4. SUMMARY

[0056] The present disclosure provides lipoprotein complexes, comprising a protein fraction (*e.g.*, an apolipoprotein fraction) and a lipid fraction, and populations thereof that are especially suited to treating and/or preventing dyslipidemia and diseases, disorders and/or conditions associated with dyslipidemia. It has been discovered that populations of complexes which have greater purity and/or homogeneity, and/or comprise particular ratios of lipids and proteins, as described herein, have increased ability to mobilize cholesterol combined with reduced risk of side effects.

[0056a] In one aspect, the present disclosure refers to a composition comprising a population of lipoprotein complexes, each comprising

(a) a lipid fraction comprising at least one neutral lipid and at least one negatively charged lipid;

(b) an apolipoprotein fraction consisting essentially of an Apolipoprotein A-I ("ApoA-I"); and

(c) a hydrophobic, lipophilic or apolar active agent, or a label;

wherein

(i) at least 95% by weight of the apolipoprotein in the composition is in complexed form;

(ii) at least 95% by weight of the lipid in the composition is in complexed form;

(iii) no more than 5% or the methionine residues in the apolipoprotein are oxidized;

(iv) the size distribution of the lipoprotein complexes is at least 95% homogeneous, as reflected by a single peak in gel permeation chromatography; and

(v) the composition does not contain any detergent.

[0056b] In another aspect, the present disclosure refers to a pharmaceutical composition comprising or consisting essentially of a composition as disclosed herein, and one or more pharmaceutically acceptable carriers, diluents and/or excipients.

[0056c] In another aspect, the present disclosure refers to a unit dosage form comprising a therapeutically effective amount of a pharmaceutical composition as disclosed herein.

[0056d] In another aspect, the present disclosure refers to use of a composition as disclosed herein in the manufacture of a medicament.

[0056e] In another aspect, the present disclosure refers to use of a composition of lipoprotein complexes as disclosed herein in the manufacture of a diagnostic agent for use in an imaging procedure.

[0057] The lipoprotein complexes comprise a protein fraction (*e.g.*, an apolipoprotein fraction) and a lipid fraction (*e.g.*, a phospholipid fraction). The protein fraction includes one or more lipid-binding proteins, such as apolipoproteins, peptides, or apolipoprotein peptide analogs or mimetics capable of mobilizing cholesterol when present in a lipoprotein complex. Non-limiting examples of such apolipoproteins and apolipoprotein peptides include preproapoliproteins, preproApoA I, proApoA-I, ApoA-I, preproApoA-II, proApoA-II, ApoA-II, preproApoA-IV, proApoA- IV, ApoA-IV, ApoA-V, preproApoE, proApoE, ApoE, preproApoA-I_M, proApoA-I_M, ApoA-I_M, preproApoA-I_P, proApoA-I_P, ApoA-I_P, preproApoA-I_Z, proApoA-I_Z, and ApoA-I_Z. The apolipoprotein(s) can be in the form of monomers, dimers, or trimers, or mixtures thereof. In a specific example, the apolipoprotein fraction consists essentially of ApoA-I, most preferably of a single isoform. ApoA-I in lipoprotein complexes can have at least 90% or at least 95% sequence identity to a protein corresponding to amino acids 25 to 267 of SEQ ID NO:1. Optionally, ApoA-I further comprises an aspartic acid at the position corresponding to the full length ApoA-I amino acid 25 of SEQ ID NO:1 (and position 1 of the mature protein). Preferably, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% of the ApoA-I is correctly processed, mature protein (*i.e.*, lacking the signal and propeptide sequences) and not oxidized, deamidated and/or truncated.

[0058] The present disclosure also provides mammalian host cells engineered to express ApoA-I, cell cultures comprising ApoA-I, and methods of producing mature, biologically active ApoA-I. It has been discovered that it is possible to engineer mammalian host cells to express large quantities of mature ApoA-I, that is substantially free of both immature ApoA-I (proApoA-I) and truncated forms of ApoA-I generated by protease degradation. These results are surprising. First, host cell machinery for protein processing could be expected to be overwhelmed by

10, 12, or 15 g/L ApoA-I in culture. The culture can be of any scale, ranging from about 150 mL to about 500L, 1000L, 2000L, 5000L, 10,000L, 25,000L, or 50,000 L or more. In varying examples, the culture volume can range from 10L to 50L, from 50L to 100L, from 100L to 150L, from 150L to 200L, from 200L to 300L, from 300L to 500L, from 500L to 1000L, from 1000L to 1500L, from 1500L to 2000L, from 2000L to 3000L, from 3000L to 5000L, from 5000L to 7500L, from 7500L to 10,000L, from 10,000L to 20,000L, from 20,000L to 40,000L, from 30,000L to 50,000L. In some instances, the culture is a large scale culture, such as 15 L, 20 L, 25 L, 30 L, 50 L, 100 L, 200L, 300L, 500L, 1000L, 5000L, 10,000L, 15,000L, 20,000L, 25,000L, up to 50,000L or more.

[0063] The mammalian host cells of the present disclosure can be grown in culture. Thus, the present disclosure further provides a mammalian cell culture, comprising a plurality of mammalian host cells as described above or in Section 6.1.2 below. The cell culture can include one or more of the following features: (a) the culture (which is optionally a large scale batch culture of at least 10 liters, at least 20 liters, at least 30 liters, at least 50 liters, at least 100 liters, 300L, 500L, 1000L, 5000L, 10,000L, 15,000L, 20,000L, 25,000L, up to 50,000L or a or a continuous culture of at least 10 liters, at least 20 liters, at least 30 liters, at least 50 liters, at least 100 liters, 300L, 500L, 1000L, 5000L, or up to 10,000L) comprises at least about 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 g/L or more of mature ApoA-I protein comprising or consisting of an amino sequence corresponding to amino acids 25 to 267 of SEQ ID NO:1; (b) at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% of the protein in the culture medium is an ApoA-I protein lacking a signal sequence; (c) at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% of the protein in the culture medium is a mature ApoA-I protein lacking a signal sequence and a propeptide sequence; and (d) at least 75%, at least 80%, at least 85%, at least 90%, at least 95% of the mature ApoA-I is not truncated, oxidized, or deamidated.

[0064] The present disclosure also provides methods of producing mature, biologically active ApoA-I. Generally, the methods comprise culturing any of the mammalian host cells described above or in Section 6.1.2 under conditions in which ApoA-I is expressed and secreted. The method can further include a step of recovering from the supernatant of a cultured mammalian host cell, and optionally purifying, the mature, biologically active ApoA-I (such as by the methods disclosed in Sections 6.1.3 and 6.1.4 below).

[0065] ApoA-I obtained or obtainable by the methods described above can further be complexed with lipid to form a lipoprotein complex as described herein, and/or incorporated into pharmaceutical compositions in therapeutically effective amounts. The pharmaceutical compositions preferably are phosphate buffered solutions that also contain sucrose and/or mannitol as excipients.

[0066] It has further been discovered that, by purifying ApoA-I using a new combination of chromatography and filtration steps, large quantities of pure ApoA-I can be produced that contains levels of host cell proteins, host cell DNA, endotoxins and truncated forms of the protein that are low enough to confer one or more attributes, including reduced risk of side effects and low or no toxicity, rendering the protein particularly suitable for therapeutic uses. Preferably, ApoA-I purified by the methods described herein is produced recombinantly in mammalian cells and is secreted into the growth medium. Accordingly, the present disclosure relates to a method of purifying ApoA-I comprising the steps of: (a) contacting an ApoA-I containing solution with an anion exchange matrix under conditions such that the ApoA-I does not bind to the matrix; (b) filtering the ApoA-I containing solution obtained in step (a) through a membrane having a pore size sufficient to remove viruses or viral particles; (c) passing the filtrate obtained in step (b) through a first reverse phase chromatography column under conditions such that the ApoA-I binds to the matrix; (d) eluting from the first reverse phase chromatography matrix a first ApoA-I containing reverse phase eluate using a gradient of increasing concentrations of an organic solvent; (e) passing the first ApoA-I reverse phase eluate from step (d) through a second reverse phase chromatography column under conditions such that the ApoA-I binds to the matrix; and (f) eluting from the second reverse phase chromatography matrix a second ApoA-I containing reverse phase eluate using a gradient of increasing concentrations of an organic solvent. The order in which the steps are performed is not critical, for example, in an exemplary example the step of filtering through a membrane to remove viruses or viral particles is performed after step (f) above, rather than after step (a).

[0067] Also provided herein is a substantially pure ApoA-I product obtained or obtainable by the purification methods described herein in which the concentration of ApoA-I is at least 10 g/L. The substantially pure ApoA-I product produced by the purification methods described herein preferably comprises less than about 10 pg of host cell DNA per mg of ApoA-I, less than about 100 ng of host cell proteins per mg of ApoA-I, and/or less than 0.1 EU of endotoxin per mg of

[0070] Most preferably the lipid fraction contains at least one neutral phospholipid and, optionally, one or more negatively charged phospholipids. In lipoprotein complexes that include both neutral and negatively charged phospholipids, the neutral and negatively charged phospholipids can have fatty acid chains with the same or different number of carbons and the same or different degree of saturation. In some instances, the neutral and negatively charged phospholipids will have the same acyl tail, for example a C16:0, or palmitoyl, acyl chain.

[0071] When the lipid component of the complex comprises or consists of neutral and negatively charged phospholipids, the weight-to-weight (wt:wt) ratio of neutral to negatively charged phospholipid(s) is preferably in a wt:wt ratio ranging from about 99:1 to about 90:10, more preferably from about 99:1 to about 95:5, and most preferably from about 98:2 to about 96:4. In one example, the neutral phospholipid(s) and the negatively charged phospholipid(s) are present in a weight-to-weight (wt:wt) ratio of neutral phospholipid(s) to negatively charged phospholipid(s) of about 97:3.

[0072] The neutral phospholipid can be natural or synthetic. Preferably, the phospholipid is a sphingomyelin ("SM"), such as palmitoyl sphingomyelin, a phytosphingomyelin, a diphytosphingomyelin, a phosphosphingolipid, or a glycosphingolipid, optionally with saturated or mono-unsaturated fatty acids with chain lengths from 16 to 26 carbon atoms. SM can be from any source. For example, the SM can be obtained from milk, egg, brain, or made synthetically. In a specific example, the SM is obtained from chicken egg ("egg SM"). In another specific example, the SM is palmitoylsphingomyelin.

[0073] Any phospholipid that bears at least a partial negative charge at physiological pH can be used as the negatively charged phospholipid. Non-limiting examples include negatively charged forms, *e.g.*, salts, of phosphatidylinositol, a phosphatidylserine, a phosphatidylglycerol and a phosphatidic acid. In a specific example, the negatively charged phospholipid is 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)], or DPPG, a phosphatidylglycerol. Preferred salts include postassium and sodium salts.

[0074] The phospholipids used to manufacture the complexes of the disclosure are preferably at least 95% pure and are more preferably at least 99% pure, and/or have levels of oxidation levels under 4 meq O/kg, more preferably under 3 meq O/kg (*e.g.*, under 2 meq O/kg). The major

initial reaction product of oxygen and fatty acids is hydroperoxide. Using an iodometric method, it is possible to measure oxidation levels by assaying the presence of peroxide in a sample.

[0075] The lipoprotein complexes of the present disclosure preferably have ratios of apolipoprotein to lipid that result in more complete and homogeneous complex formation, as shown in the examples below. The lipoprotein complexes are characterized by an apolipoprotein fraction: lipid fraction molar ratio ranging from 1:80 to 1:120, from 1:100 to 1:115, or from 1:105 to 1:110, where the apolipoprotein is expressed in ApoA-I equivalents. In specific examples, the molar ratio of the apolipoprotein fraction: lipid fraction is 1:80 to 1:90 (*e.g.*, 1:82, 1:85 or 1:87), from 1:90 to 1:100 (*e.g.*, 1:95 or 1:98), from 1:100 to 1:110 (*e.g.*, 1:105 or 1:108).

[0076] In specific examples, particularly those in which egg SM is used as the neutral lipid, the weight ratio of the apolipoprotein fraction: lipid fraction ranges from about 1:2.7 to about 1:3 (*e.g.*, 1:2.7).

[0077] The lipoprotein complexes of the present disclosure can also be used as carriers to deliver hydrophobic, lipophilic or apolar active agents. For such applications, the lipid fraction can further include, or the lipoprotein complex can be loaded with, one or more hydrophobic, lipophilic or apolar active agents, including but not limited to fatty acids, drugs, nucleic acids, vitamins, and/or nutrients. Specific examples of active agents are described in Section 6.2.

[0078] The present disclosure also provides populations of the lipoprotein complexes. Typically:

- the populations contain a plurality of lipoprotein complexes, each comprising a protein fraction and a lipid fraction;
- the protein fractions contain a lipoprotein or lipoprotein analog as described above, in Section 6.1 or in Section 6.5.3; most preferably protein fraction comprises or consists essentially of lipoprotein (*e.g.*, ApoA-I protein) that is obtained or obtainable by the methods described in Section 6.1.2 and/or purified by the methods described in Section 6.1.4;
- the lipid fractions contain a lipid as described above, in Section 6.2 or in Section 6.5.2;

(l) the population contains no more than 200 ppm, 100 ppm, 50 ppm of a non-aqueous solvent;

(m) the population does not contain any detergent (*e.g.*, cholate);

(n) the population can be at least 80%, at least 85%, at least 90% or at least 95% homogeneous, as measured by percent of the population in a single peak in gel permeation chromatography;

(o) the population is in a composition in which at least 80%, at least 85%, at least 90%, at least 95% or at least 97% of the protein is in complexed form;

(p) no more than 5%, no more than 4%, no more than 3%, no more than 2% or no more than 1% of the lipid in said population is oxidized; and

(q) no more than 15%, no more than 10%, no more than 5%, no more than 4%, no more than 3%, no more than 2% or no more than 1% of methionine and/or tryptophan residues in said population are oxidized.

[0082] In specific examples, the population has features selected from the following groups:

Group I: features (a), (b) and (e) above;

Group II: features (c), (d) and (i) above;

Group III: features (f), (j), (e), (p) and (q) above;

Group IV: features (g), (n) and (o) above;

Group V: features (l) and (m) above; and

Group VI: feature (k) above.

[0083] In certain examples, the population is characterized by one or two features independently selected from each of Group I and Group IV; optionally, the population is characterized by three features independently selected from each of Group I and Group IV. The population can additionally be characterized by one, two or three features independently selected from each of Group II and/or Group III. The population can yet be further characterized one or two features independently selected from Group V and/or one feature independently selected from Group VI.

[0084] Certain lipid and protein components can form a plurality of different but homogeneous lipoprotein complexes. Accordingly, the present disclosure also provides compositions comprising two, three, or four populations of lipoprotein complexes comprising different amounts of apolipoprotein molecules (*e.g.*, two, three or four ApoA-I molecules or ApoA-I

equivalents). In an exemplary example, a composition comprises two lipoprotein complex populations, a first population comprising lipoprotein complexes having 2 ApoA-I or ApoA-I equivalents per lipoprotein complex, a second population comprising lipoprotein complexes having 3 or 4 ApoA-I or ApoA-I equivalents per lipoprotein complex and optionally a third population comprising lipoprotein complexes having 4 or 3 ApoA-I or ApoA-I equivalents per lipoproprotein complex, respectively.

[0085] The compositions comprising two or more populations of lipoprotein complexes preferably have low levels of uncomplexed lipoprotein and/or lipid. Accordingly, preferably no more than 15%, no more than 12%, than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%, no more than 2%, or no more than 1% of the lipid in the composition is in uncomplexed form and/or no more than 15%, no more than 12%, no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%, no more than 2%, or no more than 1% of the lipoprotein in the composition is in uncomplexed form.

[0086] The disclosure provides methods for making lipoprotein complexes. The methods are based, *inter alia*, on the discovery that subjecting a suspension containing uncomplexed lipids and lipid-binding proteins or peptides to thermal cycling conditions results in the formation of lipoprotein complexes with advantageous results relative to other methods, such those in which lipoprotein complexes are produced by incubating the components at a fixed temperature.

[0087] The present disclosure provides thermal-cycling methods for preparing lipoprotein complexes, such as those described in Section 6.5.1 to 6.5.4. The methods typically comprise subjecting a suspension comprising lipid particles (a "lipid component") and lipid-binding proteins or peptides (a "protein component") to a plurality of thermal cycles until most of the protein component is incorporated into lipoprotein complexes. The methods generally entail cycling the suspension between a temperature in a first, higher, temperature range and a temperature in a second, lower, temperature range until lipoprotein complexes are formed. The high and low temperature ranges of the thermocycling process are based on the phase transition temperatures of the lipid and protein components of the lipoprotein complexes. Alternatively, where the lipid component does not exhibit a defined or discrete phase transition, as could occur when using phospholipids having unsaturated fatty acid chains or a mixture of phospholipids, the

high and low temperature ranges of the thermocycling differ by at least about 20ºC, up to about 40ºC or even more. For example, in some examples, the low and high temperature ranges differ by 20°C-30°C, 20°C-40°C, 20°C-50°C, 30°C-40°C, 30°C-50°C, 25°C-45°C, 35°C-55°C.

[0088] For a lipid, the phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. Lipoprotein complexes are typically formed in the art by incubating lipid particles and apolipoproteins at temperatures near the transition temperature of the particular lipid or mixture of lipids used. The phase transition temperature of the lipid component (which can be determined by calorimetry) +/-12°C, more preferably +/- 10°C, represents the "low" temperature range in the methods of the disclosure. In certain examples, the low temperature range is +/-3°C, +/-5°C, or +/-8°C of the phase transition temperature of the lipid component. In one specific example, the low temperature range is from no less than 5°C or no less than 10°C below to 5°C above the phase transition temperature of the lipid component.

[0089] For a protein, the phase transition temperature involves a change from the tertiary structure into the secondary structure. The phase transition temperature of the protein component +/-12°C, more preferably +/- 10°C, represents the "high" temperature range in the methods of the disclosure. In specific examples, the high temperature range is +/-3°C, +/-5°C, or +/-8°C of the phase transition temperature of the protein component. In one specific example, the low temperature range is from 10°C below to no more than 5°C, no more than 10°C, or no more than 15°C above the phase transition temperature of the protein component.

[0090] The lipid component of the starting suspension, *i.e.*, a suspension that has not yet been subjected to thermal cycling, preferably comprises particles of lipids, *e.g.*, is predominantly composed of lipids that are not complexed to lipid-binding proteins. The make-up of the lipid component is generally as described in Section 6.5.2 below.

[0091] The protein component of the starting suspension preferably contains lipid-binding peptides and/or proteins that are uncomplexed to lipids, or are combined with lipids in protein/peptide to lipid ratio that is at least 5-fold greater (*e.g.*, at least 5-fold, at least 10-fold or at least 20-fold greater) than the protein/peptide to lipid ratio in the desired complex. The make-up of the protein component is generally as described in Section 6.1 and in Section 6.5.3 below.

The protein component is preferably made according to the methods described in Section 6.1.2 and/or purified according to the methods described in Section 6.1.3 or 6.1.4.

[0092] In the methods of the disclosure, a suspension containing the protein component and lipid component is typically thermally cycled between the high temperature range and the low temperature range, preferably starting at a temperature in the high temperature range, until lipoprotein complexes are formed. Using suitable quantities of lipid and protein components (*e.g.*, as described in U.S. Patent Publication No. 2006-0217312 A1, the contents of which are incorporated herein by reference in their entireties), substantially complete complexation of the lipid and protein components can be reached after several cycles. Further details of the protein and lipid stoichiometry suitable for thermal cycling methods are described in Section 6.5.4.

[0093] The complexes produced by the methods are typically supramolecular assemblies shaped as micelles, vesicles, spherical or discoidal particles in which the protein component is physically bound to the lipid component at a specific stoichiometric range and with a homogenous size distribution. The present methods advantageously result in substantially complete complexation of the lipids and/or proteins in the starting suspension, resulting in a composition that is substantially without free lipids and/or free protein, as observed by separation methods such as chromatography. Thus, the methods of the disclosure can be performed in the absence of a purification step.

[0094] The lipid component in the starting suspension is typically in particle form. It is preferred that the particles are predominantly at least 45 nm, at least 50 nm, at least 55 nm or at least 60 nm in size ranging up to 65 nm, up to 70 nm, up to 75 nm, up to 80 nm in size, up to 100 nm, up to 120 nm, up to 150 nm, up to 200 nm, up to 250 nm, up to 300 nm, up to 500 nm, for example, in the 45 nm to 100 nm or 45 to 250 nm size range, more preferably in the 50 nm to 90 nm size range, and most preferably in the 55 nm to 75 nm size range. In a preferred example, the lipid particles are predominantly composed of egg-sphingomyelin and are 55 to 75 nm in size. In another preferred example, the lipid particles are predominantly composed of one or more synthetic sphingomyelin (*e.g.*, palmitoylsphingomyelin or phytosphingomyelin) and are 175 nm to 250 nm in size. In yet another preferred example, the lipid particles are predominantly composed of one or more synthetic lipids (*e.g.*, palmitoyl sphingomyelin or phytosphingomyelin) and are 250 nm to 1000 nm in size. In yet another preferred example,

the lipid particles are predominantly composed of one or more synthetic lipids (*e.g.*, palmitoyl sphingomyelin or phytosphingomyelin) and are 1000 nm to 4000 nm in size. The sizes referred to herein are zeta (Z) average sizes as determined by dynamic light scattering. High pressure homogenization, for example microfluidization, advantageously produces lipid particles of suitable sizes. Other methods for forming particles are disclosed in Section 6.5.2 below, and can be used as an alternative to homogenization. If such methods produce particles outside the preferred size ranges, the particles can be subject to size filtration to obtain particles of a suitable size.

[0095] The methods of preparing lipoprotein complexes described herein advantageously produce complexes that are homogeneous in their size distribution, circumventing the need for size fractionation. Moreover, the methods of the disclosure result in substantially complete incorporation (*e.g.*, at least 95%, at least 96%, at least 97%, at least 98% or at least 99%) of the starting protein into lipoprotein particles. Accordingly, the disclosure provides a composition comprising lipoprotein complexes and in which at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of the lipid-binding protein in the composition is complexed to lipid, for example as determined using gel permeation chromatography. In specific examples, the disclosure provides a composition comprising lipoprotein complexes which have 4 nm to 20 nm zeta average size, *e.g.*, a 4 nm to 20 nm zeta average size, a 4 nm to 15 nm zeta average size, a 4 nm to 12 nm zeta average size, a 5 nm to 15 nm zeta average size, a 5 nm to 12 nm zeta average size, a 5 nm to 10 nm zeta average size, a 5 nm to 20 nm zeta average size, a 6 nm to 15 nm zeta average size, or an 8 nm to 12 nm zeta average size, and in which at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of the lipid-binding protein in the composition is complexed to lipid, for example as determined using gel permeation chromatography. Subjecting lipid particles to thermal cycling with a lipoprotein according to the methods described herein typically results in a population of lipoprotein particles of 4 nm to 15 nm in size, for example a population of lipoprotein particles of 6 nm to 15 nm in size, 5 nm to 12 nm in size, or 8 nm to 12 nm in size. The size of the lipid particles subjected to thermal cycling can range from 50 nm to 250 nm. In a preferred example, the lipid particles are predominantly composed of egg-sphingomyelin and are 55 to 75 nm in size. In another preferred example, the lipid particles are predominantly composed of one or more synthetic sphingomyelin (*e.g.*, phytosphingomyelin) and are 175 nm to 250 nm in size.

[0096] One or more steps in the methods of preparing lipoprotein complexes can be carried out under an inert gas. Doing so can reduce or prevent oxidation of apolipoproteins and/or lipids, thereby reducing risk of side effects, such as liver damage. Suitable inert gases include nitrogen, helium, and argon.

[0097] The lipoprotein complexes obtained or obtainable by the methods described above are particularly suited to therapeutic uses because no further purification step is needed after the complexes are formed.

[0098] The present disclosure also provides populations of lipoprotein complexes, and pharmaceutical compositions comprising lipoprotein complexes or populations thereof, as described herein, and may optionally include one or more pharmaceutically acceptable carriers, excipients and/or diluents. In some examples, the pharmaceutical compositions are packaged in unit dosage amounts suitable for administration. For example, in some examples, the compositions comprise unit dosage amounts of dried (for example lyophilized) lipoprotein complexes packaged in sealed vials. Such compositions are suitable for reconstitution with water, physiological solution (such as saline) or buffer, and administration via injection. Such compositions may optionally include one or more anti-caking and/or anti-agglomerating agents to facilitate reconstitution of the charged complexes, or one or more buffering agents, isotonicity agents (*e.g.*, sucrose and/or mannitol), sugars or salts (*e.g.*, sodium chloride) designed to adjust the pH, osmolality and/or salinity of the reconstituted suspension. The populations of lipoprotein complexes and/or pharmaceutical compositions described above can be manufactured under conditions that minimize oxidation, thereby reducing the risk of side effects, such as liver damage, caused by oxidized products. For example, pharmaceutical compositions can be manufactured under an inert gas, such as nitrogen, helium, or argon.

[0099] For commercial applications, it is useful to make large-scale preparations of the lipoprotein complexes and pharmaceutical compositions. Accordingly, the present disclosure also provides a preparation of at least 1L, 2L, 5L or 10L and up to 15L, 20L, 30L, 50L, or more (*e.g.*, a preparation of 5L to 30L, 10L to 15L, or 30L to 50L) comprising lipoprotein complexes in an amount sufficient to achieve a concentration of lipid-binding protein of at least about 3 mg/mL, at least about 4 mg/mL, or at least about 5 mg/mL, and up to about 10 mg/mL, about 15 mg/mL, or about 20 mg/mL, preferably ranging from about 8 mg/mL to about 12 mg/mL, most

preferably about 8 mg/mL. In a specific example, the preparation has a volume of 15L to 25L and contains about 100 g to about 250 g of ApoA-I. In another specific example, the preparation has a volume of 30L to 50L and contains about 240 g to about 780 g of ApoA-I.

[0100] The lipoprotein complexes described herein are useful to treat dyslipidemic disorders in animals, most preferably in humans. Such conditions include, but are not limited to hyperlipidemia, and especially hypercholesterolemia (including heterozygous and homozygous familial hypercholesterolemia), and cardiovascular disease such as atherosclerosis (including treatment and prevention of atherosclerosis) and the myriad clinical manifestations of atherosclerosis, such as, for example, stroke, ischemic stroke, transient ischemic attack, myocardial infarction, acute coronary syndrome, angina pectoris, intermittent claudication, critical limb ischemia, valve stenosis, and atrial valve sclerosis; restenosis (*e.g.*, preventing or treating atherosclerotic plaques which develop as a consequence of medical procedures such as balloon angioplasty); and other disorders, such as endotoxemia, which often results in septic shock.

[0101] Lipoprotein complexes and compositions as described herein have been found to effect and/or facilitate cholesterol efflux when administered at doses lower than those used for other lipoprotein complexes in studies to date. See, *e.g.*, Spieker *et al.*, 2002, Circulation 105:1399-1402 (using a dose of 80 mg/kg); Nissen *et al.*, 2003, JAMA 290:2292-2300 (using doses of 15 mg/ kg or 40 mg/kg); Tardif *et al.*, 2007 JAMA 297:1675-1682 (using doses of 40 mg/kg to 80 mg/kg), using doses that range from 15 mg/kg to 80 mg/kg. Additionally, the lipoprotein complexes as described herein have been found to have reduced side effects. As shown in the examples below, it has been discovered that lipoprotein complexes of the disclosure effectively mobilize cholesterol at doses as low as 2 mg/kg, and, in contrast lipoprotein complexes previously administered to human patients, do not significantly raise the levels of triglycerides, VLDL, and liver enzymes such as transaminases (*c.f.,* Nanjee *et al.*, 1999, Arterioscler. Vasc. Throm. Biol. 19:979-89). Moreoever, the reduced side effects are observed even as doses are increased up to about 15 mg/kg (lack of triglyceride elevation) or even as high as 45 mg/kg (lack of transaminase increase) in normal subjects with normal liver and/or kidney function. Thus, the ability of the complexes of the present disclosure to be administered without side effects is preferably assessed in an indivual with normal liver function, normal kidney function, or both.

disorder. The lipoprotein complex can be administered at a dose ranging from about 0.25 mg/kg ApoA-I equivalents to about 45 mg/kg, *e.g.*, a dose of about 0.5 mg/kg to about 30 mg/kg or about 1 mg/kg ApoA-I equivalents up to about 15 mg/kg ApoA-I equivalents per injection. The dose can further be tailored to the individual being treated by selecting a dose that minimizes the increase in the level of triglycerides, VLDL-cholesterol and/or VLDL-triglyceride. In specific examples, the dose is about 3 mg/kg, about 6 mg/kg, or about 12 mg/kg.

[0104] The methods further comprise administering the lipoprotein complex at an interval ranging from 5 to 14 days, or from 6 to 12 days, such as an interval of one or two weeks. The methods can further comprise administering the lipoprotein complex 4, 5, 6, 7, 8, 9, 10, 11, 12, or up to 52 times at any of the intervals described above, and preferably at an interval of one week. For chronic conditions, more than 52 administrations can be carried out. For example, in one example, the lipoprotein complex is administered six times, with an interval of 1 week between each administration. Optionally, the methods can be preceeded by an initial induction phase where the lipoprotein complex is administered more frequently.

[0105] Complexes and/or pharmaceutical compositions thereof, can be administered parenterally, *e.g.*, intravenously. Intravenous administration can be done as an infusion over a period of time ranging from about 1 to about 24 hours, or about 1 to 4 hours, about 0.5 to 2 hours, or about 1 hour.

[0106] The examples below show small increases in triglyceride levels following administration of doses 30 mg/kg and 45 mg/kg, which is explained by the increase in VLDL and LDL resulting from the high degree of cholesterol mobilization. Those parameters can be controlled during the treatment, as they are routinely measured in hospital laboratories with standard lipid panels. Based on examples below, the dose selection can be achieved to minimize the increase in the level of triglycerides and VLDL-cholesterol and VLDL-triglyceride dependent on the patient reaction to the medicine, which allows a personalized-type medicine.

[0107] The complexes and/or compositions can be administered alone (as monotherapy) or, alternatively, they can be adjunctively administered with other therapeutic agents useful for treating and/or preventing dyslipidemia and/or its associated conditions, diseases and/or disorders. Non-limiting examples of therapeutic agents with which the negatively charged lipoprotein complexes and compositions described herein can be adjunctively administered

What Is Claimed Is:

1. A composition comprising a population of lipoprotein complexes, each comprising

(a) a lipid fraction comprising at least one neutral lipid and at least one negatively charged lipid;

(b) an apolipoprotein fraction consisting essentially of an Apolipoprotein A-I ("ApoA-I"); and

(c) a hydrophobic, lipophilic or apolar active agent, or a label;

wherein

(i) at least 95% by weight of the apolipoprotein in the composition is in complexed form;

(ii) at least 95% by weight of the lipid in the composition is in complexed form;

(iii) no more than 5% or the methionine residues in the apolipoprotein are oxidized;

(iv) the size distribution of the lipoprotein complexes is at least 95% homogeneous, as reflected by a single peak in gel permeation chromatography; and

(v) the composition does not contain any detergent.

2. The composition of claim 1, which comprises a hydrophobic, lipophilic or apolar active agent.

3. The composition of claim 2, wherein the active agent is hydrophobic.

4. The composition claim 2, wherein the active agent is lipophilic.

5. The composition of claim 2, wherein the active agent is apolar.

6. The composition of claim 2, wherein the active agent comprises a fatty acid, a drug, a nucleic acid, a vitamin, a nutrient, or a combination thereof.

7. The composition of claim 2, wherein the active agent comprises an analgesic, an anti-inflammatory agent, an anthelmintic, an anti-arrhythmic agent, an anti-bacterial agent, an anti-viral agent, an anti-coagulant, an anti-depressant, an anti-diabetic, an anti-epileptic, an anti-fungal agent, an anti-gout agent, an anti-hypertensive agent, an anti-malarial, an anti-migraine agent, an anti-muscarinic agent, an anti-neoplastic agent, an erectile dysfunction improvement agent, an immunosuppressant, an anti-protozoal agent, an anti-thyroid agent, an anxiolytic agent, a sedative, a hypnotic, a neuroleptic, a β-blocker, a cardiac inotropic agent, a corticosteroid, a diuretic, an anti-parkinsonian agent, a gastro-intestinal agent, a histamine

receptor antagonist, a keratolytic, a lipid regulating agent, an anti-anginal agent, a COX-2 inhibitor, a leukotriene inhibitor, a macrolide, a muscle relaxant, a nutritional agent, a nucleic acid, an opioid analgesic, a protease inhibitor, a sex hormone, a stimulant, a muscle relaxant, an anti-osteoporosis agent, an anti-obesity agent, a cognition enhancer, an anti-urinary incontinence agent, a nutritional oil, an anti-benign prostate hypertrophy agent, an essential fatty acid, a non-essential fatty acid, or a combination thereof.

8. The composition of claim 7, wherein the active agent comprises a nucleic acid.

9. The composition of claim 8, wherein the nucleic acid is a small interfering RNA.

10. The composition of claim 2, wherein the active agent comprises acetretin, albendazole, albuterol, aminoglutethimide, amiodarone, amlodipine, amphetamine, amphotericin B, atorvastatin, atovaquone, azithromycin, baclofen, beclomethasone, benezepril, benzonatate, betamethasone, bicalutanide, budesonide, bupropion, busulfan, butenafine, calcifediol, calcipotriene, calcitriol, camptothecin, candesartan, capsaicin, carbamezepine, carotenes, celecoxib, cerivastatin, cetirizine, chlorpheniramine, cholecalciferol, cilostazol, cimetidine, cinnarizine, ciprofloxacin, cisapride, clarithromycin, clemastine, clomiphene, clomipramine, clopidogrel, codeine, coenzyme Q10, cyclobenzaprine, cyclosporin, danazol, dantrolene, dexchlorpheniramine, diclofenac, dicoumarol, digoxin, dehydroepiandrosterone, dihydroergotamine, dihydrotachysterol, dirithromycin, donezepil, efavirenz, eposartan, ergocalciferol, ergotamine, essential fatty acid sources, etodolac, etoposide, famotidine, fenofibrate, fentanyl, fexofenadine, finasteride, fluconazole, flurbiprofen, fluvastatin, fosphenytoin, frovatriptan, furazolidone, gabapentin, gemfibrozil, glibenclamide, glipizide, glyburide, glimepiride, griseofulvin, halofantrine, ibuprofen, irbesartan, irinotecan, isosorbide dinitrate, isotretinoin, itraconazole, ivermectin, ketoconazole, ketorolac, lamotrigine, lansoprazole, leflunomide, lisinopril, loperamide, loratadine, lovastatin, L-thryroxine, lutein, lycopene, medroxyprogesterone, mifepristone, mefloquine, megestrol acetate, methadone, methoxsalen, metronidazole, miconazole, midazolam, miglitol, minoxidil, mitoxantrone, montelukast, nabumetone, nalbuphine, naratriptan, nelfinavir, nifedipine, nilsolidipine, nilutanide, nitrofurantoin, nizatidine, omeprazole, oprevelkin, oestradiol, oxaprozin, paclitaxel, paracalcitol, paroxetine, pentazocine, pioglitazone, pizofetin, pravastatin, prednisolone, probucol, progesterone, pseudoephedrine, pyridostigmine, rabeprazole, raloxifene, rofecoxib, repaglinide, rifabutine,

rifapentine, rimexolone, ritanovir, rizatriptan, rosiglitazone, saquinavir, sertraline, sibutramine, sildenafil citrate, simvastatin, sirolimus, spironolactone, sumatriptan, tacrine, tacrolimus, tamoxifen, tamsulosin, targretin, tazarotene, telmisartan, teniposide, terbinafine, terazosin, tetrahydrocannabinol, tiagabine, ticlopidine, tirofibran, tizanidine, topiramate, topotecan, toremifene, tramadol, tretinoin, troglitazone, trovafloxacin, ubidecarenone, valsartan, venlafaxine, verteporfin, vigabatrin, vitamin A, vitamin D, vitamin E, vitamin K, zafirlukast, zileuton, zolmitriptan, zolpidem, zopiclone, or a combination thereof.

11. The composition of claim 2, wherein the active agent comprises a salt of acetretin, albendazole, albuterol, aminoglutethimide, amiodarone, amlodipine, amphetamine, amphotericin B, atorvastatin, atovaquone, azithromycin, baclofen, beclomethasone, benezepril, benzonatate, betamethasone, bicalutanide, budesonide, bupropion, busulfan, butenafine, calcifediol, calcipotriene, calcitriol, camptothecin, candesartan, capsaicin, carbamezepine, carotenes, celecoxib, cerivastatin, cetirizine, chlorpheniramine, cholecalciferol, cilostazol, cimetidine, cinnarizine, ciprofloxacin, cisapride, clarithromycin, clemastine, clomiphene, clomipramine, clopidogrel, codeine, coenzyme Q10, cyclobenzaprine, cyclosporin, danazol, dantrolene, dexchlorpheniramine, diclofenac, dicoumarol, digoxin, dehydroepiandrosterone, dihydroergotamine, dihydrotachysterol, dirithromycin, donezepil, efavirenz, eposartan, ergocalciferol, ergotamine, essential fatty acid sources, etodolac, etoposide, famotidine, fenofibrate, fentanyl, fexofenadine, finasteride, fluconazole, flurbiprofen, fluvastatin, fosphenytoin, frovatriptan, furazolidone, gabapentin, gemfibrozil, glibenclamide, glipizide, glyburide, glimepiride, griseofulvin, halofantrine, ibuprofen, irbesartan, irinotecan, isosorbide dinitrate, isotretinoin, itraconazole, ivermectin, ketoconazole, ketorolac, lamotrigine, lansoprazole, leflunomide, lisinopril, loperamide, loratadine, lovastatin, L-thryroxine, lutein, lycopene, medroxyprogesterone, mifepristone, mefloquine, megestrol acetate, methadone, methoxsalen, metronidazole, miconazole, midazolam, miglitol, minoxidil, mitoxantrone, montelukast, nabumetone, nalbuphine, naratriptan, nelfinavir, nifedipine, nilsolidipine, nilutanide, nitrofurantoin, nizatidine, omeprazole, oprevelkin, oestradiol, oxaprozin, paclitaxel, paracalcitol, paroxetine, pentazocine, pioglitazone, pizofetin, pravastatin, prednisolone, probucol, progesterone, pseudoephedrine, pyridostigmine, rabeprazole, raloxifene, rofecoxib, repaglinide, rifabutine, rifapentine, rimexolone, ritanovir, rizatriptan, rosiglitazone, saquinavir, sertraline, sibutramine, sildenafil citrate, simvastatin, sirolimus, spironolactone, sumatriptan, tacrine,

tacrolimus, tamoxifen, tamsulosin, targretin, tazarotene, telmisartan, teniposide, terbinafine, terazosin, tetrahydrocannabinol, tiagabine, ticlopidine, tirofibran, tizanidine, topiramate, topotecan, toremifene, tramadol, tretinoin, troglitazone, trovafloxacin, ubidecarenone, valsartan, venlafaxine, verteporfin, vigabatrin, vitamin A, vitamin D, vitamin E, vitamin K, zafirlukast, zileuton, zolmitriptan, zolpidem, or zopiclone, or a combination thereof.

12. The composition of claim 1, which comprises a label.

13. The composition of claim 12, wherein the ApoA-I is labeled with the label.

14. The composition of claim 12, wherein one or more of the lipids in the lipid fraction are labeled with the label.

15. The composition of claim 12, wherein the label comprises a radiolabel.

16. The composition of claim 15, wherein the radiolabel comprises ^{125}I.

17. The composition of claim 15, wherein the radiolabel comprises ^{14}C.

18. The composition of claim 15, wherein the radiolabel comprises ^{3}H.

19. The composition of claim 12, wherein the label comprises a fluorescent label.

20. The composition of claim 12, wherein the label comprises an enzyme label.

21. The composition of claim 12, wherein the label comprises a dye.

22. The composition of any one of claims 1 to 21, wherein the size distribution of the lipoprotein complexes is at least 97% homogeneous, as reflected by a single peak in gel permeation chromatography.

23. The composition of any one of claims 1 to 22, wherein at least 98% by weight of the ApoA-I in the composition is untruncated ApoA-I.

24. The composition of any one of claims 1 to 23, wherein

at least 95% of the lipoprotein complexes are in the form of particles of 4 nm to 15 nm in size, 6 nm to 15 nm in size, 4 nm to 12 nm in size, 6 nm to 12 nm in size, or 8 nm to 12 in size as measured by gel permeation chromatography ("GPC") or dynamic light scattering ("DLS").

25. The composition of any one of claims 1 to 24, wherein at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% by weight of the ApoA-I in said composition is in mature form.

26. The composition of any one of claims 1 to 24, wherein no more than 25%, no more than 20%, no more than 15%, no more than 10% or no more than 5% by weight of ApoA-I in said composition is in immature form.

27. The composition of any one of claims 1 to 26, wherein

no more than 5%, no more than 3%, no more than 2% or no more than 1% of each of methionine 112 and methionine 148 in said ApoA-I in said composition is oxidized.

28. The composition of any one of claims 1 to 27, wherein no more than 15%, no more than 10%, no more than 5%, no more than 4%, no more than 3%, no more than 2% or no more than 1% of the amino acids of the ApoA-I in said composition is deamidated.

29. The composition of any one of claims 1 to 28, wherein the composition contains no more than 1 EU, no more than 0.5 EU, no more than 0.3 EU or no more than 0.1 EU of endotoxin per milligram of ApoA-I.

30. The composition of any one of claims 1 to 29, wherein the composition contains no more than 100 picograms, no more than 50 picograms, no more than 25 picograms, no more than 10 picograms or no more than 5 picograms host cell DNA per milligram of ApoA-I.

31. The composition of any one of claims 1 to 30, wherein the composition contains no more than 500 nanograms, no more than 200 nanograms, no more than 100 nanograms, no more than 50 nanograms, or no more than 20 nanograms host cell protein per milligram of ApoA-I.

32. The composition of any one of claims 1 to 31, wherein the composition contains no more than 200 pm, no more than 100 ppm, or no more than 50 ppm of a non-aqueous solvent.

33. The composition of any one of claims 1 to 32 in which no more than 15%, or no more than 10%, no more than 5% or no more than 2% by weight of the lipid in the lipid fraction in said complexes is cholesterol.

34. The composition of any one of claims 1 to 33, wherein said ApoA-I is a human ApoA-I protein.

35. The composition of any one of claims 1 to 34, wherein said ApoA-I is a recombinant ApoA-I.

36. The composition of any one of claims 1 to 35, wherein said ApoA-I has at least 95% sequence identity to a protein corresponding to amino acids 25 to 267 of SEQ ID NO: 1.

37. The composition of any one of claims 1 to 36, wherein said neutral lipid consists essentially of sphingomyelin.

38. The composition of any one of claims 1 to 37, wherein the negatively charged lipid consists essentially of 1,2-dipalmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] ("DPPG").

39. The composition of any one of claims 1 to 38, wherein the molar ratio of the components of the negatively charged lipid to the neutral lipid to the ApoA-I in the composition is 2-6:90-120:1.

40. The composition of any one of claims 1 to 39, wherein said lipid fraction consists essentially of 95 to 99 weight % neutral phospholipid and 1 to 5 weight % negatively charged phospholipid.

41. The composition claim 40, wherein said lipid fraction consists essentially of 96 to 98 weight % neutral phospholipid and 2 to 4 weight % negatively charged phospholipid.

42. The composition of claim 41, wherein said lipid fraction consists essentially of 97 weight % neutral phospholipid and 3 weight % negatively charged phospholipid.

43. The composition of claim 42, wherein the neutral lipid is natural sphingomyelin or synthetic sphingomyelin, optionally wherein the lipid has a peroxide value of less than 5 meq O/kg, less than 4 meq O/kg, less than 3 meq O/kg, or less than 2 meq O/kg.

44. The composition of claim 43 in which the sphingomyelin is egg-sphingomyelin.

45. The composition of any one of claims 42 to 44, wherein the negatively charged phospholipid comprises phosphatidylglycerol.

46. The composition of claim 45 in which the negatively charged phospholipid comprises or consists of a salt of 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DPPG).

47. The composition of any one of claims 1 to 46 which has an apolipoprotein fraction-to-phospholipid fraction ratio ranging from 1:2 to about 1:3 by weight.

48. The composition of any one of claims 1 to 47, produced by a process that does not comprise a size fractionation step for purifying the lipoprotein complexes.

49. The composition according to any one of claims 1 to 48, in which at least 97% of the lipoprotein is in complexed form.

50. The composition according to any one of claims 1 to 49, in which at least 97%, at least 98%, at least 99% or 100% of the lipid is in complexed form.

51. A pharmaceutical composition comprising or consisting essentially of a composition according to any one of claims 1 to 50, and one or more pharmaceutically acceptable carriers, diluents and/or excipients.

52. A unit dosage form comprising a therapeutically effective amount of a pharmaceutical composition of claim 51.

53. Use of a composition according to any one of claims 1 to 50 in the manufacture of a medicament.

54. Use of a composition of lipoprotein complexes according to any one of claims 12 to 21 in the manufacture of a diagnostic agent for use in an imaging procedure.

55. The use of claim 54, wherein the imaging procedure comprises a CAT scan.

56. The use of claim 54, wherein the imaging procedure comprises a MRI scan.