 1 or 2, wherein the individual has or is at risk of losing muscle strength.
8. The method of claim 7, wherein the reduced muscle strength or risk thereof is associated with inflammation, oxidative stress, immobilization, sepsis, infection, surgery, cancer, COPD, obesity, diabetes, cardiovascular disease, dementia, aging, sarcopenia, malnutrition, and/or an acute or chronic condition with a systemic pro-inflammatory state.
9. The method of any one of claims 1-8, wherein the HMB is administered to the individual in an amount of about 0.01 to 10 g/day, about 0.1 to 10 g/day, or about 0.1 to 6 g/day.
10. The method of any one of claims 1-9, wherein the at least one olive polyphenol, or more specifically, hydroxytyrosol is administered to the individual in an amount of about 0.5 to 50 mg/day, about 1 to 20 mg/day, or about 5 to 15 mg/day.
11. The method of any one of claims 1-10, wherein the individual is an adult subject.
12. The method of any one of claims 1-10, wherein the individual is a pediatric subject.
13. The method of any one of claims 1-12, wherein the HMB and the at least one olive polyphenol is administered to the individual in a nutritional composition.
14. The method of claim 13, wherein the nutritional composition is administered to the individual at least once per day, at least two times per day, or about 1-4 times per day.

15. The method of claim 13 or 14, wherein the composition comprises about 0.01 to about 10 wt %, about 0.1 to about 10 wt %, 0.1 to about 5 wt %, about 0.1 to about 3 wt %, about 0.1 to about 2 wt %, or about 0.1 to about 1.5 wt % of HMB.
16. The method of any one of claims 13-15, wherein the HMB is selected from the group consisting of alkali metal HMB, alkaline earth metal HMB, HMB free acid, HMB lactone and combinations of two or more thereof, optionally wherein the HMB is selected from the group consisting of sodium HMB, potassium HMB, magnesium HMB, chromium HMB, calcium HMB and combinations of two or more thereof.
17. The method of any one of claims 13-16, wherein the composition comprises about 0.001 to about 1 wt %, about 0.005 to about 0.5 wt %, 0.005 to about 0.25 wt %, about 0.008 to about 0.1 wt %, about 0.01 to about 0.02 wt %, or about 0.008 to about 0.025 wt % of at least one olive polyphenol, optionally wherein the at least one olive polyphenol is hydroxytyrosol.
18. The method of any one of claims 13-17, wherein the nutritional composition comprises protein comprising whey protein concentrate, whey protein isolate, whey protein hydrolysate, acid casein, sodium caseinate, calcium caseinate, potassium caseinate, casein hydrolysate, milk protein concentrate, organic milk protein concentrate, milk protein isolate, milk protein hydrolysate, nonfat dry milk, condensed skim milk, soy protein concentrate, isolated soy protein, soy protein hydrolysate, pea protein concentrate, pea protein isolate, pea protein hydrolysate, collagen protein, collagen protein isolate, L-Carnitine, taurine, lutein, rice protein concentrate, rice protein isolate, rice protein hydrolysate, fava bean protein concentrate, fava bean protein isolate, fava bean protein hydrolysate, meat protein, potato protein, chickpea protein, canola protein,

mung protein, guinea protein, amaranth protein, chia protein, hemp protein, flax seed protein, earthworm protein, insect protein, one or more amino acids and/or metabolites thereof, or a combination of two or more thereof.

19. The method of any one of any one of claims 13-18, wherein the nutritional composition comprises fat comprising coconut oil, fractionated coconut oil, soy oil, soy lecithin, corn oil, safflower oil, high oleic sunflower oil, palm olein, canola oil monoglycerides, lecithin, medium chain triglycerides, linoleic acid, alpha-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, high gamma linolenic (GLA) safflower oil, palm oil, palm kernel oil, canola oil, marine oil, fish oil, algal oil, borage oil, cottonseed oil, fungal oil, interesterified oils, transesterified oils, structured lipid, or a combination of two or more thereof.

20. The method of any one of claims 13-19, wherein the nutritional composition comprises carbohydrate comprising fiber, human milk oligosaccharide (HMO), maltodextrin, corn maltodextrin, organic corn, corn syrup, sucralose, cellulose gel, cellulose gum, gellan gum, inositol, carrageenan, fructooligosaccharide, hydrolyzed starch, glucose polymer, corn syrup solid, rice-derived carbohydrate, sucrose, glucose, lactose, honey, sugar alcohol, isomaltulose, sucromalt, pullulan, potato starch, galactooligosaccharide, oat fiber, soy fiber, corn fiber, gum arable, sodium carboxymethylcellulose, methylcellulose, guar gum, locust bean gum, konjac flour, hydroxypropyl methylcellulose, tragacanth gum, karaya gum, gum acacia, chitosan, arabinoglactin, glucomannan, xanthan gum, alginate, pectin, low methoxy pectin, high methoxy pectin, cereal beta-glucan, psyllium, inulin, cornstarch, or a combination of two or more thereof.

21. The method of any one of claims 13-20, wherein the nutritional composition comprises from about 1 to about 30 wt % of protein, from about 0.1 to about 30 wt % fat, and from about 0.5 to about 70 wt % carbohydrate.
22. The method of any one of claims 13-20, wherein the nutritional composition comprises from about 10 to about 70 wt % protein and from about 10 to about 40 wt % carbohydrate.
23. The method of any one of claims 13-22, wherein the nutritional composition is in the form of a powder.
24. The method of any one of claims 13-22, wherein the nutritional composition is in the form of a liquid.
25. The method of any one of claims 1-24, wherein maintaining skeletal muscle mobility and/or mass and/or recovery of skeletal muscle strength involves mitigating muscle protein degradation, promoting muscle protein synthesis, or a combination thereof.
26. A nutritional composition for maintaining skeletal muscle mobility and/or mass, and/or for recovery of skeletal muscle strength in an individual in need thereof comprising HMB, at least one olive polyphenol, and at least one of: at least one source of protein, at least one source of fat, at least one source of carbohydrate.
27. The nutritional composition of claim 26, wherein the at least one olive polyphenol comprises hydroxytyrosol.

28. The nutritional composition of claim 26 or 27, wherein the composition comprises about 0.01 to about 10 wt %, about 0.1 to about 10 wt %, about 0.1 to about 5 wt %, about 0.1 to about 3 wt %, about 0.1 to about 2 wt %, or about 0.1 to about 1.5 wt % of HMB.
29. The nutritional composition of any one of claims 26-28, wherein the HMB is selected from the group consisting of alkali metal HMB, alkaline earth metal HMB, HMB free acid, HMB lactone and combinations of two or more thereof, or the HMB is selected from the group consisting of sodium HMB, potassium HMB, magnesium HMB, chromium HMB, calcium HMB and combinations of two or more thereof.
30. The nutritional composition of any one of claims 26-29, wherein the composition comprises about 0.001 to about 1 wt %, about 0.005 to about 0.5 wt %, about 0.005 to about 0.25 wt %, about 0.008 to about 0.1 wt %, about 0.01 to about 0.02 wt %, or about 0.008 to about 0.025 wt % of at least one olive polyphenol, or more specifically, hydroxytyrosol.
31. The nutritional composition of any one of claims 26-30, wherein the nutritional composition comprises protein comprising whey protein concentrate, whey protein isolate, whey protein hydrolysate, acid casein, sodium caseinate, calcium caseinate, potassium caseinate, casein hydrolysate, milk protein concentrate, organic milk protein concentrate, milk protein isolate, milk protein hydrolysate, nonfat dry milk, condensed skim milk, soy protein concentrate, isolated soy protein, soy protein hydrolysate, pea protein concentrate, pea protein isolate, pea protein hydrolysate, collagen protein, collagen protein isolate, L-Carnitine, taurine, lutein, rice protein concentrate, rice protein isolate, rice protein hydrolysate, fava bean protein concentrate, fava bean protein isolate, fava bean protein hydrolysate, meat protein, potato protein, chickpea protein,

canola protein, mung protein, guinea protein, amaranth protein, chia protein, hemp protein, flax seed protein, earthworm protein, insect protein, one or more amino acids and/or metabolites thereof, or a combination of two or more thereof.

32. The nutritional composition of any one of any one of claims 26-31, wherein the nutritional composition comprises fat comprising coconut oil, fractionated coconut oil, soy oil, soy lecithin, corn oil, safflower oil, high oleic sunflower oil, palm olein, canola oil monoglycerides, lecithin, medium chain triglycerides, linoleic acid, alpha-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, high gamma linolenic (GLA) safflower oil, palm oil, palm kernel oil, canola oil, marine oil, fish oil, algal oil, borage oil, cottonseed oil, fungal oil, interesterified oils, transesterified oils, structured lipid, or a combination of two or more thereof.

33. The nutritional composition of any one of claims 26-32, wherein the nutritional composition comprises carbohydrate comprising fiber, human milk oligosaccharide (HMO), maltodextrin, corn maltodextrin, organic corn, corn syrup, sucralose, cellulose gel, cellulose gum, gellan gum, inositol, carrageenan, fructooligosaccharide, hydrolyzed starch, glucose polymer, corn syrup solid, rice-derived carbohydrate, sucrose, glucose, lactose, honey, sugar alcohol, isomaltulose, sucromalt, pullulan, potato starch, galactooligosaccharide, oat fiber, soy fiber, corn fiber, gum arable, sodium carboxymethylcellulose, methylcellulose, guar gum, locust bean gum, konjac flour, hydroxypropyl methylcellulose, tragacanth gum, karaya gum, gum acacia, chitosan, arabinogalactin, glucomannan, xanthan gum, alginate, pectin, low methoxy pectin, high methoxy pectin, cereal beta-glucan, psyllium, inulin, cornstarch, or a combination of two or more thereof.

34. The nutritional composition of any one of claims 26-33, wherein the nutritional composition comprises from about 1 to about 30 wt % of protein, from about 0.1 to about 30 wt % fat, and from about 0.5 to about 70 wt % carbohydrate.
35. The nutritional composition of any one of claims 26-31 or 33, wherein the nutritional composition comprises from about 10 to about 70 wt % protein and from about 10 to about 40 wt % carbohydrate.
36. The nutritional composition of any one of claims 26-35, wherein the nutritional composition is in the form of a powder.
37. The nutritional composition of any one of claims 26-35, wherein the nutritional composition is in the form of a liquid.
38. A method for recovery of muscle mass in an individual in need thereof, comprising administering β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol to the individual.
39. The method of claim 38, wherein the individual is undergoing muscle atrophy.
40. The method of any one of claims 38 and 39, wherein the recovery of muscle mass involves mitigating muscle protein degradation, promoting muscle protein synthesis, or a combination thereof.

41. A method for preventing decline in muscle mobility or mass in an individual in need thereof, comprising administering β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol to the individual.
42. The method of claim 41, wherein the individual is in a systemic pro-inflammatory state or catabolic state.
43. The method of any one of claims 41 and 42, wherein the preventing decline in muscle mobility or mass involves mitigating muscle protein degradation, promoting muscle protein synthesis, or a combination thereof.
44. A method for restoring muscle homeostasis during catabolic periods, preserving muscle during catabolic periods or conditions, enhancing muscle protein synthesis in conditions where protein synthesis is decreased (such as in muscle atrophy), enhancing muscle anabolism during a catabolic state, decreasing muscle protein breakdown during a catabolic state, enhancing faster recovery of muscle strength following a catabolic pro-inflammatory period, and/or reducing the recovery time for regaining muscle strength following a catabolic pro-inflammatory period, in an individual in need thereof, comprising administering β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol to the individual.
45. A method of promoting muscle regeneration in an individual in need thereof, comprising administering β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol to the individual.
46. The method of claim 45, wherein the individual in need thereof has suffered muscle damage as a result of physical activity.

47. The method of claim 45, wherein the individual in need thereof is recovering from immobilization, injury, or both.
48. The method of any one of claims 38 to 47, wherein the at least one olive polyphenol comprises or consists of hydroxytyrosol.
49. The method of any one of claims 38 to 48, wherein the individual has or is at risk of losing muscle mass.
50. The method of claim 49, wherein the reduced muscle mass or risk thereof is associated with aging, sarcopenia, hospitalization, immobilization, acquired immunodeficiency syndrome (AIDS), diabetes, cancer, chronic obstructive pulmonary disease (COPD), burn injury, nonalcoholic fatty liver disease (NAFLD), long term steroid use, inflammatory bowel disease (IBD), Crohn's disease, malnutrition, and/or an acute or chronic condition with a hypercatabolic state.
51. The method of any one of claims 38 to 50, wherein the individual suffers from diabetes.
52. The method of any one of claims 38 to 51, wherein the individual has or is at risk of muscle catabolism.
53. The method of any one of claims 38 to 52, wherein the individual has or is at risk of losing muscle strength.
54. The method of claim 53, wherein the reduced muscle strength or risk thereof is associated with inflammation, oxidative stress, immobilization, sepsis, infection, surgery,

cancer, COPD, obesity, diabetes, cardiovascular disease, dementia, aging, sarcopenia, malnutrition, and/or an acute or chronic condition with a systemic pro-inflammatory state.

55. The method of any one of claims 38 to 54, wherein the HMB is administered to the individual in an amount of about 0.01 to 10 g/day, about 0.1 to 10 g/day, or about 0.1 to 6 g/day.
56. The method of any one of claims 38 to 55, wherein the at least one olive polyphenol, optionally hydroxytyrosol, is administered to the individual in an amount of about 0.5 to 50 mg/day, about 1 to 20 mg/day, or about 5 to 15 mg/day.
57. The method of any one of claims 38 to 56, wherein the individual is an adult subject, optionally an adult subject of at least 40 years of age, an adult subject of at least 50 years of age, or an adult subject of at least 60 years of age.
58. The method of any one of claims 38 to 56, wherein the individual is a pediatric subject.
59. The method of any one of claims 38 to 58, wherein the HMB and the at least one olive polyphenol is administered to the individual in a nutritional composition.
60. The method of claim 59, wherein the nutritional composition is administered to the individual at least once per day, at least two times per day, or about 1-4 times per day.
61. The method of claim 59 or 60, wherein the composition comprises about 0.01 to about 10 wt %, about 0.1 to about 10 wt %, about 0.1 to about 5 wt %, about 0.1 to about 3 wt %, about 0.1 to about 2 wt %, or about 0.1 to about 1.5 wt % of HMB.

62. The method of any one of claims 59-61, wherein the HMB is selected from the group consisting of alkali metal HMB, alkaline earth metal HMB, HMB free acid, HMB lactone and combinations of two or more thereof, optionally wherein the HMB is selected from the group consisting of sodium HMB, potassium HMB, magnesium HMB, chromium HMB, calcium HMB and combinations of two or more thereof.
63. The method of any one of claims 59-62, wherein the composition comprises about 0.001 to about 1 wt %, about 0.005 to about 0.5 wt %, about 0.005 to about 0.25 wt %, about 0.008 to about 0.1 wt %, about 0.01 to about 0.02 wt %, or about 0.008 to about 0.025 wt % of at least one olive polyphenol, optionally wherein the at least one olive polyphenol is hydroxytyrosol.
64. The method of any one of claims 59-63, wherein the nutritional composition comprises protein comprising whey protein concentrate, whey protein isolate, whey protein hydrolysate, acid casein, sodium caseinate, calcium caseinate, potassium caseinate, casein hydrolysate, milk protein concentrate, organic milk protein concentrate, milk protein isolate, milk protein hydrolysate, nonfat dry milk, condensed skim milk, soy protein concentrate, isolated soy protein, soy protein hydrolysate, pea protein concentrate, pea protein isolate, pea protein hydrolysate, collagen protein, collagen protein isolate, L-Carnitine, taurine, lutein, rice protein concentrate, rice protein isolate, rice protein hydrolysate, fava bean protein concentrate, fava bean protein isolate, fava bean protein hydrolysate, meat protein, potato protein, chickpea protein, canola protein, mung protein, guinea protein, amaranth protein, chia protein, hemp protein, flax seed protein, earthworm protein, insect protein, one or more amino acids and/or metabolites thereof, or a combination of two or more thereof.

65. The method of any one of any one of claims 59-64, wherein the nutritional composition comprises fat comprising coconut oil, fractionated coconut oil, soy oil, soy lecithin, corn oil, safflower oil, high oleic sunflower oil, palm olein, canola oil monoglycerides, lecithin, medium chain triglycerides, linoleic acid, alpha-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, high gamma linolenic (GLA) safflower oil, palm oil, palm kernel oil, canola oil, marine oil, fish oil, algal oil, borage oil, cottonseed oil, fungal oil, interesterified oils, transesterified oils, structured lipid, or a combination of two or more thereof.
66. The method of any one of claims 59-65, wherein the nutritional composition comprises carbohydrate comprising fiber, human milk oligosaccharide (HMO), maltodextrin, corn maltodextrin, organic corn, corn syrup, sucralose, cellulose gel, cellulose gum, gellan gum, inositol, carrageenan, fructooligosaccharide, hydrolyzed starch, glucose polymer, corn syrup solid, rice-derived carbohydrate, sucrose, glucose, lactose, honey, sugar alcohol, isomaltulose, sucromalt, pullulan, potato starch, galactooligosaccharide, oat fiber, soy fiber, corn fiber, gum arable, sodium carboxymethylcellulose, methylcellulose, guar gum, locust bean gum, konjac flour, hydroxypropyl methylcellulose, tragacanth gum, karaya gum, gum acacia, chitosan, arabinoglactin, glucomannan, xanthan gum, alginate, pectin, low methoxy pectin, high methoxy pectin, cereal beta-glucan, psyllium, inulin, cornstarch, or a combination of two or more thereof.
67. The method of any one of claims 59-66, wherein the nutritional composition comprises from about 1 to about 30 wt % of protein, from about 0.1 to about 30 wt % fat, and from about 0.5 to about 70 wt % carbohydrate.

68. The method of any one of claims 59-66, wherein the nutritional composition comprises from about 10 to about 70 wt % protein and from about 10 to about 40 wt % carbohydrate.
69. The method of any one of claims 59-68, wherein the nutritional composition is in the form of a powder.
70. The method of any one of claims 59-68, wherein the nutritional composition is in the form of a liquid.
71. A nutritional composition comprising β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol for use in maintaining skeletal muscle mobility and/or mass, and/or for recovery of skeletal muscle strength in an individual.
72. The nutritional composition of claim 71, wherein the maintaining skeletal muscle mobility and/or mass, and/or for recovery of skeletal muscle strength in an individual involves mitigating muscle protein degradation, promoting muscle protein synthesis, or a combination thereof.
73. A nutritional composition comprising β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol for use in recovery of muscle mass in an individual in need thereof.
74. The nutritional composition of claim 73, wherein the individual is undergoing muscle atrophy.

75. The nutritional composition of any one of claims 73 and 74, wherein the recovery of muscle mass involves mitigating muscle protein degradation, promoting muscle protein synthesis, or a combination thereof.
76. A nutritional composition comprising β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol for use in preventing decline in muscle mobility or mass in an individual in need thereof.
77. The nutritional composition of claim 76, wherein the individual is in a systemic pro-inflammatory state or catabolic state.
78. The nutritional composition of claim 76 or 77, wherein preventing decline in muscle mobility or mass in an individual in need thereof involves mitigating muscle protein degradation, promoting muscle protein synthesis, or a combination thereof.
79. A nutritional composition comprising β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol for use in restoring muscle homeostasis during catabolic periods, preserving muscle during catabolic periods or conditions, enhancing muscle protein synthesis in conditions where protein synthesis is decreased (such as in muscle atrophy), enhancing muscle anabolism during a catabolic state, decreasing muscle protein breakdown during a catabolic state, enhancing faster recovery of muscle strength following a catabolic pro-inflammatory period, and/or reducing the recovery time for regaining muscle strength following a catabolic pro-inflammatory period, in an individual in need thereof.
80. A nutritional composition comprising β -hydroxy- β -methylbutyrate (HMB) and at least one olive polyphenol for use in promoting muscle regeneration in an individual in need thereof.

81. The nutritional composition of claim 80, wherein the individual in need thereof has suffered muscle damage as a result of physical activity.
82. The nutritional composition of claim 80, wherein the individual in need thereof is recovering from immobilization, injury, or both.
83. The nutritional composition of any one of claims 71 to 82, wherein the at least one olive polyphenol comprises or consists of hydroxytyrosol.
84. The nutritional composition of any one of claims 71 to 83, wherein the individual has or is at risk of losing muscle mass.
85. The nutritional composition of claim 84, wherein the reduced muscle mass or risk thereof is associated with aging, sarcopenia, hospitalization, immobilization, acquired immunodeficiency syndrome (AIDS), diabetes, cancer, chronic obstructive pulmonary disease (COPD), burn injury, nonalcoholic fatty liver disease (NAFLD), long term steroid use, inflammatory bowel disease (IBD), Crohn's disease, malnutrition, and/or an acute or chronic condition with a hypercatabolic state.
86. The nutritional composition of any one of claims 71 to 85, wherein the individual suffers from diabetes.
87. The nutritional composition of any one of claims 71 to 86, wherein the individual has or is at risk of muscle catabolism.

88. The nutritional composition of any one of claims 71 to 87, wherein the individual has or is at risk of losing muscle strength.
89. The nutritional composition of claim 88, wherein the reduced muscle strength or risk thereof is associated with inflammation, oxidative stress, immobilization, sepsis, infection, surgery, cancer, COPD, obesity, diabetes, cardiovascular disease, dementia, aging, sarcopenia, malnutrition, and/or an acute or chronic condition with a systemic pro-inflammatory state.
90. The nutritional composition of any one of claims 71 to 89, wherein the HMB is administered to the individual in an amount of about 0.01 to 10 g/day, about 0.1 to 10 g/day, or about 0.1 to 6 g/day.
91. The nutritional composition of any one of claims 71 to 90, wherein the at least one olive polyphenol, optionally hydroxytyrosol, is administered to the individual in an amount of about 0.5 to 50 mg/day, about 1 to 20 mg/day, or about 5 to 15 mg/day.
92. The nutritional composition of any one of claims 71 to 91, wherein the individual is an adult subject, optionally an adult subject of at least 40 years of age, an adult subject of at least 50 years of age, or an adult subject of at least 60 years of age.
93. The nutritional composition of any one of claims 71 to 91, wherein the individual is a pediatric subject.
94. The nutritional composition of any one of claims 71 to 93, wherein nutritional composition comprises at least one of a protein, fat, and carbohydrate; optionally

wherein the nutritional composition comprises at least one protein, at least one fat, and at least one carbohydrate.

95. The nutritional composition of any one of claims 71 to 94, wherein the nutritional composition is administered to the individual at least once per day, at least two times per day, or about 1-4 times per day.
96. The nutritional composition of any one of claims 71 to 95, wherein the composition comprises about 0.01 to about 10 wt %, about 0.1 to about 10 wt %, about 0.1 to about 5 wt %, about 0.1 to about 3 wt %, about 0.1 to about 2 wt %, or about 0.1 to about 1.5 wt % of HMB.
97. The nutritional composition of any one of claims 71 to 96, wherein the HMB is selected from the group consisting of alkali metal HMB, alkaline earth metal HMB, HMB free acid, HMB lactone and combinations of two or more thereof, optionally wherein the HMB is selected from the group consisting of sodium HMB, potassium HMB, magnesium HMB, chromium HMB, calcium HMB and combinations of two or more thereof.
98. The nutritional composition of any one of claims 71 to 97, wherein the composition comprises about 0.001 to about 1 wt %, about 0.005 to about 0.5 wt %, about 0.005 to about 0.25 wt %, about 0.008 to about 0.1 wt %, about 0.01 to about 0.02 wt %, or about 0.008 to about 0.025 wt % of at least one olive polyphenol, optionally wherein the at least one olive polyphenol is hydroxytyrosol.
99. The nutritional composition of any one of claims 71 to 98, wherein the nutritional composition comprises protein comprising whey protein concentrate, whey protein

isolate, whey protein hydrolysate, acid casein, sodium caseinate, calcium caseinate, potassium caseinate, casein hydrolysate, milk protein concentrate, organic milk protein concentrate, milk protein isolate, milk protein hydrolysate, nonfat dry milk, condensed skim milk, soy protein concentrate, isolated soy protein, soy protein hydrolysate, pea protein concentrate, pea protein isolate, pea protein hydrolysate, collagen protein, collagen protein isolate, L-Carnitine, taurine, lutein, rice protein concentrate, rice protein isolate, rice protein hydrolysate, fava bean protein concentrate, fava bean protein isolate, fava bean protein hydrolysate, meat protein, potato protein, chickpea protein, canola protein, mung protein, guinea protein, amaranth protein, chia protein, hemp protein, flax seed protein, earthworm protein, insect protein, one or more amino acids and/or metabolites thereof, or a combination of two or more thereof.

100. The nutritional composition of any one of claims 71 to 99, wherein the nutritional composition comprises fat comprising coconut oil, fractionated coconut oil, soy oil, soy lecithin, corn oil, safflower oil, high oleic sunflower oil, palm olein, canola oil monoglycerides, lecithin, medium chain triglycerides, linoleic acid, alpha-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, high gamma linolenic (GLA) safflower oil, palm oil, palm kernel oil, canola oil, marine oil, fish oil, algal oil, borage oil, cottonseed oil, fungal oil, interesterified oils, transesterified oils, structured lipid, or a combination of two or more thereof.

101. The nutritional composition of any one of claims 71 to 100, wherein the nutritional composition comprises carbohydrate comprising fiber, human milk oligosaccharide (HMO), maltodextrin, corn maltodextrin, organic corn, corn syrup, sucralose, cellulose gel, cellulose gum, gellan gum, inositol, carrageenan, fructooligosaccharide, hydrolyzed starch, glucose polymer, corn syrup solid, rice-derived carbohydrate, sucrose, glucose,

lactose, honey, sugar alcohol, isomaltulose, sucromalt, pullulan, potato starch, galactooligosaccharide, oat fiber, soy fiber, corn fiber, gum arable, sodium carboxymethylcellulose, methylcellulose, guar gum, locust bean gum, konjac flour, hydroxypropyl methylcellulose, tragacanth gum, karaya gum, gum acacia, chitosan, arabinoglactin, glucomannan, xanthan gum, alginate, pectin, low methoxy pectin, high methoxy pectin, cereal beta-glucan, psyllium, inulin, cornstarch, or a combination of two or more thereof.

102. The nutritional composition of any one of claims 71 to 101, wherein the nutritional composition comprises from about 1 to about 30 wt % of protein, from about 0.1 to about 30 wt % fat, and from about 0.5 to about 70 wt % carbohydrate.

103. The nutritional composition of any one of claims 71 to 101, wherein the nutritional composition comprises from about 10 to about 70 wt % protein and from about 10 to about 40 wt % carbohydrate.

104. The nutritional composition of any one of claims 71 to 103, wherein the nutritional composition is in the form of a powder.

105. The nutritional composition of any one of claims 71 to 103, wherein the nutritional composition is in the form of a liquid.