

FIELD OF THE INVENTION

[0001] The present invention relates to methods for improving muscle energy production and/or muscle strength in a subject, and/or for reducing muscle loss in a subject, and to nutritional compositions suitable for use, inter alia, to improve muscle energy production and/or muscle strength, and/or for reducing muscle loss.

BACKGROUND OF THE INVENTION

[0002] Sarcopenia is defined as any loss of skeletal muscle mass and strength secondary to aging and/or chronic disease associated with a hypercatabolism state. Sarcopenia includes both muscle loss and muscle dysfunction, which involves contractile impairment and metabolic and endocrine abnormalities. Sarcopenia affects more than 25% of men over 60 years of age and close to 20% of women over 60 years of age, and over half of both men and women over the age of 80. Although the cause of sarcopenia and the molecular pathways contributing to the age-related decrease of muscle performance are not fully understood, there is a scientific consensus that mitochondrial dysfunction and aberrant bioenergetics are key players in the development of the pathology.

[0003] Skeletal muscle fiber contraction permits locomotion and other body movements, maintenance of posture, respiration (diaphragm and intercostal muscles) and communication (verbal and facial muscles). Contraction of a muscle, which acts as a secretory organ, stimulates production, secretion, and expression of cytokines or myokines (other muscle fiber-derived peptides). Contraction-induced myokines influence crosstalk between different organs in an autocrine, endocrine, or paracrine fashion and have positive effects on glucose uptake, glucose tolerance, regulation of fat oxidation, and satellite cell proliferation. Accordingly, contractile impairment in muscle has a variety of adverse effects on an individual. Decreased

contractility leads to loss of strength (i.e., dynapenia), fatigue, disability and falls, impaired pulmonary ventilation, osteoporosis, and fractures due to reduced mechanical bone stimulation in patients with chronic disease and the elderly.

[0004] In muscle cells, the majority of the energy demand is met by the adenosine triphosphate (ATP) produced by oxidative phosphorylation. This process occurs in the mitochondria, frequently called the cell's power stations, through the generation of an electrochemical potential that is used by the ATP synthase to phosphorylate adenosine diphosphate (ADP) and produce ATP. Although muscle cells can operate at a basal level that only requires a part of their total ATP-producing capacity, there are certain circumstances in which muscle cells require a sudden burst of energy. For example, muscle cells may need to respond to stress or increased workload, in which case more ATP will be required to maintain cellular functions. The difference between basal ATP production by the mitochondria and ATP production at maximal activity is referred to as spare respiratory capacity (SRC).

[0005] SRC is one of the most important aspects of mitochondrial bioenergetics. It is well known that the energy requirements of different tissues fluctuate and that ATP synthesis is correspondingly up- or downregulated to accurately meet tissue energy demands. A cell with a larger SRC can produce more ATP to overcome more stress. This becomes particularly important in electrically excitable cells such as muscle cells, which face periods of high ATP demand to re-establish ion gradients necessary to drive muscular contraction. Mitochondrial SRC is regarded as a crucial aspect of mitochondrial function. When energy demand exceeds supply (e.g. an increase in workload), the SRC has the potential to increase supply, thus, avoiding an "ATP crisis". However, when the SRC is not sufficient to provide the required ATP, cells risk being driven into senescence or death. Several large-scale studies with skeletal muscle biopsies taken from humans ranging from 17 years of age to 91 years of age have shown an age-associated decline in mitochondrial SRC and skeletal muscle oxidative capacity.

[0006] Muscle contractile activity is intimately linked to an acceleration of ATP turnover, as well as to a coincident elevation in intracellular calcium concentration. Evidence suggests that muscle functional decline with age is associated with substantial reduction in various biochemical mitochondrial properties including respiratory capacity and ATP production leading to contractile impairment. Therefore, improving mitochondrial function by increasing the efficiency of energy production to match energy demand would optimize muscle contraction and counteract mitochondrial dysfunction associated with aging and hypercatabolic states. A need exists for conveniently providing such an improvement in mitochondrial function, and therefore improving muscle energy production and/or muscle strength, and/or reducing muscle loss, in individuals, for example, aging individuals or those suffering from an acute or chronic disease associated with a hypercatabolic state.

SUMMARY OF THE INVENTION

[0007] Accordingly, it is an object of the invention to provide a convenient means for improving muscle energy production and/or muscle strength, and/or for reducing muscle loss, in an individual in need thereof.

[0008] In one embodiment, the invention is directed to methods for improving muscle energy production and/or muscle strength in a subject, and/or for reducing muscle loss in a subject. The methods comprise administering beta-hydroxy beta-methylbutyrate (HMB) and at least one citrus flavonoid to the subject.

[0009] In another embodiment, the invention is directed to nutritional compositions comprising protein, fat, carbohydrate, HMB, and at least one citrus flavonoid.

[00010] The methods and nutritional compositions of the invention are advantageous in improving muscle energy production, improving muscle strength, and, in specific embodiments, improving muscle contractility, and/or in reducing muscle loss.

[00011] Additional aspects and advantages of the present invention will be more fully described in view of the detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[00012] The drawings illustrate certain aspects of embodiments of the invention, in which:

[00013] Fig. 1 shows the effects of HMB and flavonoid-containing citrus extract, individually, and a combination thereof on the oxygen consumption rate (OCR) in C2C12 myotubes treated with dexamethasone (DEX), as described in the Example;

[00014] Fig. 2 shows the effects of HMB and hesperidin, individually, and a combination thereof on the oxygen consumption rate (OCR) in C2C12 myotubes treated with dexamethasone (DEX), as described in the Example; and

[00015] Fig. 3 shows the effects of HMB and hesperetin, individually, and a combination thereof on the oxygen consumption rate (OCR) in C2C12 myotubes treated with dexamethasone (DEX), as described in the Example.

DETAILED DESCRIPTION

[00016] While the general inventive concepts are susceptible of embodiment in many different forms, described herein in detail are specific embodiments of the invention, with the understanding that the present disclosure is to be considered as an exemplification of the principles of the general inventive concepts. Accordingly, the general inventive concepts are not intended to be limited to the specific embodiments illustrated and described herein.

[00017] All percentages, parts and ratios as used herein, are by weight of the total composition, unless otherwise specified. All such weights as they pertain to listed ingredients are based on the active level and, therefore, do not include solvents or byproducts that may be included in commercially available materials, unless otherwise specified.

[00018] 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. 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.

[00019] Throughout this specification, when a range of values is defined with respect to a particular characteristic of the present invention, the present invention relates to and explicitly incorporates every specific subrange therein. Additionally, throughout this specification, when a group of substances is defined with respect to a particular characteristic of the present invention, the present invention relates to and explicitly incorporates every specific subgroup therein. Any specified range or group is to be understood as a shorthand way of referring to every member of a range or group individually as well as every possible subrange or subgroup encompassed therein.

[00020] The methods and nutritional compositions described herein may comprise, consist of, or consist essentially of the essential steps and elements, respectively, as described herein, as well as any additional or optional steps and elements, respectively, described herein. Any combination of method or process steps as used herein may be performed in any order, unless otherwise specified or clearly implied to the contrary by the context in which the referenced combination is made.

[00021] The various embodiments of the nutritional compositions of the invention may also be substantially free of any optional or selected ingredient or feature described herein, provided that the remaining nutritional composition still contains all of the required ingredients or features as described herein. In this context, and unless otherwise specified, the term “substantially free” means that the selected nutritional composition contains less than a functional amount of the optional ingredient, typically less than 1%, including less than 0.5%, including less than 0.1%, and also including zero percent, by weight, of such optional or selected essential ingredient.

[00022] Unless otherwise indicated herein, all exemplary embodiments, sub-embodiments, specific embodiments and optional embodiments are respective exemplary embodiments, sub-

embodiments, specific embodiments and optional embodiments to all embodiments described herein.

[00023] In a first embodiment, the invention is directed to methods for improving muscle energy production and/or muscle strength in a subject, and/or reducing muscle loss in a subject. Without being limited by theory, it is believed that the present methods improve mitochondrial function by increasing the efficiency of energy production to match energy demand, resulting in improved muscle contraction and counteracting mitochondrial dysfunction associated with, for example, aging and hypercatabolic states. Improved muscle energy production and/or muscle strength and/or reduced muscle loss result. The methods conveniently provide such improvements by administering beta-hydroxy beta-methylbutyrate (HMB) and at least one citrus flavonoid to the subject. Thus, in a second embodiment, the invention is directed to nutritional compositions comprising HMB and at least one citrus flavonoid. The inventive nutritional compositions provide a convenient means for conducting the inventive methods.

[00024] In specific embodiments, the subject is in need of improved muscle energy production or muscle strength. For example, the subject may have or be at risk of reduced muscle contractile function and/or at risk of muscle loss. As discussed above, reduced muscle contractile function can be evidenced by loss of strength, fatigue, disability and falls, impaired pulmonary ventilation, osteoporosis, and fractures due to reduced mechanical bone stimulation. Reduced muscle contractile function is often associated with sarcopenia, hospitalization, and/or an acute or chronic disease with a hypercatabolic state. Thus, in specific embodiments, the subject may be experiencing or at risk of one or more of sarcopenia, hospitalization, and/or an acute or chronic disease with a hypercatabolic state. In additional specific embodiments, the subject may be of at least 40 years of age, of at least 50 years of age, of at least 60 years of age, of at least 70 years of age, or of at least 80 years of age. Such subjects may be suffering from or at risk of experiencing reduced muscle contractile function or at risk of muscle loss. In a specific embodiment, the subject is a human subject.

[00025] Any suitable source of HMB may be employed in the methods and nutritional compositions of the invention, including the free acid, a salt, including hydrated or anhydrous salts, an ester, a lactone, or other product form that otherwise provides a bioavailable form of HMB when administered. Non-limiting examples of suitable salts of HMB for use herein include HMB salts, hydrated or anhydrous, of sodium, potassium, magnesium, chromium, calcium, or other non-toxic salt form. In a more specific embodiment, the HMB is in the form of calcium HMB, or, more specifically, calcium HMB monohydrate.

[00026] The methods and nutritional compositions as described herein employ an amount of HMB that is effective, in combination with the citrus flavonoid, to improve muscle energy production and/or muscle strength, and/or to reduce muscle loss, and, more specifically, to achieve one or more of these effects to an extent greater than that achieved with HMB alone. In specific embodiments of the methods of the invention, HMB is administered to the subject in an amount of about 0.1 to about 10 g/day, about 0.1 to about 5 g/day, about 0.5 to about 5 g/day, about 0.5 to about 3 g/day, or about 0.5 to about 1.5 g/day.

[00027] Various citrus flavonoids are suitable for use in the present methods and nutritional compositions. Examples include, but are not limited to, hesperidin, hesperetin, which is the aglycone of hesperidin, narirutin, diosmin, isonaringin, naringin, and didymin, which may be used alone or in combinations of any two or more. In a specific embodiment, the citrus flavonoid comprises hesperidin, hesperetin, or a combination thereof. Hesperidin is the major flavonoid present in sweet oranges, but is also found in other citrus fruits including lemon, lime, grapefruit, mandarin, and other classes of orange. Upon ingestion, hesperidin is hydrolyzed into hesperetin (the aglycone) by colonic microbiota prior to its absorption. The methods and nutritional compositions as described herein employ an amount of citrus flavonoid that is effective, in combination with the HMB, to improve muscle energy production and/or muscle strength, and/or to reduce muscle loss, and, more specifically, to achieve one or more of these effects to an extent greater than that achieved with the citrus flavonoid alone. In specific embodiments of the

methods of the invention, citrus flavonoid is administered in an amount of about 50 to about 1000 mg/day, about 50 to about 800 mg/day, about 50 to about 500 mg/day, about 50 to about 300 mg/day, about 100 to about 300 mg/day, or about 100 to about 500 mg/day.

[00028] The HMB and at least one citrus flavonoid may be administered simultaneously or sequentially. In a specific embodiment as discussed in detail below, the HMB and at least one citrus flavonoid are administered to the subject simultaneously in a nutritional composition. In a specific embodiment, the HMB and at least one citrus flavonoid are administered to the subject simultaneously in a nutritional composition comprising protein, fat and carbohydrate. In another specific embodiment of the inventive methods, HMB and at least one citrus flavonoid are administered to the subject daily for a period of time. In a specific embodiment, the HMB and at least one citrus flavonoid are administered to the subject one, two or three or more times per day, for a period of 5 days, 7 days, 10 days, two weeks, one month, or more. In a more specific embodiment, HMB and at least one citrus flavonoid are administered to the subject at least once per day for at least 7 days, for at least 14 day, or for at least 30 days.

[00029] In another embodiment, the invention is directed to nutritional compositions comprising protein, fat, carbohydrate, HMB, and at least one citrus flavonoid. The nutritional compositions may be liquid nutritional compositions or powdered nutritional compositions. The term "liquid nutritional composition" as used herein, unless otherwise specified, encompasses all forms of liquid nutritional compositions, including emulsified liquids, concentrated liquids intended for dilution, for example, by addition of water, ready-to-drink liquids, and liquids that are reconstituted from powdered form by addition of liquid, for example, by addition of water or juice. The nutritional compositions are suitable for consumption by a human and, in a specific embodiment are in a form suitable for oral consumption.

[00030] The nutritional compositions comprise an amount of HMB that is effective, in combination with the citrus flavonoid, to improve muscle energy production and/or muscle strength, and/or to reduce muscle loss. In specific embodiments the nutritional compositions

comprise HMB in an amount effective, in combination with the citrus flavonoid, to improve muscle energy production and/or muscle strength, and/or to reduce muscle loss, and, more specifically, to achieve one or more of these effects to an extent greater than that achieved with HMB alone. In additional specific embodiments, the nutritional compositions comprise HMB in an amount of about 0.1 to about 10 g per 237 ml serving, about 0.1 to about 5 g per 237 ml serving, about 0.5 to about 5 g per 237 ml serving, about 0.5 to about 3 g per 237 ml serving, or about 0.5 to about 1.5 g per 237 ml serving. The nutritional compositions comprise an amount of citrus flavonoid that is effective, in combination with the HMB, to improve muscle energy production and/or muscle strength, and/or to reduce muscle loss. In specific embodiments the nutritional compositions comprise citrus flavonoid in an amount effective, in combination with the HMB, to achieve one or more of these effects to an extent greater than that achieved with citrus flavonoid alone. In additional specific embodiments, the nutritional compositions comprise citrus flavonoid in an amount of about 50 to about 1000 mg per 237 ml serving, about 50 to about 800 mg per 237 ml serving, about 50 to about 500 mg per 237 ml serving, about 50 to about 300 mg per 237 ml serving, about 100 to about 300 mg per 237 ml serving, or about 100 to about 500 mg per 237 ml serving.

[00031] The protein which is contained in the nutritional composition may be any one or more proteins known for use in nutritional compositions. A wide variety of sources and types of protein can be used in the nutritional compositions. For example, the source of protein may include, but is not limited to, intact, hydrolyzed, and partially hydrolyzed protein, which may be derived from any suitable source such as milk (e.g., casein, whey), animal (e.g., meat, fish), cereal (e.g., rice, brown rice, oat, barley, etc.), vegetable (e.g., soy, corn, pea, yellow pea, fava bean, chickpea, canola, potato, mung, ancient grains such as quinoa, amaranth, and chia, hemp, flax seed, etc.), nuts (e.g., almond and cashew), and combinations of two or more thereof. The protein may also include one or a mixture of naturally occurring or synthetic amino acids (often described as free amino acids) and/or their metabolites, known for use in nutritional

products, alone or in combination with the intact, hydrolyzed, and/or partially hydrolyzed proteins described herein.

[00032] More specific examples of sources of protein which are suitable for use in the exemplary nutritional compositions described herein include, but are not limited to, whole egg powder, egg yolk powder, egg white powder, whey protein (including, but not limited to, whey protein components such as α -lactalbumin and β -lactoglobulin), whey protein concentrates, whey protein isolates, whey protein hydrolysates, acid caseins, casein protein isolates, sodium caseinates, calcium caseinates, potassium caseinates, casein hydrolysates, milk protein concentrates, milk protein isolates, milk protein hydrolysates, nonfat dry milk, condensed skim milk, whole cow's milk, partially or completely defatted milk, coconut milk, soy protein concentrates, soy protein isolates, soy protein hydrolysates, pea protein concentrates, pea protein isolates, pea protein hydrolysates, rice protein concentrate, rice protein isolate, rice protein hydrolysate, barley rice protein, fava bean protein concentrate, fava bean protein isolate, fava bean protein hydrolysate, collagen proteins, collagen protein isolates, meat proteins such as beef protein isolate and/or chicken protein isolate, potato proteins, chickpea proteins, canola proteins, mung proteins, quinoa proteins, amaranth proteins, chia proteins, hemp proteins, flax seed proteins, almond proteins, cashew proteins, earthworm proteins, insect proteins, and combinations of two or more thereof. The nutritional compositions can include any individual source of protein or a combination of any two or more sources of protein. In specific embodiments, the nutritional compositions comprise at least one milk protein, or at least one plant protein, or at least one milk protein and at least one plant protein.

[00033] The amount of the source of protein in the nutritional compositions can vary considerably depending upon, for example, the specific dietary needs of the intended user and/or the form of the composition, i.e., liquid or powder. For example, in specific embodiments, the source of protein comprises from about 1 wt% to about 30 wt% of the nutritional composition. In more specific embodiments, the source of protein comprises from about 2 wt%

to about 25 wt% of the nutritional composition, including about 2 wt% to about 20 wt%, about 2 wt% to about 15 wt%, about 5 wt% to about 20 wt%, about 5 wt% to about 25 wt%, about 10 wt% to about 25 wt%, or about 5 wt% to about 15 wt% of the nutritional composition.

[00034] The inventive nutritional compositions also include fat. The term “fat” as used herein, unless otherwise specified, refers to lipids, fats, oils, and combinations thereof. Sources of fat suitable for use in the nutritional composition include, but are not limited to, algal oil, canola oil, flaxseed oil, borage oil, safflower oil, high oleic safflower oil, high gamma-linolenic acid (GLA) safflower oil, corn oil, soy oil, sunflower oil, high oleic sunflower oil, cottonseed oil, coconut oil, fractionated coconut oil, medium chain triglycerides (MCT) oil, palm oil, palm kernel oil, palm olein, lecithin, and long chain polyunsaturated fatty acids such as docosahexanoic acid (DHA), arachidonic acid (ARA), docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA), and combinations thereof. The nutritional compositions can include any individual source of fat or a combination of two or more sources of fat.

[00035] In specific embodiments, the nutritional compositions comprise about 0.5 wt% to about 20 wt% of a source of fat. In more specific embodiments, the source of fat comprises about 0.5 wt% to about 18 wt% of the nutritional composition, including about 0.5 wt% to about 15 wt%, about 0.5 wt% to about 10 wt%, about 0.5 wt% to about 5 wt%, about 2 wt% to about 8 wt%, about 2 wt% to about 10 wt%, about 5 wt% to about 15 wt%, or about 5 wt% to about 20 wt% of the nutritional composition.

[00036] Sources of carbohydrates suitable for use in the nutritional compositions may be simple or complex, or variations, or combinations thereof. Various sources of carbohydrate may be used so long as the source is suitable for use in a nutritional composition and is otherwise compatible with any other selected ingredients or features present in the nutritional composition. Non-limiting examples of sources of carbohydrates suitable for use in the nutritional compositions include maltodextrin, hydrolyzed or modified starch, hydrolyzed or modified cornstarch, glucose polymers such as polydextrose and dextrans, corn syrup, corn syrup solids,

rice-derived carbohydrates such as rice maltodextrin, brown rice mild powder and brown rice syrup, sucrose, glucose, fructose, lactose, high fructose corn syrup, honey, sugar alcohols (e.g., maltitol, erythritol, sorbitol), isomaltulose, sucromalt, pullulan, potato starch, corn starch, fructooligosaccharides, galactooligosaccharides, oat fiber, soy fiber, gum arabic, sodium carboxymethylcellulose, methylcellulose, guar gum, gellan gum, locust bean gum, konjac flour, hydroxypropyl methylcellulose, tragacanth gum, karaya gum, gum acacia, chitosan, arabinogalactans, glucomannan, xanthan gum, alginate, pectin, low methoxy pectin, high methoxy pectin, cereal beta-glucans, carrageenan, psyllium, Fibersol™, fruit puree, vegetable puree, isomalto-oligosaccharides, monosaccharides, disaccharides, human milk oligosaccharides (HMOs), tapioca-derived carbohydrates, inulin, other digestion-resistant starches, and artificial sweeteners, and combinations of two or more thereof. The nutritional compositions may include any individual source of carbohydrate or a combination of two or more sources of carbohydrate.

[00037] In specific embodiments, a source of carbohydrate is present in an amount from about 5 wt% to about 75 wt% of the nutritional compositions. In more specific embodiments, the source of carbohydrate is present in an amount from about 5 wt% to about 70 wt% of the nutritional composition, including 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 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. In a specific embodiment, wherein the nutritional composition is a liquid, the source of carbohydrate comprises about 5 wt% to about 30 wt% of the nutritional composition. In more specific liquid embodiments, the carbohydrate comprises about 5 wt% to about 25 wt%, about 5 wt% to about 20 wt%, about 5 wt% to about 15 wt%, about 10 wt% to about 25 wt%, about 10 wt% to about 20 wt%, about 15 wt% to about 25 wt%, or about 15 wt% to about 30 wt% of the nutritional composition. In another specific embodiment, wherein the nutritional composition is a powder, the source of carbohydrate comprises about 25

wt% to about 75 wt% of the nutritional composition. In more specific powder embodiments, the carbohydrate comprises about 30 wt% to about 70 wt%, about 35 wt% to about 65 wt%, about 40 wt% to about 65 wt%, about 40 wt% to about 70 wt%, about 50 wt% to about 70 wt%, or about 50 wt% to about 75 wt% of the nutritional composition.

[00038] The relative amounts of the sources of protein, fat and carbohydrate in the nutritional compositions can vary considerably depending upon, for example, the specific dietary needs of the intended user and/or the form of the composition, i.e., liquid or powder. In a specific embodiment, the nutritional compositions comprise a source of protein in an amount of about 2 wt % to about 25 wt %, a source of carbohydrate in an amount of about 5 wt % to about 75 wt %, and a source of fat in an amount of about 0.5 wt % to about 20 wt %, based on the weight of the nutritional composition. In a specific embodiment, the nutritional composition comprises a source of protein in an amount of about 2 wt% to about 25 wt%, a source of carbohydrate in an amount of about 5 wt% to about 30 wt%, and a source of fat in an amount of about 0.5 wt% to about 10 wt%, based on the weight of the nutritional composition, and, more specifically, such composition is in liquid form. In another specific embodiment, the nutritional composition comprises a source of protein in an amount of about 10 wt% to about 25 wt%, a source of carbohydrate in an amount of about 40 wt% to about 70 wt%, and a source of fat in an amount of about 5 wt% to about 20 wt%, based on the weight of the nutritional composition, and, more specifically, such composition is in powder form.

[00039] 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.

[00040] The nutritional composition may further comprise one or more additional components that may 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, thickening agents, stabilizers, and so forth.

[00041] Additionally, the nutritional composition may further include vitamins or related nutrients, non-limiting examples of which include vitamin A, vitamin B12, vitamin C, vitamin D, vitamin K, thiamine, riboflavin, pyridoxine, niacin, folic acid, pantothenic acid, biotin, choline, inositol, salts and derivatives thereof, and combinations thereof. Water soluble vitamins may be added in the form of a water-soluble vitamin (WSV) premix and/or oil-soluble vitamins may be added in one or more oil carriers as desired.

[00042] In additional embodiments, the nutritional composition may further include one or more minerals, non-limiting examples of which include calcium, phosphorus, magnesium, zinc, manganese, sodium, potassium, molybdenum, chromium, chloride, and combinations thereof.

[00043] In additional embodiments, the nutritional composition may further include one or more probiotics. The term "probiotic" as used herein refers to a microorganism such as a bacteria or yeast that survives the digestive process to confer a health benefit to the subject. Examples of probiotics that can be included in the nutritional compositions, either alone or in combination, include, but are not limited to, *Bifidobacterium* (B.), such as *B. breve*, *B. infantis*, *B. lactis*, *B. bifidum*, *B. longum*, and *B. animalis*, and *Lactobacillus* (L.), such as *L. rhamnosus*, *L. acidophilus*, *L. fermentum*, *L. reuteri*, *Streptococcus thermophilus*, *Akkermansia*, *Bacteroides*, *Enterococcus*, *Eubacterium*, *Fecalibacterium*, *Roseburia*, and/or *Saccharomyces*.

[00044] The nutritional compositions may be formed using any techniques known in the art. In one embodiment, the nutritional compositions may be formed by (a) preparing an aqueous solution comprising protein and carbohydrate; (b) preparing an oil blend comprising fat and oil-soluble components; and (c) mixing together the aqueous solution and the oil blend to form an emulsified liquid nutritional composition. The HMB and citrus flavonoid 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. Alternatively, a powder product can be formed by dry blending ingredients. 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.

[00045] The improvements provided by the inventive methods are demonstrated in the following Example.

EXAMPLE

[00046] The present example evaluated the effect of HMB, a flavonoid-containing citrus extract, hesperidin and hesperetin each individually, and the effect of combinations of HMB with each of the flavonoid-containing citrus extract, hesperidin, and hesperetin, respectively, on muscle mitochondria dysfunction caused by exposure to the glucocorticoid dexamethasone (DEX) using the well-established mouse muscle cell line, C2C12 myotubes. Dexamethasone is known to trigger protein degradation in muscle and is used to induce mitochondrial dysfunction as encountered in aging and/or hypercatabolic conditions.

[00047] C2C12 myoblasts were grown in high-glucose Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum, 4 mM glutamine, 100 units/ml penicillin, and 0.1 mg/ml streptomycin at 37°C in humidified 95% air with 5% CO₂. After reaching 90% confluence, the cells were induced to differentiate in differentiation media of high-glucose DMEM supplemented with 2% horse serum. Cells were differentiated in the presence of, individually, (1) HMB (50 µM), (2) flavonoid-containing citrus extract (20 µM), (3) hesperidin (5 µM), (4) hesperetin (0.1 µM), and combinations of (5) HMB (50 µM) and flavonoid-containing citrus extract (20 µM), (6) HMB (50 µM) and hesperidin (5 µM), and (7), HMB (50 µM) and hesperetin (0.1 µM), for 24 hours. Subsequently, the cells, as well as a samples of cells incubated without any HMB or citrus flavonoid (negative controls), were incubated with dexamethasone (DEX, 5 µM) in the last 24 hours of differentiation.

[00048] To assess alterations in oxidative metabolism, the oxygen consumption rate (OCR), which is an indirect index of mitochondrial metabolism, was measured using an Agilent Seahorse XFe24 Extracellular Flux Analyzer (Agilent, Santa Clara, CA, USA). The test employs sequential addition of oligomycin (1 μ M), proton ionophore carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP, 0.5 μ M) and rotenone and antimycin A (0.5 μ M), and measures the key parameters of mitochondrial respiration including spare respiratory capacity (SRC). The terms “reserve respiratory capacity” and “spare respiratory capacity” are used to describe the amount of extra ATP that can be produced by oxidative phosphorylation in case of a sudden increase in energy demand on the cell. This is especially needed during hypercatabolic and inflammatory states that lead to muscle atrophy. These respiratory parameters of mitochondrial function were calculated by the equipment’s software.

[00049] All results were normalized to a positive Control group of cells which were incubated without any HMB, citrus flavonoid, or DEX, and expressed as mean $\pm$ SEM. The normalized results are presented in Figs. 1-3. Statistical analyses were carried out using Student’s t test. Probability values less than 0.05 were considered as statistically significant and are indicated with different letters (a, b, c) in Figs. 1-3.

[00050] As shown in Figs. 1-3, the negative control DEX-treated (5 μ M) C2C12 myotubes showed a general impairment in SRC, specifically a 48% reduction in SRC, as compared with the positive Control group which did not have any HMB, citrus flavonoid, or DEX treatment. SRC values near to that of the Control group were obtained by the individual treatments of the DEX-treated cells with HMB (50 μ M), flavonoid-containing citrus extract (20 μ M), hesperidin (5 μ M) and hesperetin (0.1 μ M), showing that the individual treatments substantially protected cells from the reduction caused by DEX, with HMB providing equal or better protection individually than any of flavonoid-containing citrus extract (20 μ M), hesperidin (5 μ M), and hesperetin, individually. Surprisingly, however, cells treated with the combinations of HMB and respectively,

one of the three citrus flavonoid sources provided a significantly improved and unexpected protective SRC, reversing the negative effect caused by DEX and improving the SRC.

[00051] Specifically, C2C12 grown in presence of the combination of HMB (50 μ M) and flavonoid-containing citrus extract (20 μ M) (Fig. 1) showed a significant increase of SRC of about 55 % with respect to the DEX-treated negative control. Additionally, the increase in SRC was higher than the effect observed with each one of the individual ingredients (+21%). Similar results were observed when cells were grown in presence of the combination of HMB (50 μ M) and hesperidin (5 μ M) (Fig. 2), showing an increase in SRC of about +50% with respect to DEX-treated cells and about +23% in comparison with the effects observed with each of the individual ingredients.

[00052] The most improved effects were observed with cells incubated in the presence of the combination of HMB (50 μ M) and hesperetin (0.1 μ M), which, as noted, is the aglycone form of hesperidin. SRC was increased about 100 % with respect to the DEX-treated negative control cells, about 50% with respect to the effects of individual ingredients, and about 40% with respect to the positive Control group.

[00053] This data shows that, in mitochondrial dysfunction induced by DEX treatment, the synergistic combination of HMB and citrus flavonoid provides the high values for mitochondrial respiratory capacity, allowing cells to supply an incremental ATP request and adapt to an increase of energy demand due to an increase in cellular activity. These results show that the combination of HMB and citrus flavonoid can prevent or restore the loss of mitochondrial function needed for improved muscle contraction during a condition of stress associated with muscle loss, for example in connection with sarcopenia, hospitalization, or an acute or chronic disease with a hypercatabolic state.

[00054] While the present application 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 methods or compositions, 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.

What is claimed is:

1. A method for improving muscle energy production and/or muscle strength in a subject, and/or for reducing muscle loss in a subject, comprising administering beta-hydroxy beta-methylbutyrate (HMB) and at least one citrus flavonoid to the subject.
2. The method of claim 1, wherein the subject has or is at risk of reduced muscle strength.
3. The method of claim 2, wherein the reduced muscle strength or risk thereof is associated with sarcopenia, hospitalization, and/or an acute or chronic disease with a hypercatabolic state.
4. The method of any one of claims 1-3, wherein the subject is of at least 40 years of age, of at least 50 years of age, or of at least 60 years of age.
5. The method of any one of claims 1-4, wherein the HMB comprises a salt of HMB or HMB free acid, or comprises calcium HMB monohydrate.
6. The method of any one of claims 1-5, wherein the at least one citrus flavonoid comprises hesperidin, hesperetin, diosmin, narirutin, isonaringin, naringin, didymin, or a combination of two or more thereof.
7. The method of any one of claims 1-6, wherein the HMB is administered to the subject in an amount of about 0.1 to about 10 g/day, about 0.1 to about 5 g/day, about 0.5 to about 5 g/day, about 0.5 to about 3 g/day, or about 0.5 to about 1.5 g/day.
8. The method of any one of claims 1-7, wherein the at least one citrus flavonoid is administered to the subject in an amount of about 50 to about 1000 mg/day, about 50 to about 800 mg/day, about 50 to about 500 mg/day, about 50 to about 300 mg/day, about 100 to about 300 mg/day, or about 100 to about 500 mg/day.
9. The method of any one of claims 1-8, wherein the HMB and at least one citrus flavonoid are administered to the subject at least once per day for at least 7 days.

10. The method of any one of claims 1-9, wherein the HMB and the at least one citrus flavonoid are administered to the subject in a nutritional composition comprising protein, fat and carbohydrate.
11. A nutritional composition comprising protein, fat, carbohydrate, beta-hydroxy beta-methylbutyrate (HMB), and at least one citrus flavonoid.
12. The nutritional composition of claim 11, wherein the HMB comprises a salt of HMB or HMB free acid, or comprises calcium HMB monohydrate.
13. The nutritional composition of claim 11 or 12, wherein the at least one citrus flavonoid comprises hesperidin, hesperetin, diosmin, narirutin, isonaringin, naringin, didymin, or a combination of two or more thereof.
14. The nutritional composition of any one of claims 11-13, comprising the HMB in an amount of about 0.1 to about 10 g, about 0.1 to about 5 g, about 0.5 to about 5 g, about 0.5 to about 3 g, or about 0.5 to about 1.5 g, per 237 ml serving.
15. The nutritional composition of any one of claims 11-14, comprising the at least one citrus flavonoid in an amount of about 50 to about 1000 mg, about 50 to about 800 mg, about 50 to about 500 mg, about 50 to about 300 mg, about 100 to about 300 mg, or about 100 to about 500 mg, per 237 ml serving.
16. The nutritional composition of any one of claims 11-15, wherein the protein is from a source comprising whole egg powder, egg yolk powder, egg white powder, whey protein, whey protein concentrate, whey protein isolate, whey protein hydrolysate, acid casein, casein protein isolate, sodium caseinate, calcium caseinate, potassium caseinate, casein hydrolysate, milk protein concentrate, milk protein isolate, milk protein hydrolysate, nonfat dry milk, condensed skim milk, whole cow's milk, partially or completely defatted milk, coconut milk, soy protein concentrate, soy protein isolate, soy protein hydrolysate, pea protein concentrate, pea protein

isolate, pea protein hydrolysate, rice protein concentrate, rice protein isolate, rice protein hydrolysate, barley rice protein, fava bean protein concentrate, fava bean protein isolate, fava bean protein hydrolysate, collagen protein, collagen protein isolate, meat protein, potato protein, chickpea protein, canola protein, mung protein, quinoa protein, amaranth protein, chia protein, hemp protein, flax seed protein, almond protein, cashew protein, earthworm protein, insect protein, free amino acid and/or a metabolite thereof, or a combination of two or more thereof.

17. The nutritional composition of claim 16, comprising at least one milk protein and at least one plant protein.

18. The nutritional composition of any one of claims 11-17, wherein the fat is from a source comprising algal oil, canola oil, flaxseed oil, borage oil, safflower oil, high oleic safflower oil, high gamma-linolenic acid (GLA) safflower oil, corn oil, soy oil, sunflower oil, high oleic sunflower oil, cottonseed oil, coconut oil, fractionated coconut oil, medium chain triglycerides (MCT) oil, palm oil, palm kernel oil, palm olein, lecithin, docosahexanoic acid, arachidonic acid, docosapentaenoic acid, eicosapentaenoic acid, or a combination of two or more thereof.

19. The nutritional composition of any one of claims 11-18, wherein the carbohydrate is from a source comprising maltodextrin, hydrolyzed or modified starch, hydrolyzed or modified cornstarch, polydextrose, dextrin, corn syrup, corn syrup solids, rice-derived carbohydrate, sucrose, glucose, fructose, lactose, high fructose corn syrup, honey, sugar alcohol, isomaltulose, sucromalt, pullulan, potato starch, corn starch, fructooligosaccharide, galactooligosaccharide, oat fiber, soy fiber, gum arabic, sodium carboxymethylcellulose, methylcellulose, guar gum, gellan gum, locust bean gum, konjac flour, hydroxypropyl methylcellulose, tragacanth gum, karaya gum, gum acacia, chitosan, arabinogalactans, glucomannan, xanthan gum, alginate, pectin, low methoxy pectin, high methoxy pectin, cereal beta-glucans, carrageenan, psyllium, fruit puree, vegetable puree, isomalto-oligosaccharide,

monosaccharide, disaccharide, human milk oligosaccharide (HMO), tapioca-derived carbohydrate, inulin, artificial sweetener, or a combination of two or more thereof.

20. The nutritional composition of any one of claims 11-19, comprising a source of protein in an amount of about 2 wt% to about 25 wt%, a source of carbohydrate in an amount of about 5 wt% to about 30 wt%, and a source of fat in an amount of about 0.5 wt% to about 10 wt%, based on the weight of the nutritional composition, and the nutritional composition is in liquid form; or comprising a source of protein in an amount of about 10 wt% to about 25 wt%, a source of carbohydrate in an amount of about 40 wt% to about 70 wt%, and a source of fat in an amount of about 5 wt% to about 20 wt%, based on the weight of the nutritional composition, and the nutritional composition is in powder form.