

**METHODS FOR ENHANCING MUSCLE PERFORMANCE OR REDUCING CHRONIC
FATIGUE BY ADMINISTERING BOVINE MILK-DERIVED EXOSOMES**

FIELD OF THE INVENTION

[0001] The present invention relates to a method of enhancing muscle performance in a subject in need of improved physical performance by administering an exosome-enriched product comprising intact bovine milk-derived exosomes to the subject in need thereof. The present invention also relates to a method of reducing chronic fatigue in a subject who is recovering or has recovered from a viral infection by administering an exosome-enriched product comprising intact bovine milk-derived exosomes to the subject.

BACKGROUND OF THE INVENTION

[0002] Skeletal muscle is the most abundant tissue in the body. The mass and functionality of skeletal muscle are key determinants of strength, endurance and physical performance throughout a lifespan. Unfortunately, muscle is particularly susceptible to advancing age. From the age of 35-40 years in adult humans, muscle mass typically starts to decline progressively by about 0.4-1.0% per year, with a dramatic acceleration in muscle mass decline after age 65. The age-related loss of muscle mass and strength in otherwise healthy, aging individuals is referred to as sarcopenia. 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] 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 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).

[0004] 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.

[0005] Mitochondrial SRC is regarded as a crucial aspect of mitochondrial function. When the SRC is not enough 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] Like skeletal muscle, cardiac muscle is also rich in mitochondria. The SRC in cardiac muscle cells has been reported to be depleted under conditions of severe cardiac stress, such as pressure overload, ischemia and cardiac failure. This lowered capacity makes the heart more vulnerable to bioenergetic exhaustion and thereby increases the risk of inducing cardiac muscle death and organ failure. It is thus desirable to find new treatments that may help restore compromised mitochondrial activity in subjects suffering from, for example, sarcopenia and/or chronic or acute cardiac damage.

[0007] In addition to the above, muscle has also been shown to harbor and supply anti-viral stem T-cells. Many viruses, such as coronavirus, modulate bioenergetics and redox regulation of the immune system and other cells which they infect in order to enhance their own replication. The viruses are able to induce excessive stress in these systems when the mitochondria are already sub-optimally functional. Following the recovery from a viral infection, many individuals suffer from long term effects, including fatigue. While the relationship between mitochondrial function and immunity is a focus of ongoing research, it would be desirable to find new treatments that help restore compromised mitochondrial activity in subjects who are recovering or have recovered from a viral infection.

[0008] Targeting mitochondria with specific dietary factors would be a convenient way to enhance muscle performance in subjects suffering from, or at risk of suffering from, various conditions, particularly sarcopenia and cardiac muscle injury. It would also be a convenient way to reduce chronic fatigue during or following the recovery from a viral infection. Therefore, it is desirable to develop nutritional intervention strategies to enhance muscle performance in subjects in need of improved physical performance, and more particularly in subjects suffering from or at

risk of suffering from sarcopenia and/or chronic or acute cardiac damage, and/or to reduce chronic fatigue in subjects who are recovering or have recovered from a viral infection, such as COVID-19.

SUMMARY OF THE INVENTION

[0009] It is therefore an object of the invention to provide a method which enhances muscle performance in a subject in need of improved physical performance.

[0010] It is another object of the invention to provide a method which reduces chronic fatigue in a subject who is recovering or has recovered from a viral infection.

[0011] The present invention is directed to a method of enhancing muscle performance in a subject in need of improved physical performance, comprising administering an exosome-enriched product comprising intact bovine milk-derived exosomes to the subject in need thereof.

[0012] The present invention is also directed to a method of reducing chronic fatigue in a subject who is recovering or has recovered from a viral infection, comprising administering an exosome-enriched product comprising intact bovine milk-derived exosomes to the subject.

[0013] The methods of the invention are advantageous in providing a convenient manner to improve mitochondrial function, and thereby improve muscle performance, in a subject in need of improved physical performance. The methods are useful in the prevention or treatment of conditions that are hallmarked by a reduction in spare respiratory capacity, including sarcopenia and chronic or acute cardiac damage.

[0014] The improved mitochondrial function afforded by the methods of the invention is also advantageous in that it reduces chronic fatigue during or following recovery from a viral illness associated with mitochondrial dysfunction, for example COVID-19. These and additional advantages of the inventive methods will be more fully apparent in view of the detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The drawings are illustrative of certain embodiments of the invention and exemplary in nature and are not intended to limit the invention defined by the claims, wherein:

[0016] FIG. 1 illustrates the effect of bovine-milk derived exosomes on maximal respiratory capacity in C2C12 myoblasts incubated with an exosome-enriched product containing intact bovine milk-derived exosomes, as described in Example 2.

[0017] FIG. 2 illustrates the effect of bovine-milk derived exosomes on spare respiratory capacity in C2C12 myoblasts incubated with an exosome-enriched product containing intact bovine milk-derived exosomes, as described in Example 2.

[0018] FIG 3. illustrates a flow diagram of a membrane filtration process coupled to spray-drying or freeze-drying to produce a lactose-free exosome-enriched product from cheese whey, as described in Example 1.

DETAILED DESCRIPTION

[0019] 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.

[0020] 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.

[0021] 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.

[0022] 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.

[0023] 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.

[0024] All percentages are percentages by weight unless otherwise indicated.

[0025] The term “chronic fatigue” as used herein refers to extreme fatigue that lasts for an extended period of time, for example, one week, one month or more.

[0026] The term “elderly subject” as used herein refers to a human adult about 50 years of age or older.

[0027] The term “enhancing muscle performance” as used herein refers to enhancing muscle strength, endurance, flexibility, power, and/or mass. Muscle strength is defined as the amount of force that a muscle can produce in a single effort. Muscle power is defined as the ability of the muscle to exert a maximal force in as short a time as possible. Muscular endurance is defined as the ability of a muscle to exert force against resistance over time. Flexibility is defined as the range of movement in a joint or series of joints, and length in muscles that cross the joints to induce a bending movement or motion. Muscle mass is defined as the weight of the muscles in the body and includes smooth muscles, skeletal muscles and water contained in the muscles.

[0028] The term “exosome-enriched product comprising bovine milk-derived exosomes” as used herein, unless otherwise specified, refers to a product in which exosomes have been substantially separated from other bovine milk components such as lipids, cells, and debris, and are concentrated in an amount higher than that found in bovine milk. The exosomes are small, extracellular vesicles and account for a minor percentage of milk’s total solids content. In specific embodiments, the exosome-enriched product is provided in a liquid form or a powdered form and also contains co-isolated milk solids.

[0029] The term “intact exosomes” as used herein refers to exosomes in which the vesicle membrane is not ruptured and/or otherwise degraded and the endogenous cargo, i.e., the bioactive agents, therapeutics (e.g. miRNA), and/or other biomolecules which are inherently present in a bovine milk-derived exosome, are retained therein in active form.

[0030] The term “powdered exosomes” as used herein, unless otherwise specified, refers to a dry powder that contains exosomes which have been isolated from bovine milk. The isolated exosomes are dried to form a dry powder. As the isolated fluid containing the exosomes also contains co-isolated milk solids as described above, the powdered exosomes also contain such other milk solids in the resulting powder.

[0031] As indicated above, the present invention is directed to a method of enhancing muscle performance in a subject in need of improved physical performance. The method comprises administering an exosome-enriched product comprising intact bovine milk-derived exosomes to the subject in need thereof.

[0032] The present invention is also directed to a method of reducing chronic fatigue in a subject recovering or recovered from a viral infection. The method comprises administering an exosome-enriched product comprising intact bovine milk-derived exosomes to the subject. In a specific embodiment, the subject is recovering or has recovered from a viral infection resulting in chronic fatigue. In a further specific embodiment, the viral infection resulting in chronic fatigue is selected from the group consisting of Epstein-Barr virus, human herpes virus 6, and coronavirus. In another specific embodiment, the coronavirus is COVID-19.

[0033] Without wishing to be bound by any particular theory, the methods of the present invention enhance muscle performance and/or reduce chronic fatigue by improving mitochondrial function via administration of intact bovine milk-derived exosomes to the subject in need thereof. The present inventors have surprisingly discovered that intact bovine milk-derived exosomes significantly enhance both maximal respiratory capacity and spare respiratory capacity and can thus be administered to a subject to improve mitochondrial function. The improved mitochondrial function results in improved muscle performance, and/or reduced chronic fatigue. Treating mitochondrial dysfunction may thus be an effective way to alleviate fatigue following a viral infection.

[0034] As indicated above, the methods of the invention are therefore useful in the prevention or treatment of conditions that are hallmarked by a reduction in spare respiratory capacity,

including sarcopenia and chronic or acute cardiac damage, and/or by other mitochondrial dysfunction, for example, viral infections such as COVID-19. The enriched product of intact bovine milk-derived exosomes is typically obtained from a whey fraction of bovine milk. By way of example, the whey-containing bovine milk fraction may comprise cheese whey. Generally, the exosomes are obtained from a whey-containing bovine milk fraction using gentle procedures which do not disrupt the exosome vesicle membrane, thereby leaving the exosomes intact and active bioactive agents contained within the exosome structure.

[0035] Various methods may be employed to isolate exosomes with care being exercised to avoid disruption of the lipid membrane. Fresh bovine milk, refrigerated bovine milk, thawed frozen bovine milk, or otherwise preserved bovine milk, or any bovine milk fraction containing exosomes, for example, cheese whey, may be employed as a source of exosomes. Isolating the exosomes may comprise performing the isolation immediately upon obtaining milk from a bovine. By way of example, isolating the exosomes may comprise performing the isolation within about 1 day, or about 2 days, or about 3 days, or about 4 days, or about 5 days, or about 6 days, or about 7 days from the time of obtaining the milk from a bovine. The exosomes may be isolated within about 10 days, or within about 14 days from the time of obtaining milk from a bovine. Additionally, the bovine milk may be frozen and then thawed for processing for isolating exosomes, with the bovine milk preferably having been frozen within about 1 day, or about 2 days, or about 3 days, or about 4 days, or about 5 days, or about 6 days, or about 7 days from the time of obtaining the milk from a bovine. Thawed milk is preferably processed immediately upon thawing. The fresh bovine milk may be subjected to the processing within about 5 days of obtaining the milk from a bovine, or thawed bovine milk which is subjected to processing is thawed from bovine milk that was frozen within about 5 days of obtaining the milk from a bovine.

[0036] As mentioned above, a whey-containing bovine milk fraction or, specifically, cheese whey, may serve as a source of exosomes. Cheese whey is the liquid by-product of milk after the formation of curd during the cheese-making or casein manufacturing process. Since cheese whey has already been separated from the casein fraction during the cheese manufacture process, cheese whey has very low casein content. Furthermore, cheese whey advantageously retains more than 50% of milk nutrients, including lactose, fat, proteins, mineral salts, and, surprisingly, a significant number of exosomes that were originally present in the milk in intact form. In addition to these benefits, cheese whey is less expensive than raw milk, and thus using cheese whey as a starting material significantly reduces costs for production of an exosome-enriched product. As

such, cheese whey is a novel and promising source for isolating milk exosomes and producing exosome-enriched products.

[0037] In a specific embodiment, the cheese whey is obtained by applying an enzyme or enzyme mixture, and more specifically a protease enzyme, for example chymosin, to milk to hydrolyze casein peptide bonds, thus allowing for enzymatic coagulation of casein in the milk. Thus, when the protease enzyme cleaves the protein, it causes the casein in the milk to coagulate and form a gel structure. The casein protein gel network and milk fat then contract together and form curd. The resulting liquid that is separated from the curd is often referred to as sweet whey or cheese whey, typically has a pH from about 6.0 to about 6.5, and comprises whey proteins, lactose, minerals, water, fat and other low level components.

[0038] As indicated above, it is important that the enzyme or enzyme mixture is capable of destabilizing the casein protein in the milk fraction by cleaving peptides which stabilize the casein protein in the milk. Therefore, any proteolytic enzyme suitable for this purpose may be used to obtain cheese whey. In a preferred embodiment, however, the cheese whey is provided by adding rennet enzyme to bovine milk, resulting in enzymatic coagulation of casein. Rennet enzyme is commonly used in the cheese making process and comprises a set of enzymes which are produced in the stomachs of ruminant mammals. These enzymes normally include chymosin, pepsin, and lipase. The rennet enzyme mix destabilizes the casein protein in the bovine milk fraction by proteolytically cleaving peptides which stabilize the protein in the milk. As indicated above, the casein in the milk coagulates and contracts with milk fat to form the cheese curd. The remaining liquid, i.e., the sweet cheese whey, comprises whey proteins, lactose, minerals, water, fat, and other low level components.

[0039] By way of example, a gentle procedure of obtaining an exosome-enriched product containing intact bovine milk-derived exosomes may comprise physical methods and/or chemical methods. In one embodiment, an exosome-enriched product is obtained by cascade membrane filtration. In a specific embodiment, the exosome-enriched product is lactose-free. In a specific embodiment, sweet cheese whey, which may be obtained as described in the preceding paragraph, is processed using tandem multiple ceramic filtration steps. In a specific embodiment, a multiple filtration process employs, successively, membranes with cut offs which gradually decrease in size with each filtration step. In a specific embodiment, the method of processing sweet cheese whey is subjected to microfiltration (MF), ultrafiltration (UF) and diafiltration (DF). In one more specific embodiment, as shown in **FIG. 3**, the process employs, successively, MF,

UF and DF membranes with cut offs of about 1.4 μm , 0.14 μm and 10 kDa to provide an exosome-enriched product.

[0040] In a specific embodiment, the exosome-enriched product resulting from successive filtration steps may be pasteurized to provide storage stability. For example, the exosome-enriched product may be heated, for example, at about 70°C for about 15 seconds, to ensure microbiological stability in order to yield a pasteurized fraction. Other pasteurization conditions will be apparent to those skilled in the art and may be employed.

[0041] With or without pasteurization, the exosome-enriched product may be used as is or subjected to additional processing steps to provide a desired physical form. In one embodiment, the exosome-enriched product, optionally pasteurized, may be converted to a powder form. In more specific embodiments, the exosome-enriched product can be spray-dried, freeze dried, or otherwise converted to powder form. In one specific embodiment, the exosome-enriched product may be spray dried, for example, at 185°C/85°C, to obtain an exosome-enriched product in the form of a spray-dried powder (SP). Prior to spray drying, the exosome-enriched product may be subjected to an optional evaporation step to increase the solids content of the product and therefore reduce the time and/or energy demand for the spray drying process. Other spray drying conditions will be apparent to those skilled in the art and may be employed. Alternatively, the exosome-enriched product may be freeze-dried, for example at -50°C and 0.5 mbar vacuum to obtain an exosome-enriched freeze-dried powder (FP). Other freeze drying conditions will be apparent to those skilled in the art and may be employed.

[0042] In a specific embodiment, the exosome-enriched product comprises at least 0.001 wt% exosomes. In another specific embodiment, the exosome-enriched product comprises at least about 0.001 wt%, 0.01 wt%, 1 wt%, 5 wt%, 10 wt%, 15 wt%, 20 wt%, 25 wt%, 30 wt%, 35 wt%, 40 wt%, 45 wt%, or 50 wt% exosomes. In a further embodiment, the exosome-enriched product comprises at least about 10^8 exosomes per gram of the exosome-enriched product as measured by a nanotracking procedure. Briefly, nanoparticle tracking analysis (NTA) can be used to determine exosome diameter and concentration. The principle of NTA is based on the characteristic movement of nanosized particles in solution according to the Brownian motion. The trajectory of the particles in a defined volume is recorded by a camera that is used to capture the scatter light upon illumination of the particles with a laser. The Stokes-Einstein equation is used to determine the size of each tracked particle. In addition to particle size, this technique also allows determination of particle concentration.

[0043] In another specific embodiment, the exosome-enriched product comprises from about 10^8 to about 10^{14} exosomes per gram of the exosome-enriched product. In yet a more specific embodiment, the exosome-enriched product comprises from about 10^9 to about 10^{13} exosomes per gram of the exosome-enriched product. In another specific embodiment, the exosome-enriched product contains at least about a three-fold increase in the number of exosomes, as compared to a raw whey-containing bovine milk fraction. In another specific embodiment, the exosome-enriched product contains a 3-fold to 50-fold increase in the number of exosomes, as compared to a raw whey-containing bovine milk fraction, for example cheese whey.

[0044] In another embodiment, greater than 90% of the bovine milk-derived exosomes are from about 10 nanometers to about 250 nanometers in diameter, or from about 20 to 200 nm in diameter, or from about 50 to 150 nm in diameter.

[0045] In another embodiment, at least about 50 wt% of the exosomes in the exosome-enriched product are intact. In a specific embodiment, at least about 55, 60, 65, 70, 75, 80, 85, 90, or 95 wt% of the exosomes in the exosome-enriched product are intact.

[0046] In a specific embodiment, the exosome-enriched product is administered in the form of an exosome-enriched powder. In another embodiment, the exosome-enriched product is administered in the form of an exosome-enriched liquid. The exosome-enriched product can be administered to the subject in either form.

[0047] In another specific embodiment, the exosome-enriched product comprising intact bovine milk-derived exosomes is administered to the subject at a dose of about 0.01 to about 30 g. More specifically, the dosage of the exosome-enriched product comprising the intact bovine milk-derived exosomes may be from about 0.1 to about 30 g, from about 0.1 to about 15 g, or from about 1 to about 15 g. The exosome-enriched product comprising the intact bovine milk-derived exosomes can be administered to a subject at any of the above dosages 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. By way of example, the dosage of the exosome-enriched product comprising the intact bovine milk-derived exosomes may be from about 0.01 to about 30 g/day, from about 0.1 to about 30 g/day, from about 0.1 to about 15 g/day, or from about 1 to about 15 g/day.

[0048] In another embodiment, the exosome-enriched product comprising the intact bovine milk-derived exosomes is administered to the subject orally.

[0049] In a specific embodiment, the subject is a human. In other embodiments, the subject is a human adult 40 years of age or older. By way of example, the subject may be an aging human adult, for example, over 45 years of age, over 50 years of age, over 55 years of age, over 60 years of age, over 65 years of age, over 70 years of age, over 75 years of age, over 80 years of age, over 85 years of age, or older.

[0050] In a specific embodiment, the muscle is skeletal muscle. In another specific embodiment, the muscle is cardiac muscle.

[0051] In another specific embodiment, the exosome-enriched product comprising intact bovine milk-derived exosomes is administered to the subject in a nutritional composition comprising protein, carbohydrate, and/or fat. In another embodiment, the nutritional composition comprises protein, carbohydrate, fat, and one or more nutrients selected from the group consisting of vitamins, minerals, and trace minerals.

[0052] Non-limiting examples of vitamins include vitamin A, vitamin B12, vitamin C, vitamin D, vitamin E, vitamin K, thiamine, riboflavin, pyridoxine, niacin, folic acid, pantothenic acid, biotin, choline, inositol, and/or salts and derivatives thereof, and combinations thereof. 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.

[0053] In another specific embodiment, the nutritional composition comprises about 0.001 to about 30 wt%, about 0.001 to about 10 wt%, about 0.001 to about 5 wt%, about 0.001 to about 1 wt%, about 0.01 to about 30 wt%, about 0.01 to about 10 wt%, about 0.01 to about 5 wt%, about 0.01 to about 1 wt%, about 0.1 to about 30 wt%, about 0.1 to about 10 wt%, about 0.1 to about 5 wt%, about 0.1 to about 1 wt%, about 1 to about 30 wt%, about 1 to about 10 wt%, or about 1 to about 5 wt% of the exosome-enriched product comprising the intact bovine milk-derived exosomes, based on the weight of the nutritional composition. In a specific embodiment, the nutritional composition comprises from about 0.001 to about 10 wt% of the intact bovine milk-derived exosomes, based on the weight of the nutritional composition.

[0054] In view of the exosome-enriched product also containing whey protein, the exosome-enriched product may be the sole source of protein in the nutritional composition. Nevertheless, additional protein sources can be included in the nutritional composition. In one embodiment, the protein comprises whole egg powder, egg yolk powder, egg white powder, whey protein, 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, fava bean protein concentrate, fava bean protein isolate, fava bean protein hydrolysate, collagen proteins, collagen protein isolates, meat proteins, potato proteins, chickpea proteins, canola proteins, mung proteins, quinoa proteins, amaranth proteins, chia proteins, hemp proteins, flax seed proteins, earthworm proteins, insect proteins, one or more amino acids and/or metabolites thereof, or combinations of two or more thereof.

[0055] The one or a mixture of amino acids, which may be described as free amino acids, can be any amino acid known for use in nutritional products. 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.

[0056] 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), β -hydroxy- β -methylbutyrate (HMB), and combinations of two or more thereof.

[0057] The nutritional composition may comprise protein in an amount from about 1 wt% to about 30 wt% of the nutritional composition. More specifically, the protein may be present in an amount from about 1 wt% to about 25 wt% of the nutritional composition, including 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 25 wt%, or about 10 wt% to about 20 wt% of the nutritional composition. Even more specifically, the protein comprises from about 1 wt% to about 5 wt% of the nutritional composition, or from about 20 wt% to about 30 wt% of the nutritional composition.

[0058] In another embodiment, the carbohydrate comprises maltodextrin, hydrolyzed starch, modified starch, hydrolyzed cornstarch, modified cornstarch, polydextrose, dextrins, corn syrup,

corn syrup solids, rice maltodextrin, brown rice mild powder, brown rice syrup, sucrose, glucose, fructose, lactose, high fructose corn syrup, honey, 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, arabinoglactins, glucomannan, xanthan gum, alginate, pectin, low methoxy pectin, high methoxy pectin, cereal beta-glucans, carrageenan, psyllium, fiber, fruit puree, vegetable puree, isomalto-oligosaccharides, monosaccharides, disaccharides, human milk oligosaccharides (HMOs), tapioca-derived carbohydrates, inulin, and artificial sweeteners, or combinations of two or more thereof.

[0059] The nutritional composition may comprise carbohydrate in an amount from about 5 wt% to about 75 wt% of the nutritional composition. More specifically, the carbohydrate may be 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.

[0060] In another embodiment, the fat comprises 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, long chain polyunsaturated fatty acids, or combinations of two or more thereof.

[0061] The nutritional composition may comprise fat in an amount of from about 0.5 wt% to about 30 wt% of the nutritional composition. More specifically, the fat may be present in an amount from about 0.5 wt% to about 10 wt%, about 1 wt% to about 30 wt% of the nutritional composition, including about 1 wt% to about 20 wt%, about 1 wt% to about 15 wt%, about 1 wt% to about 10 wt%, about 1 wt% to about 5 wt%, about 3 wt% to about 30 wt%, about 5 wt% to about 30 wt%, about 5 wt% to about 25 wt%, about 5 wt% to about 20 wt%, about 5 wt% to about 10 wt%, or about 10 wt% to about 20 wt% of the nutritional composition.

[0062] 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. In a specific embodiment, the nutritional composition comprises a source of protein in an amount of about 2 wt% to about 20 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.

[0063] In one embodiment, the nutritional composition is a liquid nutritional composition and comprises from about 1 to about 15 wt% of protein, from about 0.5 to about 10 wt% fat, and from about 5 to about 30 wt% carbohydrate, based on the weight of the nutritional composition.

[0064] In another embodiment, the nutritional composition is a powder nutritional composition and comprises from about 10 to about 30 wt% of protein, from about 5 to about 15 wt% fat, and from about 30 wt% to about 65 wt% carbohydrate, based on the weight of the nutritional composition.

[0065] In a specific embodiment, the nutritional composition comprises at least one protein comprising milk protein concentrate and/or soy protein isolate, at least one fat comprising canola oil, corn oil, coconut oil and/or marine oil, and at least one carbohydrate comprising maltodextrin, sucrose, and/or short-chain fructooligosaccharide.

[0066] 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, thickening agents, stabilizers, and so forth.

[0067] 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.

[0068] The nutritional composition may be formed using any techniques known in the art. In one embodiment, the nutritional composition 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 intact exosomes may be added at any time as desired in the process, for example, to the aqueous solution or to the emulsified blend. The intact exosomes may be dry blended in powder form with one or more dry ingredients, for example, for combined addition to a liquid composition or if a powdered nutritional product is desirable.

[0069] In a specific embodiment, the nutritional composition is administered in the form of a powder. In another specific embodiment, the nutritional composition is administered in the form of a liquid. The nutritional composition can be administered to the subject in either form.

[0070] When the nutritional composition is a powder, for example, a serving size is from about 40 g to about 60 g, such as 45 g, or 48.6 g, or 50 g, to be administered as a powder or to be reconstituted in from about 1 ml to about 500 ml of liquid.

[0071] 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, 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 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 237 ml, or about 500 ml.

[0072] In specific embodiments, the nutritional compositions comprising bovine milk-isolated exosomes are administered to a subject once or multiple times daily or weekly. In specific embodiments, the nutritional composition is 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 specific embodiments, the nutritional composition is administered once or twice daily for a period of at least one week, at least two weeks, at least three weeks, or at least four weeks.

[0073] The following Examples demonstrate aspects of the inventive methods and are provided solely for the purpose of illustration. The Examples are not to be construed as limiting of the

general inventive concepts, as many variations thereof are possible without departing from the spirit and scope of the general inventive concepts.

EXAMPLES

[0074] **Example 1:** Preparation and Characterization of Exosome-enriched Products

[0075] This example describes a method of preparing an exosome-enriched product from cheese whey. The cheese whey was provided by adding rennet enzyme to bovine milk, resulting in enzymatic coagulation of casein and production of sweet cheese whey, as described above.

[0076] An exosome-enriched product containing about 10^8 to 10^{14} intact bovine milk-derived exosomes per gram of the exosome-enriched product was prepared by cascade membrane filtration. First, 1,000 L of sweet cheese whey was processed using tandem multiple ceramic filtration steps. With reference to **FIG. 3**, the first microfiltration MF step employed a membrane with a molecular weight cut off of 1.4 μm , which yielded a first retentate R1 and a first permeate P1. The first permeate P1 was then subjected to a ultrafiltration step UF with a molecular weight cut off of 0.14 μm , which yielded a second retentate R2 and second permeate P2. About 5 volumes of water was added to one volume of the second retentate R2, and the diluted retentate was then passed through the 0.14 μm UF membrane again to remove at least part of the lactose and minerals. The resulting retentate R3 was then combined with an equal volume of water and diafiltered using a 10 kDa membrane to produce a fourth retentate R4. The retentate R4 was diluted with a volume of water five times that of the fourth retentate R4 and diafiltered a second time using the 10 kDa membrane to yield a concentrated retentate, R5. The lactose-free exosome-enriched product R5 was pasteurized at 70°C for 15 seconds to ensure microbiological stability in order to yield a pasteurized exosome-enriched product R6. A portion of the pasteurized exosome-enriched product R6 was subjected to evaporation at about 65°C to increase the solids content up to 17-18% and then spray-dried at 185°C/85°C to obtain a exosome-enriched spray-dried product, SP. Another portion of the pasteurized exosome-enriched product R6 was freeze dried at -50°C and 0.5 mbar to obtain a exosome-enriched freeze-dried product, FP.

[0077] The starting cheese whey, the second retentate R2, and the exosome-enriched products comprising intact bovine milk derived exosomes prepared as described above were analyzed to determine lactose and protein content, as set forth in Table 1 below.

[0078] **Table 1.** *Lactose and protein composition of the exosome-enriched product.*

Fractions	Protein % (by Milkoscan)	Protein % (by LECO)	Lactose %	Total Solids %
W	1.39±0.02	0.93	4.48±0.01	6.33±0.03
R2	1.82±0.01	1.13	3.41±0.02	5.62±0.01
R6	5.63±0.04	6.87	0	7.10±0.03
SP	-	80.34	0	Powder
FP	-	78.45	0	Powder

Composition analysis of different fractions and exosome-enriched powders:

W= cheese whey. R2 =final exosome-enriched liquid fraction. R6 =final exosome-enriched liquid fraction. SP=spray-dried powder. FP=freeze-dried powder.

[0079] The amount of fat, protein, lactose, and total solids of the collected samples from each of the fractions referred to in Table 1 were determined by Fourier transform infrared (FTIR) spectroscopy using a Bentley Instruments Dairy Spec FT (Bentley Instruments, Inc., Chaska, MN, USA). The Bentley Instruments Dairy Spec FT captures the complete infrared absorption spectrum of the milk sample for component analysis. This particular technology exceeds the IDF 141C:2000 Standard and ICAR requirements for Milk Component Measurement and uses AOAC approved methodology, thus providing a non-destructive, reliable and precise measurement.

[0080] The results presented in Table 1 surprisingly demonstrate that the pasteurized exosome-enriched product R6, the spray-dried exosome-enriched product SP, and the freeze-dried exosome-enriched product FP were all lactose-free. Further, the protein content in the pasteurized exosome-enriched product R6 increased almost 7 times with respect to the cheese whey starting material, and about 6 times with respect to the exosome-enriched second retentate R2. In addition, about 80% of the dry matter of the powders was protein and about 15% of the dry matter was fat, which is consistent with the lipid-protein nature of exosomes.

[0081] In order to gain further insight on the exosome content of the pasteurized exosome-enriched product R6 and the exosome-enriched SP and FP powders, a Western blot analysis was performed to detect the presence of the exosome-specific marker TSG101. The exosome-enriched product R6 and the exosome-enriched SP and FP powders showed the TSG101 band of interest at around 50 kDa. Notably, the TSG101 biomarker was not detectable in cheese whey, despite equal amounts of protein being loaded per lane. This indicates that the pasteurized exosome-enriched product R6 and the exosome-enriched SP and FP powders produced according to the process described above are significantly enriched in milk exosomes.

[0082] Transmission electron microscopy (TEM) was also used for purposes of assessing the presence of exosomes in the pasteurized exosome-enriched product R6, and in the exosome-enriched SP and FP powders. TEM is a technique which can be used for the direct visualization of nanosized structures, such as exosomes. The application of uranyl acetate as a negative dye was used to study the impact of thermal treatments, such as pasteurization, evaporation, spray-drying, and freeze-drying, on the exosome structure of the exosomes in the pasteurized lactose-free exosome-enriched product R6, and in the final lactose-free exosome-enriched SP and FP products. Briefly, the uranyl acetate acts as a negative dye, which stains the background and leaves the intact vesicular structures, such as intact exosomes, unstained and highly visible.

[0083] The lactose-free exosome-enriched SP and FP powders prepared as described above were resuspended in water and 3 microliters of each sample were placed on a Formvar® coated grid and stained with 2% uranyl acetate for 5 minutes. The exosome-enriched R5 and R6 products, prepared as described above, were placed undiluted on a Formvar® coated grid and stained with 2% uranyl acetate for 5 minutes. The samples were visualized at a magnification of x25,000. TEM images of the R5 and R6 exosome-enriched products, and the exosome-enriched SP and FP powders showed that the intact exosomes were present at high concentration. Remarkably, none of the thermal treatments that were applied led to significant exosome damage. These results demonstrate that the process described above can isolate and stabilize a significant amount intact milk exosomes from cheese whey.

[0084] The exosome-enriched products comprising intact bovine milk-derived exosomes prepared as described above were also analyzed to determine nucleic acid content. More specifically, the exosome-enriched SP and FP powders and the pasteurized exosome-enriched product R6 were analyzed in order to determine their total RNA content (µg), total miRNA content (µg), and miRNA as a percentage of the total RNA, as set forth in Table 2 below. 10 mg of each sample were extracted and analyzed using a Bioanalyzer 2100/ Eukaryote Total RNA Nano Chip. The exosome-enriched SP and FP powders and the pasteurized exosome-enriched product R6 displayed high amounts of both RNA and miRNA, however the exosome-enriched SP powder showed higher miRNA content than the exosome-enriched FP powder. This indicates that spray-drying may be a better stabilization strategy for providing an exosome-enriched product in powder form.

[0085] **Table 2.** *Nucleic acid composition of the exosome-enriched product.*

	Total RNA content (μ g)	miRNA % (of total nucleic acids)	miRNA content (μ g)
R6	5.50	67.1	3.68
SP	5.09	72.5	3.69
FP	2.51	76.1	1.91

[0086] The exosome-enriched products comprising intact bovine milk-derived exosomes were also analyzed to determine lipid composition. Ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry analysis (UPLC-TOF-MS) was performed to analyze the lipid content of the lactose-free exosome-enriched products described above. The results are set forth in Table 3 below and are expressed as a percentage of total lipids.

[0087] **Table 3.** *Lipid composition of the lactose-free exosome-enriched product.*

LIPID SPECIE	R6		SP		FP	
	% of total lipids	mg/g powder	% of total lipids	mg/g powder	% of total lipids	mg/g powder
Triacylglycerols	76.9	NA (liquid)	70.6	117.4	70.7	75.1
Phosphatidylcholine	6.4	NA (liquid)	7.5	12.5	7.9	8.3
Phosphatidylethanolamine	5.0	NA (liquid)	6.7	11.1	6.0	6.3
Sphingomyelin	3.8	NA (liquid)	4.9	8.2	6.6	7.0
Gangliosides (GD3)	1.9	NA (liquid)	2.7	4.4	2.4	2.5
Phosphatidylserine	1.8	NA (liquid)	2.0	3.4	2.0	2.1
Cholesterol esters	1.2	NA (liquid)	1.4	2.4	1.1	1.2
Ceramide dihexoside	0.9	NA (liquid)	1.3	2.2	1.0	1.1
Dihydrosphingomyelin	0.6	NA (liquid)	0.8	1.3	0.8	0.8
Cholesterol (free)	0.4	NA (liquid)	0.5	0.8	0.4	0.4
Ceramide monohexoside	0.3	NA (liquid)	0.4	0.7	0.4	0.4
Ether-linked phosphatidylethanolamine	0.2	NA (liquid)	0.2	0.4	0.2	0.2
Ceramide	0.1	NA (liquid)	0.2	0.3	0.2	0.2
Gangliosides (GM3)	0.1	NA (liquid)	0.2	0.3	0.1	0.1
Phosphatidylinositol	0.1	NA (liquid)	0.2	0.3	0.1	0.1
Lysophosphatidylethanolamine	0.1	NA (liquid)	0.1	0.2	0.1	0.1
Lysophosphatidylcholine	0.1	NA (liquid)	0.1	0.2	0.1	0.1
Dihydroceramide	0.1	NA (liquid)	0.1	0.1	0.1	0.1
Free fatty acids	Traces	Traces	Traces	Traces	Traces	Traces
Total lipid content (1%, on a dry basis)	NA		NA		NA	

[0088] The protein compositions of the exosome-enriched products were also determined. Specifically, the protein composition was determined by LC-MS/MS and mass spectra were searched in Proteome Discoverer v1.4 (database Bos Taurus, Uniprot 06/19 + Proteomics contaminants database). The results of several proteins of interest are set forth in Table 4 and surprisingly demonstrate that caseins were present at very low levels (e.g., only 0.04% of a α -S2-casein was detected). In addition, the results demonstrate that significant amounts of bioactive proteins (i.e., lactoferrin and immunoglobulins) were detected. The results are expressed as % of total proteins identified.

[0089] **Table 4.** *Protein composition of the lactose-free exosome-enriched product.*

PROTEIN	R6		SP		FP	
	% of total proteins	mg/g powder	% of total proteins	mg/g powder	% of total proteins	mg/g powder
β -lactoglobulin	56.40	NA (liquid)	58.97	471.76	59.24	473.92
Serum albumin	12.12	NA (liquid)	11.77	94.16	12.32	98.56
Antibodies (IgG, IgM, IgA)	6.33	NA (liquid)	6.63	53.04	6.42	51.36
α -lactalbumin	5.00	NA (liquid)	4.55	36.4	4.59	36.72
Lactoferrin	3.53	NA (liquid)	3.12	24.96	3.18	25.44
Butyrophilin	2.18	NA (liquid)	1.90	15.2	1.06	8.48
Lactadherin	1.67	NA (liquid)	1.45	11.6	1.52	12.16
Xanthine dehydro-genase/oxidase	1.45	NA (liquid)	1.36	10.88	1.40	11.2
Transferrin	1.27	NA (liquid)	1.05	8.4	0.98	7.84
Lactoperoxidase	0.54	NA (liquid)	0.53	4.24	0.59	4.72
Vitamin D-binding protein	0.17	NA (liquid)	0.15	1.2	0.15	1.2
Osteopontin	0.18	NA (liquid)	0.15	1.2	0.15	1.2
α -S1-casein	0.29	NA (liquid)	0.13	1.04	0.21	1.68
κ -casein	0.06	NA (liquid)	0.04	0.32	0.05	0.4
β -casein	0.04	NA (liquid)	0.03	0.24	0.03	0.24
α -S2-casein	0.02	NA (liquid)	0.01	0.08	0.01	0.08
Total casein	0.41	NA (liquid)	0.21	1.68	0.30	2.4
Total protein content (1%, on a dry basis)	NA		NA		NA	

[0090] **Example 2:** Enhanced Maximal Respiratory Capacity and Mitochondrial SRC in C2C12 Myoblasts Incubated with Intact Bovine Milk-Derived Exosomes

[0091] This example demonstrates that an exosome-enriched product containing intact bovine milk-derived exosomes enhances maximal respiratory capacity and mitochondrial SRC in C2C12 myoblasts. Mitochondrial function was analyzed by measuring the oxygen consumption rate

(OCR) in differentiated C2C12 myotubes incubated with either powdered bovine milk-derived exosomes resuspended in phosphate buffer saline (PBS) or with PBS alone using the SeaHorse XFe24 flux analyzer with XF Cell Mito Stress kit in accordance with manufacturer instructions.

[0092] C2C12 myoblasts were grown at 37°C in High Glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 4 mM of glutamine, 100 units/mL penicillin and 0.1 mg/mL streptomycin in a humidified atmosphere of 95% air and 5% CO₂. The C2C12 myoblasts were seeded (10⁴ cells/cm²) in a 24-well culture plate (V28) (SeaHorse Bioscience (Billerica, MA, USA)). When the myoblasts reached about 80% confluence, they were induced to differentiate into myotubes by exchanging the growth medium with a differentiation medium of High Glucose DMEM with 2% Horse serum (HS), 4 mM of glutamine, 100 units/mL penicillin and 0.1 mg/mL streptomycin. The differentiation medium was replaced every 24 hours.

[0093] After 72 hours of differentiation, the myotubes were incubated with either the spray dried (SP) exosome-enriched product prepared as described above, which was resuspended in PBS (1 µg/ml exosome protein), or with the same volume of PBS for 24 hours. Following treatment, the cells were washed twice and the media was replaced with adapted FAO Assay Medium (Krebs Henseleit Buffer (KHB): 111 mM NaCl, 4.7 mM KCL, 1.25 mM CaCl, 2 mM MgSO₄, 1.2 mM Na H₂PO₄ supplemented with 5 mM HEPES, 0.5 mM L-Carnitine, 1 mM sodium pyruvate and 2 mM glutamine) and the pH was adjusted to 7.4 ± 0.05 using 0.1 M NaOH. The cells were incubated for 45 min at 37°C without CO₂. Following incubation, glucose 10 mM was added to each well. The final volume for each well was 500 µl. All experiments were performed at 37°C.

[0094] Mitochondrial function was analyzed by measuring the OCR in the C2C12 myotubes using the SeaHorse XFe24 flux analyzer with XF Cell Mito Stress kit in accordance with manufacturer instructions. A measurement of basal respiration was taken and recorded. Ionophore carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP) (1.5 mM) was then injected to measure maximal respiratory capacity (MAX), which was also recorded. FCCP mimics a physiological "energy demand" by stimulating the respiratory chain to operate at maximum capacity, so the OCR observed after the addition of the ionophore corresponds to the maximal

respiration level. The FCCP-stimulated OCR can then be used to calculate SRC, which, as described above, is defined as the difference between maximal respiration and basal respiration.

[0095] As shown in **FIG. 1** and **FIG. 2**, compared to untreated C2C12 myotubes, bovine milk-derived exosome-treated myotubes increased both maximal respiratory capacity (**FIG. 1**) and SRC (**FIG. 2**) by about 21% and about 64%, respectively. The increased values for both MAX and SRC indicate improved mitochondrial performance, with the latter being particularly important. As indicated above, a cell with larger SRC can produce more ATP to maintain adequate levels of energetic molecules and overcome more stress.

[0096] All results were normalized to the control group and expressed as mean $\pm$ SEM. Statistical analyses were carried out using Student's t-test and *p*-values less than 0.05 (*) were considered as statistically significant. The experiment was repeated independently six times with four internal replicates.

[0097] These results thus indicate that the intact bovine milk exosomes can improve mitochondrial function and therefore muscle performance, which is particularly relevant for the prevention, treatment or recovery of conditions that are hallmarked by a reduction in SRC, such as sarcopenia and chronic or acute cardiac damage. As indicated above, treating mitochondrial dysfunction is also an effective way to alleviate chronic fatigue following a viral infection. The indication that the intact bovine milk exosomes can improve mitochondrial function is particularly relevant for improving chronic fatigue associated with the recovery from a viral infection.

[0098] 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.

WHAT IS CLAIMED IS:

1. A method of enhancing muscle performance in a subject in need of improved physical performance, comprising administering an exosome-enriched product comprising intact bovine milk-derived exosomes to the subject in need thereof.
2. A method of reducing chronic fatigue in a subject recovering or recovered from a viral infection, comprising administering an exosome-enriched product comprising intact bovine milk-derived exosomes to the subject .
3. The method of claim 2, wherein the viral infection is selected from the group consisting of Epstein-Barr virus, human herpes virus 6, and coronavirus.
4. The method of claim 3, wherein the coronavirus is COVID-19.
5. The method of any one of claims 1-4, wherein the intact bovine milk-derived exosomes are sourced from a whey-containing bovine milk fraction.
6. The method of any one of claims 1-5, wherein the exosome-enriched product comprises at least 0.001 wt% exosomes.
7. The method of any one of claims 1-6, wherein greater than 90% of the bovine milk-derived exosomes are from about 10 nanometers to about 250 nanometers in diameter.
8. The method of any one of claims 1-7, wherein at least about 50 wt% of the exosomes in the exosome-enriched product are intact.

9. The method of claim 8, wherein at least about 55, 60, 65, 70, 75, 80, 85, 90, or 95 wt% of the exosomes in the exosome-enriched product are intact.
10. The method of any one of claims 1-9, wherein the exosome-enriched product comprises at least 0.001 wt% exosomes, wherein at least about 50 wt% of exosomes in the exosome-enriched product are intact, and/or wherein the exosome-enriched product is lactose-free.
11. The method of any one of claims 1-10, wherein the exosome-enriched product is administered in the form of an exosome-enriched powder.
12. The method of any one of claims 1-10, wherein the exosome-enriched product is administered in the form of an exosome-enriched liquid.
13. The method of any one of claims 1-12, wherein the exosome-enriched product comprising the intact bovine milk-derived exosomes is administered to the subject at a dose of about 0.01 to about 30 g.
14. The method of any one of claims 1-13, wherein the exosome-enriched product comprising the intact bovine milk-derived exosomes is administered to the subject orally.
15. The method of any one of claims 1-14, wherein the subject is a human adult 50 years of age or older.
16. The method of any one of claims 1 and 5-15, wherein the muscle is skeletal muscle.

17. The method of any one of claims 1 and 5-16, wherein the muscle is cardiac muscle.
18. The method of any one of claims 1-17, wherein the exosome-enriched product comprising intact bovine milk-derived exosomes is administered to the subject in a nutritional composition comprising protein, carbohydrate, and/or fat.
19. The method of claim 18, wherein the nutritional composition comprises protein, carbohydrate, fat, and one or more nutrients selected from the group consisting of vitamins and minerals.
20. The method of claim 18 or claim 19, wherein the nutritional composition comprises from about 0.001 to about 30 wt% of the exosome-enriched product comprising the intact bovine milk-derived exosomes, based on the weight of the nutritional composition.
21. The method of any one of claims 18-20, wherein the protein comprises whole egg powder, egg yolk powder, egg white powder, whey protein, 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, fava bean protein concentrate, fava bean protein isolate, fava bean protein hydrolysate, collagen proteins, collagen protein isolates, meat proteins, potato proteins, chickpea proteins, canola proteins, mung proteins, quinoa proteins, amaranth proteins, chia proteins, hemp proteins, flax seed proteins, earthworm proteins, insect

proteins, one or more amino acids and/or metabolites thereof, or combinations of two or more thereof.

22. The method of claim 21, wherein the one or more amino acids and/or metabolites thereof comprise one or more branched chain amino acids or metabolites thereof.
23. The method of claim 22, wherein the one or more branched chain amino acids or metabolites thereof comprise alpha-hydroxy-isocaproic acid (HICA), keto isocaproate (KIC), β -hydroxy- β -methylbutyrate (HMB), and combinations of two or more thereof.
24. The method of any one of claims 18-23, wherein the nutritional composition comprises from about 1 wt% to about 30 wt%, from about 1 wt% to about 25 wt%, from about 1 to about 20 wt%, from about 1 to about 15 wt%, from about 1 to about 10 wt%, or from about 10 wt% to about 30 wt% protein, based on the weight of the nutritional composition.
25. The method of any one of claims 18-24, wherein the carbohydrate comprises maltodextrin, hydrolyzed starch, modified starch, hydrolyzed cornstarch, modified cornstarch, polydextrose, dextrans, corn syrup, corn syrup solids, rice maltodextrin, brown rice mild powder, brown rice syrup, sucrose, glucose, fructose, lactose, high fructose corn syrup, honey, 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, arabinoglactins, glucomannan, xanthan gum, alginate, pectin, low methoxy pectin, high methoxy pectin, cereal beta-glucans, carrageenan, psyllium, fiber, fruit puree, vegetable puree, isomalto-oligosaccharides, monosaccharides, disaccharides, human milk

oligosaccharides, tapioca-derived carbohydrates, inulin, and artificial sweeteners, or combinations of two or more thereof.

26. The method of any one of claims 18-25, wherein the nutritional composition comprises from about 5 wt% to about 75 wt%, from about 5 wt% to about 70 wt%, from about 5 wt% to about 65 wt%, from about 5 wt% to about 50 wt%, from about 5 wt% to about 40 wt%, from about 5 wt% to about 30 wt%, from about 5 wt% to about 25 wt%, from about 10 wt% to about 65 wt%, from about 20 wt% to about 65 wt%, from about 30 wt% to about 65 wt%, from about 40 wt% to about 65 wt%, or from about 15 wt% to about 25 wt% carbohydrate, based on the weight of the nutritional composition.
27. The method of any one of claims 18-26, wherein the fat comprises 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, long chain polyunsaturated fatty acids, or combinations of two or more thereof.
28. The method of any one of claims 18-27, wherein the nutritional composition comprises from 0.5 wt% to 20 wt%, from about 0.5 to about 15 wt%, from about 0.5 to about 10 wt%, from about 0.5 to about 5 wt%, or from about 5 to about 15 wt% fat, based on the weight of the nutritional composition.
29. The method of any one of claims 18-28, wherein the nutritional composition is administered in the form of a powder.

30. The method of any one of claims 18-29, wherein the nutritional composition is administered in the form of a liquid.
31. The method of claim 30, wherein the nutritional composition comprises from about 1 to about 15 wt% of protein, from about 0.5 to about 10 wt% fat, and from about 5 to about 30 wt% carbohydrate, based on the weight of the nutritional composition.
32. The method of claim 29, wherein the nutritional composition comprises from about 10 to about 30 wt% of protein, from about 5 to about 15 wt% fat, and from about 30 wt% to about 65 wt% carbohydrate, based on the weight of the nutritional composition.
33. The method of any one of claims 18-32, wherein the nutritional composition comprises at least one protein comprising milk protein concentrate and/or soy protein isolate, at least one fat comprising canola oil, corn oil, coconut oil and/or marine oil, and at least one carbohydrate comprising maltodextrin, sucrose, and/or short-chain fructooligosaccharide.