have a tendency to form colloidal aggregates that are around 20 to 600 nm in size. The size of exosomes and casein aggregates thus overlaps. In view of this overlap, size-based isolation techniques, which are common in the dairy industry, tend to co-purify exosomes and casein. Accordingly, improved methods of isolating bovine milk exosomes are desirable.

[0005] Insulin sensitivity resistance, prediabetes, and diabetes are conditions of glucose dysregulation. Lifestyle modifications and insulin sensitizers are among the treatment strategies available, however these often fail and insulin therapy can become necessary. Dietary management can improve metabolic health and lead to weight loss. Planning for weight maintenance over time, sustaining and improving muscle mass, and taking vitamins and minerals can improve quality of life. Unfortunately, in many cases, lifestyle modifications are inadequate to fully treat such conditions. New compositions and methods for improving insulin sensitivity are therefore needed in order to treat insulin resistance, prediabetes, and diabetes.

SUMMARY ~~OF THE INVENTION~~

[0005a] In one aspect, the invention is directed to a method of obtaining an exosome-enriched product, the method comprising: providing a whey-containing bovine milk fraction; conducting a first centrifugation, comprising centrifuging the whey-containing bovine milk fraction to obtain a whey middle fraction; conducting a second centrifugation, comprising centrifuging the whey middle fraction at an increased speed as compared with the speed of the first centrifugation to obtain a concentrated whey fraction; filtering the concentrated whey fraction to obtain a filtered whey fraction; and conducting a third centrifugation, comprising centrifuging the filtered whey fraction at an increased speed as compared with the speed of the second centrifugation to obtain an exosome-enriched product, wherein the exosome-enriched product comprises intact exosomes and less than 5 wt% casein based on the total weight of protein in the exosome-enriched product.

[0005b] In another aspect, the invention is directed to a method of obtaining an exosome-enriched product, the method comprising: conducting a first centrifugation, comprising centrifuging cheese whey to obtain a whey middle fraction; conducting a second centrifugation, comprising centrifuging the whey middle fraction at an increased speed as compared with the speed of the first centrifugation to obtain a concentrated whey fraction; filtering the concentrated whey fraction to obtain a filtered whey fraction; and conducting a third centrifugation, comprising centrifuging the filtered whey fraction at an increased speed as compared with the speed of the second centrifugation to obtain the exosome-enriched product, wherein the exosome-enriched product comprises intact exosomes and less than 5 wt% casein based on the total weight of protein in the exosome-enriched product.

[0005c] In another aspect, the invention is directed to an exosome-enriched product obtained by the method disclosed herein.

[0005d] In another aspect, the invention is directed to a bovine milk-derived exosome-enriched product comprising bovine milk-derived intact exosomes and less than 5 wt% casein based on the total weight of protein in the exosome-enriched product.

[0005e] In another aspect, the invention is directed to a nutritional composition comprising protein, carbohydrate, and/or fat, and bovine milk-derived exosomes, wherein the exosomes are provided by addition of the exosome-enriched product disclosed herein.

[0005f] In another aspect, the invention is directed to a use of a nutritional composition comprising the exosome-enriched product disclosed herein, or the nutritional composition disclosed herein, in the manufacture of a medicament for improving insulin sensitivity in an individual at risk of developing or having insulin-resistance, prediabetes, or diabetes.

[0005g] In another aspect, the invention is directed to a nutritional composition comprising the exosome-enriched product disclosed herein, or the nutritional composition disclosed herein, for use in therapy.

[0006] In one embodiment, the invention is directed to a method of obtaining an exosome-enriched product from a bovine milk fraction. The method comprises providing a whey-containing bovine milk fraction, a first centrifugation which comprises centrifuging the whey-containing bovine milk fraction to obtain a whey middle fraction, a second centrifugation which comprises centrifuging the whey middle fraction at an increased speed as compared with the speed of the first centrifugation to obtain a concentrated whey fraction, filtering the concentrated whey fraction to obtain a filtered whey fraction, and a third centrifugation which comprises centrifuging the filtered whey fraction at an increased speed as compared with the speed of the second centrifugation to obtain an exosome-enriched product, wherein the exosome-enriched product comprises intact exosomes and less than 5 wt% casein based on the total weight of protein in the exosome-enriched product.

WHAT IS CLAIMED IS:

1. A method of obtaining an exosome-enriched product, the method comprising:

providing a whey-containing bovine milk fraction;

conducting a first centrifugation, comprising centrifuging the whey-containing bovine milk fraction to obtain a whey middle fraction;

conducting a second centrifugation, comprising centrifuging the whey middle fraction at an increased speed as compared with the speed of the first centrifugation to obtain a concentrated whey fraction;

filtering the concentrated whey fraction to obtain a filtered whey fraction; and

conducting a third centrifugation, comprising centrifuging the filtered whey fraction at an increased speed as compared with the speed of the second centrifugation to obtain an exosome-enriched product, wherein the exosome-enriched product comprises intact exosomes and less than 5 wt% casein based on the total weight of protein in the exosome-enriched product.

2. The method of claim 1, wherein the whey-containing bovine milk fraction is provided by lowering the pH of a bovine milk product to precipitate milk solids, and removing the milk solids.

3. The method of claim 2, wherein the pH of the milk product is lowered to about 3.0 to 4.6.

4. A method of obtaining an exosome-enriched product, the method comprising:

conducting a first centrifugation, comprising centrifuging cheese whey to obtain a whey middle fraction;

conducting a second centrifugation, comprising centrifuging the whey middle fraction at an increased speed as compared with the speed of the first centrifugation to obtain a concentrated whey fraction;

filtering the concentrated whey fraction to obtain a filtered whey fraction; and

conducting a third centrifugation, comprising centrifuging the filtered whey fraction at an increased speed as compared with the speed of the second centrifugation to obtain the exosome-enriched product, wherein the exosome-enriched product comprises intact exosomes and less than 5 wt% casein based on the total weight of protein in the exosome-enriched product.

5. The method of claim 4, wherein the cheese whey is sweet cheese whey and has a pH from about 6.0 to about 6.5.

6. The method of claim 5, wherein the sweet cheese whey comprises liquid separated from curd formed by subjecting bovine milk to at least one enzyme which destabilizes casein.

7. The method of any one of the preceding claims, wherein the second centrifugation comprises centrifuging the whey middle fraction at least two times to obtain the concentrated whey fraction.

8. The method of any one of the preceding claims, wherein the exosome-enriched product comprises at least 10 wt% exosomes.

9. The method of any one of the preceding claims, wherein at least about 50 wt% of exosomes in the exosome-enriched product are intact.

10. The method of any one of the preceding claims, 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.

11. The method of any one of the preceding claims, wherein the exosome-enriched product comprises less than 4, 3, 2, 1, 0.5, or 0.1 wt% casein based on the total weight of protein in the exosome-enriched product.

12. The method of any one of the preceding claims, wherein the third centrifugation comprises ultracentrifuging the filtered whey fraction to obtain a pellet containing intact exosomes.

13. The method of claim 12, further comprising incubating the pellet containing exosomes in aqueous medium to dissolve the pellet without disrupting exosome membranes to provide an exosome suspension.

14. The method of claim 13, further comprising drying the exosome suspension.

15. The method of claim 14, wherein the drying comprises freeze drying the exosome suspension.

16. The method of any one of the preceding claims, wherein the exosome-enriched product is an exosome-enriched powder.

17. An exosome-enriched product obtained by the method of any one of the preceding claims.

18. A bovine milk-derived exosome-enriched product comprising bovine milk-derived intact exosomes and less than 5 wt% casein based on the total weight of protein in the exosome-enriched product.

19. The bovine milk-derived exosome-enriched product of claim 18, wherein the exosome-enriched product comprises less than 4, 3, 2, 1, 0.5, or 0.1 wt% casein based on the total weight of protein in the exosome-enriched product.

20. The bovine milk-derived exosome-enriched product of claim 18 or claim 19, wherein the exosome-enriched product comprises at least 10 wt% exosomes.

21. The bovine milk-derived exosome-enriched product of any one of claims 18-20, wherein at least about 50 wt% of exosomes in the exosome-enriched product are intact.

22. The bovine milk-derived exosome-enriched product of claim 21, 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.

23. The bovine milk-derived exosome-enriched product of any one of claims 18-22, wherein the exosome-enriched product is a powder.

24. A nutritional composition comprising protein, carbohydrate, and/or fat, and bovine milk-derived exosomes, wherein the exosomes are provided by addition of the exosome-enriched product of any one of claims 17-23.

25. The nutritional composition of claim 24, comprising from about 0.001 to about 10 wt % of the bovine milk-derived exosomes, based on the weight of the nutritional composition.

26. The nutritional composition of claim 24 or 25, wherein the protein comprises whey protein concentrate, whey protein isolate, whey protein hydrolysate, milk protein concentrate, milk protein isolate, milk protein hydrolysate, soy protein concentrate, soy protein isolate, soy protein hydrolysate, pea protein concentrate, pea protein isolate, pea protein hydrolysate, fava bean protein, or combinations of two or more thereof.

27. The nutritional composition of any one claims 24-26, wherein the carbohydrate comprises fiber, human milk oligosaccharides (HMOs), maltodextrin, corn maltodextrin, corn syrup, sucralose, cellulose gel, cellulose gum, gellan gum, carrageenan, fructooligosaccharides, or combinations of two or more thereof.

28. The nutritional composition of any one of claims 24-27, wherein the fat comprises coconut oil, fractionated coconut oil, soy oil, soy lecithin, corn oil, safflower oil sunflower oil, palm olein, canola oil monoglycerides, lecithin, at least one omega-3 fatty acid, anola oil, medium chain triglycerides, one or more fatty acids such as linoleic acid, alpha-linolenic acid, ARA, EPA, and/or DHA, or combinations of two or more thereof.

29. The nutritional composition of claim 28, wherein the at least one omega-3 fatty acid of the composition is selected from the group consisting of eicosapentaenoic acid, docosahexaenoic acid, arachidonic acid, and alpha-linolenic acid.

30. The nutritional composition of any one of claims 28-29, wherein the nutritional composition is in the form of a powder.

31. The nutritional composition of any one of claims 28-30, wherein the nutritional composition is in the form of a liquid.

32. Use of a nutritional composition comprising the exosome-enriched product of any one of claims 17-23, or the nutritional composition of any one of claims 24-31, in the manufacture of a medicament for improving insulin sensitivity in an individual at risk of developing or having insulin-resistance, prediabetes, or diabetes. ~~A method of improving insulin sensitivity in an individual at risk of developing or having insulin-resistance, prediabetes, or diabetes, comprising:~~

~~administering to the individual a nutritional composition comprising the exosome-enriched product of any one of claims 17-23, or the nutritional composition of any one of claims 24-31.~~

33. A nutritional composition comprising the exosome-enriched product of any one of claims 17-23, or the nutritional composition of any one of claims 24-31, for use in therapy.