METHODS FOR IMPROVING THE STRUCTURE AND FUNCTION OF ARTERIOLES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of and priority to USSN 60/634,318, filed on December 6, 2004, which is incorporated herein by reference in its entirety for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This work was supported, in part, by Grant No: HL30568 from the National Heart Blood Lung Institute of the National Institutes of Health. The Government of the United States of America may have certain rights in this invention.

FIELD OF THE INVENTION

[0003] This invention pertains to the field of vascular medicine. In particular, this invention provides novel methods of improving arteriole structure and function and thereby mitigating pathologies associated with impaired circulation.

BACKGROUND OF THE INVENTION

[0004] Arterioles, as described herein, are vessels in the arterial circulation (as opposed to the venous circulation) with a diameter (after perfusion fixation or in vivo) of < 200 μM. There is an extensive literature on changes in arterioles associated with hypertension, aging, subarachnoid hemorrhage, multi-infarct dementia, Alzheimer's disease, and chronic kidney disease as well as in other conditions. It appears that a variety of pathological conditions can result in thickening of these arterioles accompanied by a loss of normal vasoreactivity.

[0005] The normal response to a fall in blood pressure is vasodilation to allow resistance to decrease and maintain forward flow. Failure to be able to vasodilate arterioles in the face of a fall in blood pressure may result in a fall in blood flow to the target organ. If the target organ is the brain, the fall in forward blood flow can result in an infarct in the region of the arterioles involved.

[0006] Since the arterioles are so small they ordinarily serve a small area of the brain and therefore the infarct is small and may only be perceived as a "Senior Moment". However, we believe the accumulation of such a series of insults over time may lead to significant end organ damage.

[0007] The major treatments for prevention of such end organ damage include blood pressure control and control of plasma glucose and lipid levels. The use of certain agents (e.g., statins) to improve the structure and function of small to large arteries has been known and assumed to relate to the ability of these agents to lower cholesterol and reduce inflammatory cell infiltration into the arteries (*see, e.g.,* Schonbeck *et al.* (2004) *Circulation* 109(21 Suppl 1): II18-26).

SUMMARY OF THE INVENTION

[0008] The present invention relates to the unexpected finding that vessels smaller than even the smallest arteries (i.e. arterioles) thicken, become dysfunctional and cause end organ damage to tissues as diverse as the brain and the kidney. This invention provides a method to improve the structure and function of arterioles and preserve the function of end organs such as the brain and kidney.

[0009] Thus, in certain embodiments, this invention provides methods of improving arteriole structure and/or function. The methods typically involve administering to a mammal in need thereof one or more of the active agents described herein typically, in a dosage sufficient to improve arteriole structure or function. In various embodiments the arteriole is an arteriole in kidney and/or brain, and/or in alveoli. The mammal can be a human, *e.g.,* a patient in need of such therapeutic or prophylactic treatment or a non-human. Thus, both medical and veterinary applications are considered. In various embodiments the mammal is a human diagnosed as having memory loss or impaired learning and/or impaired kidney function, and/or impaired alveolar (lung) function. In certain embodiments the mammal is a human not diagnosed as having or at risk for atherosclerosis and/or associated pathology and/or not under treatment for atherosclerosis and/or associated pathology. In various embodiments the active agent (*e.g.,* peptide and/or peptide mimetic and/or lipid) is in a unit dosage formulation. In various embodiments the active agent(s) are formulated for administration by a route selected from the group consisting of oral administration, nasal administration, rectal administration, intraperitoneal injection, and intravascular injection,

subcutaneous injection, transcutaneous administration, and intramuscular injection. In various embodiments the method of administration is by a route selected from the group consisting of oral administration, nasal administration, rectal administration, intraperitoneal injection, and intravascular injection, subcutaneous injection, transcutaneous administration, and intramuscular injection. In certain embodiments the active agent(s) are selected from the group consisting of D4F, L4F, reverse D4F, reverse L4F, circularly permuted D4F, circularly permuted L4F, circularly permuted reverse L4F, circularly permuted reverse D4F, and DMPC. In various embodiments the active agent(s) are provided in combination with a pharmaceutically acceptable excipient.

[0010] In certain embodiments this invention also provides an active agent as described herein for use in the prophylaxis or treatment of arterioles having impaired structure or function. Also provided is the use of an active agent as described herein for the manufacture of a medicament for the prophylaxis or treatment of arterioles having impaired structure or function.

[0011] Also provided are kits for the treatment of a condition characterized by abnormal arteriole structure or function. The kits typically comprise one or more containers containing the active agent(s) described herein and instructional materials teaching the use of the active agent(s) in the treatment of a condition characterized by abnormal arteriole structure or function. . In various embodiments the active agent (*e.g.*, peptide and/or peptide mimetic and/or lipid) is in a unit dosage formulation. In various embodiments the active agent(s) are formulated for administration by a route selected from the group consisting of oral administration, nasal administration, rectal administration, intraperitoneal injection, and intravascular injection, subcutaneous injection, transcutaneous administration, and intramuscular injection. In certain embodiments the active agent(s) are selected from the group consisting of D4F, L4F, reverse D4F, reverse L4F, circularly permuted D4F, circularly permuted L4F, circularly permuted reverse L4F, circularly permuted reverse D4F, and DMPC. In various embodiments the active agent(s) are provided in combination with a pharmaceutically acceptable excipient.

DEFINITIONS

[0012] The terms "isolated", "purified", or "biologically pure" when referring to an isolated polypeptide refer to material that is substantially or essentially free from

components that normally accompany it as found in its native state. With respect to nucleic acids and/or polypeptides the term can refer to nucleic acids or polypeptides that are no longer flanked by the sequences typically flanking them in nature. Chemically synthesized polypeptides are "isolated" because they are not found in a native state (*e.g.* in blood, serum, *etc.*). In certain embodiments, the term "isolated" indicates that the polypeptide is not found in nature.

[0013] The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residues is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

[0014] The term "an amphipathic helical peptide" refers to a peptide comprising at least one amphipathic helix (amphipathic helical domain). Certain amphipathic helical peptides of this invention can comprise two or more (*e.g.* 3, 4, 5, *etc.*) amphipathic helices.

[0015] The term " class A amphipathic helix" refers to a protein structure that forms an α-helix producing a segregation of a polar and nonpolar faces with the positively charged residues residing at the polar-nonpolar interface and the negatively charged residues residing at the center of the polar face (*see, e.g.,* " Segrest *et al.* (1990) *Proteins: Structure, Function, and Genetics* 8: 103-117).

[0016] "Apolipoprotein J" (apo J) is known by a variety of names including clusterin, TRPM2, GP80, and SP 40,40 (Fritz (1995) Pp 112 In: *Clusterin: Role in Vertebrate Development, Function, and Adaptation* (Harmony JAK Ed.), R.G. Landes, Georgetown, TX,). It was first described as a heterodimeric glycoprotein and a component of the secreted proteins of cultured rat Sertoli cells (Kissinger *et al.* (1982) *Biol Reprod*; 27:233240). The translated product is a single-chain precursor protein that undergoes intracellular cleavage into a disulfide-linked 34kDa αsubunit and a 47 kDa βsubunit Collard and Griswold (187) *Biochem.*, 26: 3297-3303). It has been associated with cellular injury, lipid transport, apoptosis and it may be involved in clearance of cellular debris caused by cell injury or death. Clusterin has been shown to bind to a variety of molecules with high affinity including lipids, peptides, and proteins and the hydrophobic probe 1-anilino-8-naphthalenesulfonate (Bailey *et al.* (2001) *Biochem.*, 40: 11828-11840).

[0017] The class G amphipathic helix is found in globular proteins, and thus, the name class G. The feature of this class of amphipathic helix is that it possesses a random distribution of positively charged and negatively charged residues on the polar face with a narrow nonpolar face. Because of the narrow nonpolar face this class does not readily associate with phospholipid (*see,* Segrest *et al.* (1990) *Proteins: Structure, Function, and Genetics.* 8: 103-117; *also see* Erratum (1991) *Proteins: Structure, Function and Genetics*, 9: 79). Several exchangeable apolipoproteins possess similar but not identical characteristics to the G amphipathic helix. Similar to the class G amphipathic helix, this other class possesses a random distribution of positively and negatively charged residues on the polar face. However, in contrast to the class G amphipathic helix which has a narrow nonpolar face, this class has a wide nonpolar face that allows this class to readily bind phospholipid and the class is termed G* to differentiate it from the G class of amphipathic helix (*see* Segrest *et al.* (1992) *J. Lipid Res.*, 33: 141-166; also see Anantharamaiah *et al.* (1993) Pp. 109-142 In: *The Amphipathic Helix,* Epand, R.M. Ed CRC Press, Boca Raton, Florida). Computer programs to identify and classify amphipathic helical domains have been described by Jones *et al.*(1992) *J. Lipid Res.* 33: 287-296) and include, but are not limited to the helical wheel program (WHEEL or WHEEL/SNORKEL), helical net program (HELNET, HELNET/SNORKEL, HELNET/Angle), program for addition of helical wheels (COMBO or COMBO/SNORKEL), program for addition of helical nets (COMNET, COMNET/SNORKEL, COMBO/SELECT, COMBO/NET), consensus wheel program (CONSENSUS, CONSENSUS/SNORKEL), and the like.

[0018] The term "ameliorating" when used with respect to "ameliorating one or more symptoms of atherosclerosis" refers to a reduction, prevention, or elimination of one or more symptoms characteristic of atherosclerosis and/or associated pathologies. Such a reduction includes, but is not limited to a reduction or elimination of oxidized phospholipids, a reduction in atherosclerotic plaque formation and rupture, a reduction in clinical events such as heart attack, angina, or stroke, a decrease in hypertension, a decrease in inflammatory protein biosynthesis, reduction in plasma cholesterol, and the like.

[0019] The term "enantiomeric amino acids" refers to amino acids that can exist in at least two forms that are nonsuperimposable mirror images of each other. Most amino acids (except glycine) are enantiomeric and exist in a so-called L-form (L amino acid) or D-form (D amino acid). Most naturally occurring amino acids are "L" amino acids. The terms

"D amino acid" and "L amino acid" are used to refer to absolute configuration of the amino acid, rather than a particular direction of rotation of plane-polarized light. The usage herein is consistent with standard usage by those of skill in the art. Amino acids are designated herein using standard 1-letter or three-letter codes, *e.g.* as designated in Standard ST.25 in the Handbook On Industrial Property Information and Documentation.

[0020] The term "protecting group" refers to a chemical group that, when attached to a functional group in an amino acid (*e.g.* a side chain, an alpha amino group, an alpha carboxyl group, *etc.*) blocks or masks the properties of that functional group. Preferred amino-terminal protecting groups include, but are not limited to acetyl, or amino groups. Other amino-terminal protecting groups include, but are not limited to alkyl chains as in fatty acids, propeonyl, formyl and others. Preferred carboxyl terminal protecting groups include, but are not limited to groups that form amides or esters.

[0021] The phrase "protect a phospholipid from oxidation by an oxidizing agent" refers to the ability of a compound to reduce the rate of oxidation of a phospholipid (or the amount of oxidized phospholipid produced) when that phospholipid is contacted with an oxidizing agent (*e.g.* hydrogen peroxide, 13-(S)-HPODE, 15-(S)-HPETE, HPODE, HPETE, HODE, HETE, *etc.*).

[0022] The terms "low density lipoprotein" or "LDL" is defined in accordance with common usage of those of skill in the art. Generally, LDL refers to the lipid-protein complex which when isolated by ultracentrifugation is found in the density range d = 1.019 to d = 1.063.

[0023] The terms "high density lipoprotein" or "HDL" is defined in accordance with common usage of those of skill in the art. Generally "HDL" refers to a lipid-protein complex which when isolated by ultracentrifugation is found in the density range of d = 1.063 to d = 1.21.

[0024] The term "Group I HDL" refers to a high density lipoprotein or components thereof (*e.g.* apo A-I, paraoxonase, platelet activating factor acetylhydrolase, *etc.*) that reduce oxidized lipids (*e.g.* in low density lipoproteins) or that protect oxidized lipids from oxidation by oxidizing agents.

[0025] The term "Group II HDL" refers to an HDL that offers reduced activity or no activity in protecting lipids from oxidation or in repairing (*e.g.* reducing) oxidized lipids.

[0026] The term "HDL component" refers to a component (*e.g.* molecules) that comprises a high density lipoprotein (HDL). Assays for HDL that protect lipids from oxidation or that repair (*e.g.* reduce oxidized lipids) also include assays for components of HDL (*e.g.* apo A-I, paraoxonase, platelet activating factor acetylhydrolase, *etc.*) that display such activity.

[0027] The term "human apo A-I peptide" refers to a full-length human apo A-I peptide or to a fragment or domain thereof comprising a class A amphipathic helix.

[0028] A "monocytic reaction" as used herein refers to monocyte activity characteristic of the "inflammatory response" associated with atherosclerotic plaque formation. The monocytic reaction is characterized by monocyte adhesion to cells of the vascular wall (*e.g.* cells of the vascular endothelium), and/or chemotaxis into the subendothelial space, and/or differentiation of monocytes into macrophages.

[0029] The term "absence of change" when referring to the amount of oxidized phospholipid refers to the lack of a detectable change, more preferably the lack of a statistically significant change (*e.g.* at least at the 85%, preferably at least at the 90%, more preferably at least at the 95%, and most preferably at least at the 98% or 99% confidence level). The absence of a detectable change can also refer to assays in which oxidized phospholipid level changes, but not as much as in the absence of the protein(s) described herein or with reference to other positive or negative controls.

[0030] The following abbreviations are used herein: PAPC: L-α-1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine; POVPC: 1-palmitoyl-2-(5-oxovaleryl)-*sn*-glycero-3-phosphocholine; PGPC: 1-palmitoyl-2-glutaryl-*sn*-glycero-3-phosphocholine; PEIPC: 1-palmitoyl-2-(5,6-epoxyisoprostane E_2)-*sn*-glycero-3-phosphocholine; ChC18:2: cholesteryl linoleate; ChC18:2-OOH: cholesteryl linoleate hydroperoxide; DMPC: 1,2-ditetradecanoyl-rac-glycerol-3-phosphocholine; PON: paraoxonase; HPF: Standardized high power field; PAPC: L-α-1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine; BL/6: C57BL/6J; C3H:C3H/HeJ.

[0031] The term "conservative substitution" is used in reference to proteins or peptides to reflect amino acid substitutions that do not substantially alter the activity (specificity (*e.g.* for lipoproteins))or binding affinity (*e.g.* for lipids or lipoproteins)) of the molecule. Typically conservative amino acid substitutions involve substitution one amino acid for another amino acid with similar chemical properties (*e.g.* charge or hydrophobicity). The following six groups each contain amino acids that are typical conservative substitutions for one another: 1) Alanine (A), Serine (S), Threonine (T); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

[0032] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. With respect to the peptides of this invention sequence identity is determined over the full length of the peptide.

[0033]. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0034] Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally* Ausubel *et al., supra*).

[0035] One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle (1987) *J. Mol. Evol.* 35:351-360. The method used is similar to the method described by Higgins & Sharp (1989) *CABIOS* 5: 151-153. The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. For example, a reference sequence can be compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps.

[0036] Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to

calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

[0037] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul (1993) *Proc. Natl. Acad. Sci. USA* ,90: 5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0038] A "circularly permuted protein" is one in the natural/original termini of the protein are joined and the resulting circular protein is opened at another point to create new C- and N- termini. The circularly permuted protein need not be created by an actual joining of termini and opening at another point, but may be synthesized/expressed *de novo* having a sequence identical to a circularly permuted variant. Two proteins are related by a circular permutation (CP) when a fragment in the C-terminal part of a first protein matches a fragment in the N-terminal part of a second protein and a fragment in the N-terminal part of the first protein matches a fragment in the C-terminal part of the second protein. Methods of identifying circular permutations are known to those of skill in the art (*see, e.g.*, Uliel *et al.* (1999) *Bioinformatics*, 15(11): 930-936).

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] Figures 1A, 1B, and 1C show that mice with an absence of LDL receptors (LDLR-/-) have thickened brain arterioles compared to wild-type mice (WT). Figure 1A: wall thickness of small arterioles (15-40 μm diameter); Figure 1B: wall thickness in medium arterioles (41-80 μm diameter); Figure 1C: wall thickness in large arterioles (81-160 μm diameter).

[0040] Figure 2 shows the results from a T-maze continuous alteration task (T-CAT) in LDR -/- mice on Western diet treated with D4F and "scrambled" D4F.

[0041] Figure 3 shows the results from a T-maze continuous alteration task (T-CAT) in LDR -/- mice on Western diet treated with D4F and scrambled (sc D-4F) expressed as percentage alteration.

[0042] Figure 4 illustrates improvement in a T-maze test by treatment with D4F.

[0043] Figure 5 shows that oral DMPC improves vasoreactivity in LDLreceptor null mice on a Western diet.

[0044] Figures 6A - 6C show that Brain arteriolar wall thickness (measured on H&E sections) is increased in LDL receptor null mice on chow (n=4) compared to wild-type mice on chow (n=4) and is further increased with addition of the Western diet (n=4). Figure 6A shows vessel wall thickness for arterioles with lumens 15-40 μm in diameter. Figure 6B shows vessel wall thickness for arterioles with lumens 41-80 μm in diameter. Figure 6C shows vessel wall thickness for vessels with lumens 81-160 μm in diameter. The bar graphs show the Mean ± SEM. WT, wild-type mice; LDL-/- Chow, LDL receptor null mice on a chow diet; LDL-/- Western, LDL receptor null mice on a Western diet for six weeks.

[0045] Figures 7A- 7G show that brain arteriolar wall thickness (measured on H&E sections) is decreased in LDL receptor null mice on a Western diet treated with D-4F but not scrambled D - 4F. LDL receptor null mice were fed a Western diet for six weeks and received either D-4F at 300 μg/mL in their drinking water (n=15) or scrambled D-4F (Sc D-4F) at 300 μg/mL in their drinking water (n=15). Figure 7A shows vessel wall thickness for arterioles with lumens 10-20 μm in diameter. Figure 7B shows vessel wall thickness for arterioles with lumens 2 1-50 μm in diameter. Figure 7C shows vessel wall thickness for arterioles 5 1-100 μm in diameter. Figure 7D shows the arteriolar lumen diameters for the

mice. Figure 7E shows the wall thickness divided by the diameter of the lumen for arterioles 10-20 μm in diameter. Figure 7F shows the wall thickness divided by the diameter of the lumen for arterioles 2 1-50 μm in diameter. Figure 7G shows the wall thickness divided by the diameter of the lumen for arterioles 51-100 μm in diameter. The bar graphs show the Mean ± SEM for 15 mice in each group.

[0046] Figures 8A- 8E show that brain arteriolar smooth muscle α-actin is increased by feeding LDL receptor null mice a Western diet and is reduced by treatment with D-4F but not scrambled D-4F. Figure 8A: LDL receptor null mice were fed a chow (n= 10) or Western diet (WD) (n=10) for six weeks and their brain arterioles were stained for smooth muscle α-actin and the wall to lumen ratio calculated for each arteriole. The values shown are the Mean ± SEM for 10 mice in each group. Figures 8B-8E. The brain arterioles of the mice described in Figure 7 and Panel A of this Figure were stained for smooth muscle α-actin. Figure 8B: Examples of brain arterioles stained for smooth muscle α-actin. Figure 8C: Wall to lumen ratio of arterioles with lumens 10-20 μm in diameter from mice in Figure 7. Figure 8D: Wall to lumen ratio of arterioles with lumens 2 1-50 μm in diameter from mice in Figure 7. Figure 8E: Wall to lumen ratio of arterioles 5 1-100 μm in diameter from mice in Figure 7. The bar graphs show the Mean ± SEM for 15 mice in each group.

[0047] Figures 9A-9G shows performance of LDL receptor null mice in the T-maze continuous alternation task (T-CAT) as a function of diet and treatment. Figure 9A-9D: The mice described in Figure 8A were tested by T-CAT for spatial memory performance. Figure 9A: The number of spontaneous alternations was determined. The data are the Mean ± SEM from 15 trials with 10 mice in each group. Figure 9B: The percent spontaneous alternations were determined. The data are the Mean ± SEM from 15 trials with 10 mice in each group. Figure 9C: The spontaneous alternation rate different from the 50% random choice was determined. The data are the Mean ± SEM from 15 trials with 10 mice in each group. Figure 9D: The time to complete the trials was determined. The data are the Mean ± SEM from 15 trials with 10 mice in each group. Figures 9E–9G: The mice described in Figures 7 and 8B – 8E were tested by T-CAT for spatial memory performance as described above. Figure 9E: The number of spontaneous alternations was determined. The data are the Mean ± SEM from 15 trials with 15 mice in each group. Figure 9F: The percent spontaneous alternations were determined. The data are the Mean ± SEM from 15 trials with

15 mice in each group. Figure 9G: The spontaneous alternation rate different from the 50% random choice was determined. The data are the Mean ± SEM from 15 trials with 15 mice in each group.

DETAILED DESCRIPTION

[0048] This invention pertains to the surprising finding that vessels smaller than even the smallest arteries (*i.e.*, arterioles) thicken, become dysfunctional and cause end organ damage to tissues as diverse as the brain and the kidney. This invention provides a method to improve the structure and function of arterioles and preserve the function of end organs such as the brain and kidney.

[0049] We reasoned that if the abnormalities that we had studied in large arteries would extend to small arteries and even to smaller vessels, the arterioles. Arterioles are are vessels of less than about 200μm more typically less than about 100 μm in diameter. We also hypothesized that if the beneficial effects of an apoA-I mimetic peptide (D-4F) were seen in both large arteries (Navab *et al.* (2002) *Circulation,* 105: 290-292; Van Lenten *et al.* (2002) *Circulation*, 106: 1127-1132) and small arteries (Ou *et al.* (2003) *Circulation*, 107: 2337-2341), the beneficial effect might also be seen in arterioles. We report here that the walls of brain arterioles ranging from 10 to 100 μm in diameter are thickened in LDL receptor null mice compared to wild-type, that the thickening is worsened with a Western diet and associated with decreased performance in the T-maze continuous alternation task. The increase in brain arteriolar wall thickness is due in part to an increase in brain arteriolar smooth muscle α-actin content and was significantly improved with D-4F treatment. It appears that treatment of LDL-receptor null mice fed a Western Diet with D-4F reduces brain arteriolar wall thickness independent of plasma lipids and arteriolar lumen diameter and improves spatial memory.

[0050] Accordingly, it is believed that use of D-4F, L-4F and other active agents described herein peptides are effective to improve the structure and/or function of arterioles and thereby to ameliorate one or more symptoms of a condition characterized by impaired arteriole structure and /or function. Such conditions include, for example, impaired neurological function (e.g., associated with Alzheimer's disease, Parkinsons disease, age related memory loss, stroke associated memory loss, Benswanger's disease, and the like), impaired kidney function, impaired alveolar function, and the like.

[0051] In certain embodiments the present invention thus provides novel methods for improving the structure and function of arterioles by administering one or more agents that sequester, and/or remove, and/or destroy inflammatory lipids and convert pro-inflammatory high density lipoproteins (HDL) to anti-inflammatory or render anti-inflammatory HDL more anti-inflammatory. These active agents include certain peptides containing a class A amphipathic helix (*see, e.g.,* U.S. Patent 6,664,230, PCT Publications WO 2002/15923, and WO 2004/034977, and copending applications USSN: 09/896,841, 10/187,215, 10/273,386, and 10/423,830 which are incorporated herein by reference), peptides containing a G* amphipathic helix (*see, e.g.,* PCT publication WO 03/086,326, and copending US application USSN 10/120,508, which are incorporated herein by reference), short peptides and non-peptides with a molecular weight of less than 900 daltons that have a solubility in ethyl acetate of at least 4 mg/mL, and which are soluble in aqueous buffer at pH 7.0 and when contacted with a phospholipid in an aqueous environment, form particles with a diameter of approximately 7.5 nm and form stacked bilayers with a bilayer dimension on the order of 3.4 to 4.1 nm with spacing between the bilayers in the stack of approximately 2 nm (*see, e.g.,* PCT/US2004/026288, copending U.S. applications USSN 10/649,378, and 10/913,800 and copending U.S. application USSN 60/600,925, respectively, which are incorporated herein by reference); and oral synthetic phospholipids in which the sn-1 and sn-2 positions are identical and contain at least 3 carbons (*see, e.g.,* copending U.S. Applications 09/539,569 and 09/994,227, and PCT publication WO 01/75168, which are incorporated herein by reference).

[0052] In certain embodiments, this invention is practiced by administering to a mammal (*e.g.,* a human) one or more of the active agents described herein (peptides, peptide mimetics, lipids, small organic molecules, *etc.*). The agent(s) are preferably administered in a dose or a dosage regimen sufficient to improve the structure and/or function of arterioles, preferably arterioles having a diameter less than about 200 μm, more preferably having a diameter less than about 160 μm, still more preferably having a diameter less than about 80 μm, and most preferably having a diameter less than about 50 μm or 40 μm.

I. Active Agents.

[0053] A wide variety of active agents are suitable for the treatment of one or more of the indications discussed above. These agents include, but are not limited to class A amphipathic helical peptides, class A amphipathic helical peptide mimetics of apoA-I having aromatic or aliphatic residues in the non-polar face, small peptides including penta-peptides, tetrapeptides, tripeptides, dipeptides and pairs of amino acids, Apo-J (G* peptides), and peptide mimetics, e.g., as described below.

A) Class A amphipathic helical peptides.

[0054] In certain embodiments, the activate agents for use in the method of this invention include class A amphipathic helical peptides, *e.g.* as described in U.S. Patent 6,664,230, and PCT Publications WO 02/15923 and WO 2004/034977. It was discovered that peptides comprising a class A amphipathic helix ("class A peptides"), in addition to being capable of mitigating one or more symptoms of atherosclerosis are also useful in the treatment of one or more of the other indications described herein.

[0055] Class A peptides are characterized by formation of an α-helix that produces a segregation of polar and non-polar residues thereby forming a polar and a nonpolar face with the positively charged residues residing at the polar-nonpolar interface and the negatively charged residues residing at the center of the polar face (*see, e.g.,* Anantharamaiah (1986) *Meth. Enzymol*, 128: 626-668). It is noted that the fourth exon of apo A-I, when folded into 3.667 residues/turn produces a class A amphipathic helical structure.

[0056] One class A peptide, designated 18A (*see, e.g.,* Anantharamaiah (1986) *Meth. Enzymol*, 128: 626-668) was modified as described herein to produce peptides orally administratable and highly effective at inhibiting or preventing one or more symptoms of atherosclerosis and/or other indications described herein.. Without being bound by a particular theory, it is believed that the peptides of this invention may act *in vivo* may by picking up seeding molecule(s) that mitigate oxidation of LDL.

[0057] We determined that increasing the number of Phe residues on the hydrophobic face of 18A would theoretically increase lipid affinity as determined by the computation described by Palgunachari *et al.* (1996) *Arteriosclerosis, Thrombosis, &*

Vascular Biology 16: 328-338. Theoretically, a systematic substitution of residues in the nonpolar face of 18A with Phe could yield six peptides. Peptides with an additional 2, 3 and 4 Phe would have theoretical lipid affinity (λ) values of 13, 14 and 15 units, respectively. However, the λ values jumped four units if the additional Phe were increased from 4 to 5 (to 19 λ units). Increasing to 6 or 7 Phe would produce a less dramatic increase (to 20 and 21 λ units, respectively).

[0058] A number of these class A peptides were made including, the peptide designated 4F, D4F, 5F, and D5F, and the like. Various class A peptides inhibited lesion development in atherosclerosis-susceptible mice. In addition, the peptides show varying, but significant degrees of efficacy in mitigating one or more symptoms of the various pathologies described herein. A number of such peptides are illustrated in Table 1.

[0059] Table 1. Illustrative class A amphipathic helical peptides for use in this invention.

Peptide Name	Amino Acid Sequence	SEQ ID NO.
18A	D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F	1
2F	Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-NH_2	2
3F	Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-NH_2	3
3F14	Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2	4
4F	Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2	5
5F	Ac-D-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH_2	6
6F	Ac-D-W-L-K-A-F-Y-D-K-F-F-E-K-F-K-E-F-F-NH_2	7
7F	Ac-D-W-F-K-A-F-Y-D-K-F-F-E-K-F-K-E-F-F-NH_2	8
	Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-F-F-NH_2	9
	Ac-D-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-A-F-NH_2	10
	Ac-D-W-L-K-A-F-Y-D-K-V-F-E-K-L-K-E-F-F-NH_2	11
	Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-F-K-E-F-F-NH_2	12
	Ac-D-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH_2	13
	Ac-E-W-L-K-L-F-Y-E-K-V-L-E-K-F-K-E-A-F-NH_2	14
	Ac-E-W-L-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2	15
	Ac-E-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-F-F-NH_2	16
	Ac-E-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-A-F-NH_2	17
	Ac-E-W-L-K-A-F-Y-D-K-V-F-E-K-L-K-E-F-F-NH_2	18
	Ac-E-W-L-K-A-F-Y-D-K-V-A-E-K-F-K-E-F-F-NH_2	19

Ac-E-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH_2	20
AC-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-NH_2	21
Ac-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2	22
Ac-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2	23
Ac-A-F-Y-D-K-F-F-E-K-F-K-E-F-F-NH_2	24
Ac-A-F-Y-D-K-F-F-E-K-F-K-E-F-F-NH_2	25
Ac-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2	26
Ac-A-F-Y-D-K-V-A-E-K-L-K-E-F-F-NH_2	27
Ac-A-F-Y-D-K-V-F-E-K-F-K-E-A-F-NH_2	28
Ac-A-F-Y-D-K-V-F-E-K-L-K-E-F-F-NH_2	29
Ac-A-F-Y-D-K-V-A-E-K-F-K-E-F-F-NH_2	30
Ac-K-A-F-Y-D-K-V-F-E-K-F-K-E-F-NH_2	31
Ac-L-F-Y-E-K-V-L-E-K-F-K-E-A-F-NH_2	32
Ac-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2	33
Ac-A-F-Y-D-K-V-A-E-K-L-K-E-F-F-NH_2	34
Ac-A-F-Y-D-K-V-F-E-K-F-K-E-A-F-NH_2	35
Ac-A-F-Y-D-K-V-F-E-K-L-K-E-F-F-NH_2	36
Ac-A-F-Y-D-K-V-A-E-K-F-K-E-F-F-NH_2	37
Ac-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH_2	38
Ac-D-W-L-K-A-L-Y-D-K-V-A-E-K-L-K-E-A-L-NH_2	39
Ac-D-W-F-K-A-F-Y-E-K-V-A-E-K-L-K-E-F-F-NH_2	40
Ac-D-W-F-K-A-F-Y-E-K-F-F-E-K-F-K-E-F-F-NH_2	41
Ac-E-W-L-K-A-L-Y-E-K-V-A-E-K-L-K-E-A-L-NH_2	42
Ac-E-W-L-K-A-F-Y-E-K-V-A-E-K-L-K-E-A-F-NH_2	43
Ac-E-W-F-K-A-F-Y-E-K-V-A-E-K-L-K-E-F-F-NH_2	44
Ac-E-W-L-K-A-F-Y-E-K-V-F-E-K-F-K-E-F-F-NH_2	45
Ac-E-W-L-K-A-F-Y-E-K-F-F-E-K-F-K-E-F-F-NH_2	46
Ac-E-W-F-K-A-F-Y-E-K-F-F-E-K-F-K-E-F-F-NH_2	47
Ac-D-F-L-K-A-W-Y-D-K-V-A-E-K-L-K-E-A-W-NH_2	48
Ac-E-F-L-K-A-W-Y-E-K-V-A-E-K-L-K-E-A-W-NH_2	49
Ac-D-F-W-K-A-W-Y-D-K-V-A-E-K-L-K-E-W-W-NH_2	50
Ac-E-F-W-K-A-W-Y-E-K-V-A-E-K-L-K-E-W-W-NH_2	51
Ac-D-K-L-K-A-F-Y-D-K-V-F-E-W-A-K-E-A-F-NH_2	52
Ac-D-K-W-K-A-V-Y-D-K-F-A-E-A-F-K-E-F-L-NH_2	53
Ac-E-K-L-K-A-F-Y-E-K-V-F-E-W-A-K-E-A-F-NH_2	54
Ac-E-K-W-K-A-V-Y-E-K-F-A-E-A-F-K-E-F-L-NH_2	55
Ac-D-W-L-K-A-F-V-D-K-F-A-E-K-F-K-E-A-Y-NH_2	56
Ac-E-K-W-K-A-V-Y-E-K-F-A-E-A-F-K-E-F-L-NH_2	57
Ac-D-W-L-K-A-F-V-Y-D-K-V-F-K-L-K-E-F-F-NH_2	58

Ac-E-W-L-K-A-F-V-Y-E-K-V-F-K-L-K-E-F-F-NH_2 59

Ac-D-W-L-R-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-NH_2 60

Ac-E-W-L-R-A-F-Y-E-K-V-A-E-K-L-K-E-A-F-NH_2 61

Ac-D-W-L-K-A-F-Y-D-R-V-A-E-K-L-K-E-A-F-NH_2 62

Ac-E-W-L-K-A-F-Y-E-R-V-A-E-K-L-K-E-A-F-NH_2 63

Ac-D-W-L-K-A-F-Y-D-K-V-A-E-R-L-K-E-A-F-NH_2 64

Ac-E-W-L-K-A-F-Y-E-K-V-A-E-R-L-K-E-A-F-NH_2 65

Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-L-R-E-A-F-NH_2 66

Ac-E-W-L-K-A-F-Y-E-K-V-A-E-K-L-R-E-A-F-NH_2 67

Ac-D-W-L-K-A-F-Y-D-R-V-A-E-R-L-K-E-A-F-NH_2 68

Ac-E-W-L-K-A-F-Y-E-R-V-A-E-R-L-K-E-A-F-NH_2 69

Ac-D-W-L-R-A-F-Y-D-K-V-A-E-K-L-R-E-A-F-NH_2 70

Ac-E-W-L-R-A-F-Y-E-K-V-A-E-K-L-R-E-A-F-NH_2 71

Ac-D-W-L-R-A-F-Y-D-R-V-A-E-K-L-K-E-A-F-NH_2 72

Ac-E-W-L-R-A-F-Y-E-R-V-A-E-K-L-K-E-A-F-NH_2 73

Ac-D-W-L-K-A-F-Y-D-K-V-A-E-R-L-R-E-A-F-NH_2 74

Ac-E-W-L-K-A-F-Y-E-K-V-A-E-R-L-R-E-A-F-NH_2 75

Ac-D-W-L-R-A-F-Y-D-K-V-A-E-R-L-K-E-A-F-NH_2 76

Ac-E-W-L-R-A-F-Y-E-K-V-A-E-R-L-K-E-A-F-NH2 77

D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-**-P-**D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F 78

D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-F-F-**-P-**D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-F-F 79

D-W-F-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-**-P-**D-W-F-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F 80

D-K-L-K-A-F-Y-D-K-V-F-E-W-A-K-E-A-F-**-P-**D-K-L-K-A-F-Y-D-K-V-F-E-W-L-K-E-A-F 81

D-K-W-K-A-V-Y-D-K-F-A-E-A-F-K-E-F-L-**-P-**D-K-W-K-A-V-Y-D-K-F-A-E-A-F-K-E-F-L 82

D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-**-P-**D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F 83

D-W-L-K-A-F-V-Y-D-K-V-F-K-L-K-E-F-F-**-P-**D-W-L-K-A-F-V-Y-D-K-V-F-K-L-K-E-F-F 84

D-W-L-K-A-F-Y-D-K-F-A-E-K-F-K-E-F-F-**-P-**D-W-L-K-A-F-Y-D-K-F-A-E-K-F-K-E-F-F 85

Ac-E-W-F-K-A-F-Y-E-K-V-A-E-K-F-K-E-A-F-NH_2 86

Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-NH_2 87

Ac-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-NH_2 88

Ac-F-K-A-F-Y-E-K-V-A-E-K-F-K-E-NH_2 89

NMA-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-NH_2 90

NMA-F-K-A-F-Y-E-K-V-A-E-K-F-K-E-NH_2 91

NMA-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2	92
NMA-E-W-F-K-A-F-Y-E-K-V-A-E-K-F-K-E-A-F-NH_2	93
NMA-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2	94
NMA-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-NH_2	95
Ac-D-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH_2	96
NMA-D-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH_2	
Ac-E-W-L-K-A-F-Y-E-K-V-F-E-K-F-K-E-F-F-NH_2	97
NMA-E-W-L-K-A-F-Y-E-K-V-F-E-K-F-K-E-F-F-NH_2	
Ac-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH_2	98
NMA-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH_2	
Ac-A-F-Y-E-K-V-F-E-K-F-K-E-F-F-NH_2	99
NMA-A-F-Y-E-K-V-F-E-K-F-K-E-F-F-NH_2	
Ac-D-W-L-K-A-F-Y-D-K-V-F-E-K-F-NH_2	100
NMA-D-W-L-K-A-F-Y-D-K-V-F-E-K-F-NH_2	
Ac-E-W-L-K-A-F-Y-E-K-V-F-E-K-F-NH_2	101
NMA-E-W-L-K-A-F-Y-E-K-V-F-E-K-F-NH_2	
Ac-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-NH_2	102
NMA-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-NH_2	
Ac-L-K-A-F-Y-E-K-V-F-E-K-F-K-E-NH_2	103
NMA-L-K-A-F-Y-E-K-V-F-E-K-F-K-E-NH_2	

[1]Linkers are underlined.

NMA is N-Methyl Anthranilyl.

[0060] In certain preferred embodiments, the peptides include variations of 4F ((SEQ ID NO:5 in Table 1, also known as L-4F, where all residues are L form amino acids) or D-4F where one or more residues are D form amino acids). In any of the peptides described herein, the C-terminus, and/or N-terminus, and/or internal residues can be blocked with one or more blocking groups as described herein.

[0061] While various peptides of Table 1, are illustrated with an acetyl group or an N-methylanthranilyl group protecting the amino terminus and an amide group protecting the carboxyl terminus, any of these protecting groups may be eliminated and/or substituted with another protecting group as described herein. In particularly preferred embodiments, the peptides comprise one or more D-form amino acids as described herein. In certain embodiments, every amino acid (*e.g.*, every enantiomeric amino acid) of the peptides of Table 1 is a D-form amino acid.

[0062] It is also noted that Table 1 is not fully inclusive. Using the teachings provided herein, other suitable class A amphipathic helical peptides can routinely be produced (*e.g.*, by conservative or semi-conservative substitutions (*e.g.*, D replaced by E), extensions, deletions, and the like). Thus, for example, one embodiment utilizes truncations of any one or more of peptides shown herein (*e.g.*, peptides identified by SEQ ID Nos:2-20 and 39- in Table 1). Thus, for example, SEQ ID NO:21 illustrates a peptide comprising 14 amino acids from the C-terminus of 18A comprising one or more D amino acids, while SEQ ID NOS:22-38 illustrate other truncations.

[0063] Longer peptides are also suitable. Such longer peptides may entirely form a class A amphipathic helix, or the class A amphipathic helix (helices) can form one or more domains of the peptide. In addition, this invention contemplates multimeric versions of the peptides (*e.g.*, concatamers). Thus, for example, the peptides illustrated herein can be coupled together (directly or through a linker (*e.g.*, a carbon linker, or one or more amino acids) with one or more intervening amino acids). Illustrative polymeric peptides include 18A-Pro-18A and the peptides of SEQ ID NOs:78-85, in certain embodiments comprising one or more D amino acids, more preferably with every amino acid a D amino acid as described herein and/or having one or both termini protected.

[0064] It will also be appreciated that, in addition to the peptide sequences expressly illustrated herein, this invention also contemplates retro and retro-inverso forms of each of these peptides. In retro forms, the direction of the sequence is reversed. In inverse forms, the chirality of the constituent amino acids is reversed (*i.e.*, L form amino acids become D form amino acids and D form amino acids become L form amino acids). In the retro-inverso form, both the order and the chirality of the amino acids is reversed. Thus, for example, a retro form of the D4F peptide (DWFKAFYDKVAEKFKEAF, SEQ ID NO:5), where the amino terminus is at the aspartate (D) and the carboxyl terminus is at the phenylalanine (F), has the same sequence, but the amino terminus is at the phenylalanine and the carobxy terminus is at the aspartate (*i.e.*, FAEKFKEAVKDYFAKFWD, SEQ ID NO: 444). Where the D4F peptide comprises all D amino acids, the restro-inverso form will have the sequence shown above (SEQ ID NO:444) and comprise all L form amino acids. Thus, for example, 4F and Rev-4F are mirror images of each other with identical segregation of the polar and nonpolar faces with the positively charged residues residing at the polar-nonpolar interface and the negatively charged residues residing at the center of the

polar face. These mirror images of the same polymer of amino acids are identical in terms of the segregation of the polar and nonpolar faces with the positively charged residues residing at the polar-nonpolar interface and the negatively charged residues residing at the center of the polar face. For a discussion of retro and retro-inverso peptides (*see, e.g.,* Chorev and Goodman, (1995) *TibTech,* 13: 439-445).

[0065] Where reference is made to a sequence and orientation is not expressly indicated, the sequence can be viewed as representing the amino acid sequence in the amino to carboxyl orientation, the retro form (*i.e.,* the amino acid sequence in the carboxyl to amino orientation), the retro form where L amino acids are replaced with D amnino acids or D amino acids are replaced with L amino acids, and the retro-inverso form where both the order is reversed and the amino acid chirality is reversed.

[0066] It will also be appreciated that, in addition to the peptide sequences expressly illustrated herein, this invention also contemplates circular permutations (CP) of such peptides and/or circular permutations of the retro-, inverso-, or retroinverso forms of such peptides. A circular permutation of a peptide is a peptide that has an amino acid sequence identical to that produced by joining the amino and carboxyl termini of the original peptide and opening the circular peptide thus formed to form new amino and carboxyl termini.

B) Other class A amphipathic helical peptide mimetics of apoA-I having Aromatic or aliphatic residues in the non-polar face.

[0067] In certain embodiments, this invention also provides modified class A amphipathic helix peptides. Certain preferred peptides incorporate one or more aromatic residues at the center of the nonpolar face, *e.g.,* $3F^{C\pi}$, (as present in 4F), or with one or more aliphatic residues at the center of the nonpolar face, *e.g.,* $3F^{I\pi}$, *see, e.g.,* Table 2. Without being bound to a particular theory, we believe the central aromatic residues on the nonpolar face of the peptide $3F^{C\pi}$, due to the presence of π electrons at the center of the nonpolar face, allow water molecules to penetrate near the hydrophobic lipid alkyl chains of the peptide-lipid complex, which in turn would enable the entry of reactive oxygen species (such as lipid hydroperoxides) shielding them from the cell surface. Similarly, we also believe the peptides with aliphatic residues at the center of the nonpolar face, *e.g.,* $3F^{I\pi}$, will act similarly but not quite as effectively as $3F^{C\pi}$.

[0068] Preferred peptides will convert pro-inflammatory HDL to anti-inflammatory HDL or make anti-inflammatory HDL more anti-inflammatory, and/or decrease LDL-induced monocyte chemotactic activity generated by artery wall cells equal to or greater than D4F or other peptides shown in Table 1.

[0069] Table 2. Examples of certain preferred peptides.

Name	Sequence	SEQ ID NO
($3F^{C\pi}$)	Ac-DKWKAVYDKFAEAFKEFL-NH_2	104
($3F^{I\pi}$)	Ac-DKLKAFYDKVFEWAKEAF-NH_2	105

C) **Smaller peptides.**

[0070] It was also a surprising discovery that certain small peptides consisting of a minimum of three amino acids preferentially (but not necessarily) with one or more of the amino acids being the D-stereoisomer of the amino acid, and possessing hydrophobic domains to permit lipid protein interactions, and hydrophilic domains to permit a degree of water solubility also possess significant anti-inflammatory properties and are useful in treating one ore more of the pathologies described herein. The "small peptides" typically range in length from 2 amino acids to about 15 amino acids, more preferably from about 3 amino acids to about 10 or 11 amino acids, and most preferably from about 4 to about 8 or 10 amino acids. In various embodiments the peptides are typically characterized by having hydrophobic terminal amino acids or terminal amino acids rendered hydrophobic by the attachment of one or more hydrophobic "protecting" groups. Various "small peptides" are described in copending applications USSN 10/649,378, filed August 26, 2003, and in USSN 10/913,800, filed on August 6, 2004, and in PCT Application PCT/US2004/026288.

[0071] In certain embodiments, the peptides can be characterized by Formula I, below:

$$X^1\text{-}X^2\text{-}X^3{}_n\text{-}X^4 \qquad \text{I}$$

where, n is 0 or 1, X^1 is a hydrophobic amino acid and/or bears a hydrophobic protecting group, X^4 is a hydrophobic amino acid and/or bears a hydrophobic protecting group; and when n is 0 X^2 is an acidic or a basic amino acid; when n is 1: X^2 and X^3 are independently an acidic amino acid, a basic amino acid, an aliphatic amino acid, or an aromatic amino acid

such that when X^2 is an acidic amino acid; X^3 is a basic amino acid, an aliphatic amino acid, or an aromatic amino acid; when X^2 is a basic amino acid; X^3 is an acidic amino acid, an aliphatic amino acid, or an aromatic amino acid; and when X^2 is an aliphatic or aromatic amino acid, X^3 is an acidic amino acid, or a basic amino acid.

[0072] Longer peptides (*e.g.*, up to 10, 11, or 15 amino acids) are also contemplated within the scope of this invention. Typically where the shorter peptides (*e.g.*, peptides according to formula I) are characterized by an acidic, basic, aliphatic, or aromatic amino acid, the longer peptides are characterized by acidic, basic, aliphatic, or aromatic domains comprising two or more amino acids of that type.

1) Functional properties of active small peptides.

[0073] It was a surprising finding of this invention that a number of physical properties predict the ability of small peptides (*e.g.*, less than 10 amino acids, preferably less than 8 amino acids, more preferably from about 3 to about 5 or 6 amino acids) of this invention to render HDL more anti-inflammatory and to mitigate atherosclerosis and/or other pathologies characterized by an inflammatory response in a mammal. The physical properties include high solubility in ethyl acetate (*e.g.*, greater than about 4 mg/mL), and solubility in aqueous buffer at pH 7.0. Upon contacting phospholipids such as 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), in an aqueous environment, the particularly effective small peptides induce or participate in the formation of particles with a diameter of approximately 7.5 nm (± 0.1 nm), and/or induce or participate in the formation of stacked bilayers with a bilayer dimension on the order of 3.4 to 4.1 nm with spacing between the bilayers in the stack of approximately 2 nm, and/or also induce or participate in the formation of vesicular structures of approximately 38 nm). In certain preferred embodiments, the small peptides have a molecular weight of less than about 900 Da.

[0074] Thus, in certain embodiments, this invention contemplates small peptides that ameliorate one or more symptoms of an indication/pathology described herein, *e.g.*, an inflammatory condition, where the peptide(s): ranges in length from about 3 to about 8 amino acids, preferably from about 3 to about 6, or 7 amino acids, and more preferably from about 3 to about 5 amino acids; are soluble in ethyl acetate at a concentration greater than about 4mg/mL; are soluble in aqueous buffer at pH 7.0; when contacted with a phospholipid in an aqueous environment, form particles with a diameter of approximately 7.5 nm and/or

form stacked bilayers with a bilayer dimension on the order of 3.4 to 4.1 nm with spacing between the bilayers in the stack of approximately 2 nm; have a molecular weight less than about 900 daltons; convert pro-inflammatory HDL to anti-inflammatory HDL or make anti-inflammatory HDL more anti-inflammatory; and do not have the amino acid sequence Lys-Arg-Asp-Ser (SEQ ID NO:235), especially in which Lys-Arg-Asp and Ser are all L amino acids. In certain embodiments, these small peptides protect a phospholipid against oxidation by an oxidizing agent.

[0075] While these small peptides need not be so limited, in certain embodiments, these small peptides can include the small peptides described below.

2) Tripeptides.

[0076] It was discovered that certain tripeptides (3 amino acid peptides) can be synthesized that show desirable properties as described herein (*e.g.*, the ability to convert pro-inflammatory HDL to anti-inflammatory HDL, the ability to decrease LDL-induced monocyte chemotactic activity generated by artery wall cells, the ability to increase pre-beta HDL, *etc.*). In certain embodiments, the peptides are characterized by formula I, wherein N is zero, shown below as Formula II:

$$X^1\text{-}X^2\text{-}X^4 \qquad \text{II}$$

where the end amino acids (X^1 and X^4) are hydrophobic either because of a hydrophobic side chain or because the side chain or the C and/or N terminus is blocked with one or more hydrophobic protecting group(s) (*e.g.*, the N-terminus is blocked with Boc-, Fmoc-, nicotinyl-, *etc.*, and the C-terminus blocked with (*t*Bu)-O*t*Bu, *etc.*). In certain embodiments, the X^2 amino acid is either acidic (*e.g.*, aspartic acid, glutamic acid, *etc.*) or basic (*e.g.*, histidine, arginine, lysine, *etc.*). The peptide can be all L- amino acids or include one or more or all D-amino acids.

[0077] Certain preferred tripeptides of this invention include, but are not limited to the peptides shown in Table 3.

[0078] Table 3. Examples of certain preferred tripeptides bearing hydrophobic blocking groups and acidic, basic, or histidine central amino acids.

X^1	X^2	X^3	X^4	SEQ ID NO
Boc-Lys(εBoc)	Arg		Ser(*t*Bu)-O*t*Bu	106
Boc-Lys(εBoc)	Arg		Thr(*t*Bu)-O*t*Bu	107
Boc-Trp	Arg		Ile-O*t*Bu	108
Boc-Trp	Arg		Leu-O*t*Bu	109
Boc-Phe	Arg		Ile –O*t*Bu	110
Boc-Phe	Arg		Leu-O*t*Bu	111
Boc-Lys(εBoc)	Glu		Ser(*t*Bu)-O*t*Bu	112
Boc-Lys(εBoc)	Glu		Thr(*t*Bu)-O*t*Bu	113
Boc-Lys(εBoc)	Asp		Ser(*t*Bu)-O*t*Bu	114
Boc-Lys(εBoc)	Asp		Thr(*t*Bu)-O*t*Bu	115
Boc-Lys(εBoc)	Arg		Ser(*t*Bu)-O*t*Bu	116
Boc-Lys(εBoc)	Arg		Thr(*t*Bu)-O*t*Bu	117
Boc-Leu	Glu		Ser(*t*Bu)-O*t*Bu	118
Boc-Leu	Glu		Thr(*t*Bu)-O*t*Bu	119
Fmoc-Trp	Arg		Ser(*t*Bu)-O*t*Bu	120
Fmoc-Trp	Asp		Ser(*t*Bu)-O*t*Bu	121
Fmoc-Trp	Glu		Ser(*t*Bu)-O*t*Bu	122
Fmoc-Trp	Arg		Ser(*t*Bu)-O*t*Bu	123
Boc-Lys(εBoc)	Glu		Leu-*Ot*Bu	124
Fmoc-Leu	Arg		Ser(*t*Bu)-O*t*Bu	125
Fmoc-Leu	Asp		Ser(*t*Bu)-O*t*Bu	126
Fmoc-Leu	Glu		Ser(*t*Bu)-O*t*Bu	127
Fmoc-Leu	Arg		Ser(*t*Bu)-O*t*Bu	128
Fmoc-Leu	Arg		Thr(*t*Bu)-O*t*Bu	129
Boc-Glu	Asp		Tyr(*t*Bu)-O*t*Bu	130
Fmoc-Lys(εFmoc)	Arg		Ser(*t*Bu)-O*t*Bu	131
Fmoc-Trp	Arg		Ile-O*t*Bu	132
Fmoc-Trp	Arg		Leu-O*t*Bu	133
Fmoc-Phe	Arg		Ile-O*t*Bu	134
Fmoc-Phe	Arg		Leu-O*t*Bu	135
Boc-Trp	Arg		Phe-O*t*Bu	136
Boc-Trp	Arg		Tyr-O*t*Bu	137
Fmoc-Trp	Arg		Phe-O*t*Bu	138
Fmoc-Trp	Arg		Tyr-O*t*Bu	139
Boc-Orn(δBoc)	Arg		Ser(*t*Bu)-O*t*Bu	140
Nicotinyl Lys(εBoc)	Arg		Ser(*t*Bu)-O*t*Bu	141
Nicotinyl Lys(εBoc)	Arg		Thr(*t*Bu)-O*t*Bu	142
Fmoc-Leu	Asp		Thr(*t*Bu)-OtBu	143
Fmoc-Leu	Glu		Thr(*t*Bu)-OtBu	144
Fmoc-Leu	Arg		Thr(*t*Bu)-OtBu	145

Fmoc-norLeu	Arg	Ser(*t*Bu)-OtBu	146
Fmoc-norLeu	Asp	Ser(*t*Bu)-OtBu	147
Fmoc-norLeu	Glu	Ser(*t*Bu)-OtBu	148
Fmoc-Lys(εBoc)	Arg	Ser(*t*Bu)-OtBu	149
Fmoc-Lys(εBoc)	Arg	Thr(*t*Bu)-OtBu	150
Fmoc-Lys(εBoc)	Glu	Ser(*t*Bu)-OtBu	151
Fmoc-Lys(εBoc)	Glu	Thr(*t*Bu)-OtBu	152
Fmoc-Lys(εBoc)	Asp	Ser(*t*Bu)-OtBu	153
Fmoc-Lys(εBoc)	Asp	Thr(*t*Bu)-OtBu	154
Fmoc-Lys(εBoc)	Glu	Leu-*OtBu*	155
Fmoc-Lys(εBoc)	Arg	Leu-*OtBu*	156
Fmoc-Lys(εFmoc)	Arg	Thr(*t*Bu)-OtBu	157
Fmoc- Lys(εFmoc)	Glu	Ser(*t*Bu)-OtBu	158
Fmoc- Lys(εFmoc)	Glu	Thr(*t*Bu)-OtBu	159
Fmoc- Lys(εFmoc)	Asp	Ser(*t*Bu)-OtBu	160
Fmoc- Lys(εFmoc)	Asp	Thr(*t*Bu)-OtBu	161
Fmoc- Lys(εFmoc)	Arg	Ser(*t*Bu)-OtBu	162
Fmoc- Lys(εFmoc))	Glu	Leu-*OtBu*	163
Boc-Lys(εFmoc)	Asp	Ser(*t*Bu)-OtBu	164
Boc-Lys(εFmoc)	Asp	Thr(*t*Bu)-OtBu	165
Boc-Lys(εFmoc)	Arg	Thr(*t*Bu)-OtBu	166
Boc-Lys(εFmoc)	Glu	Leu-*OtBu*	167
Boc-Orn(δFmoc)	Glu	Ser(*t*Bu)-OtBu	168
Boc-Orn(δFmoc)	Asp	Ser(*t*Bu)-OtBu	169
Boc-Orn(δFmoc)	Asp	Thr(*t*Bu)-OtBu	170
Boc-Orn(δFmoc)	Arg	Thr(*t*Bu)-OtBu	171
Boc-Orn(δFmoc)	Glu	Thr(*t*Bu)-OtBu	172
Fmoc-Trp	Asp	Ile-OtBu	173
Fmoc-Trp	Arg	Ile-OtBu	174
Fmoc-Trp	Glu	Ile-OtBu	175
Fmoc-Trp	Asp	Leu-OtBu	176
Fmoc-Trp	Glu	Leu-OtBu	177
Fmoc-Phe	Asp	Ile-OtBu	178
Fmoc-Phe	Asp	Leu-OtBu	179
Fmoc-Phe	Glu	Leu-OtBu	180
Fmoc-Trp	Arg	Phe-OtBu	181
Fmoc-Trp	Glu	Phe-OtBu	182
Fmoc-Trp	Asp	Phe-OtBu	183
Fmoc-Trp	Asp	Tyr-OtBu	184
Fmoc-Trp	Arg	Tyr-OtBu	185
Fmoc-Trp	Glu	Tyr-OtBu	186

Fmoc-Trp	Arg	Thr(*t*Bu)-OtBu	187
Fmoc-Trp	Asp	Thr(*t*Bu)-OtBu	188
Fmoc-Trp	Glu	Thr(*t*Bu)-OtBu	189
Boc-Phe	Arg	norLeu-OtBu	190
Boc-Phe	Glu	norLeu-OtBu	191
Fmoc-Phe	Asp	norLeu-OtBu	192
Boc-Glu	His	Tyr(*t*Bu)-O*t*Bu	193
Boc-Leu	His	Ser(*t*Bu)-O*t*Bu	194
Boc-Leu	His	Thr(*t*Bu)-O*t*Bu	195
Boc-Lys(εBoc)	His	Ser(*t*Bu)-O*t*Bu	196
Boc-Lys(εBoc)	His	Thr(*t*Bu)-O*t*Bu	197
Boc-Lys(εBoc)	His	Leu-*OtBu*	198
Boc-Lys(εFmoc)	His	Ser(*t*Bu)-OtBu	199
Boc-Lys(εFmoc)	His	Thr(*t*Bu)-OtBu	200
Boc-Lys(εFmoc)	His	Leu-*OtBu*	201
Boc-Orn(δBoc)	His	Ser(*t*Bu)-O*t*Bu	202
Boc-Orn(δFmoc)	His	Thr(*t*Bu)-OtBu	203
Boc-Phe	His	Ile –O*t*Bu	204
Boc-Phe	His	Leu-O*t*Bu	205
Boc-Phe	His	norLeu-OtBu	206
Boc-Phe	Lys	Leu-O*t*Bu	207
Boc-Trp	His	Ile-O*t*Bu	208
Boc-Trp	His	Leu-O*t*Bu	209
Boc-Trp	His	Phe-O*t*Bu	210
Boc-Trp	His	Tyr-O*t*Bu	211
Boc-Phe	Lys	Leu-O*t*Bu	212
Fmoc- Lys(εFmoc)	His	Ser(*t*Bu)-OtBu	213
Fmoc- Lys(εFmoc)	His	Thr(*t*Bu)-OtBu	214
Fmoc- Lys(εFmoc)	His	Leu-O*t*Bu	215
Fmoc-Leu	His	Ser(*t*Bu)-O*t*Bu	216
Fmoc-Leu	His	Thr(*t*Bu)-OtBu	217
Fmoc-Lys(εBoc)	His	Ser(*t*Bu)-OtBu	218
Fmoc-Lys(εBoc)	His	Thr(*t*Bu)-OtBu	219

Fmoc-Lys(εBoc)	His	Leu-*OtBu*	220
Fmoc-Lys(εFmoc)	His	Ser(*t*Bu)-O*t*Bu	221
Fmoc-Lys(εFmoc)	His	Thr(*t*Bu)-OtBu	222
Fmoc-norLeu	His	Ser(*t*Bu)-OtBu	223
Fmoc-Phe	His	Ile-O*t*Bu	224
Fmoc-Phe	His	Leu-O*t*Bu	225
Fmoc-Phe	His	norLeu-OtBu	226
Fmoc-Trp	His	Ser(*t*Bu)-O*t*Bu	227
Fmoc-Trp	His	Ile-O*t*Bu	228
Fmoc-Trp	His	Leu-O*t*Bu	229
Fmoc-Trp	His	Phe-O*t*Bu	230
Fmoc-Trp	His	Tyr-O*t*Bu	231
Fmoc-Trp	His	Thr(*t*Bu)-OtBu	232
Nicotinyl Lys(εBoc)	His	Ser(*t*Bu)-O*t*Bu	233
Nicotinyl Lys(εBoc)	His	Thr(*t*Bu)-O*t*Bu	234

[0079] While the peptides of Table 3 are illustrated with particular protecting groups, it is noted that these groups may be substituted with other protecting groups as described herein and/or one or more of the shown protecting group can be eliminated.

3) Small peptides with central acidic and basic amino acids.

[0080] In certain embodiments, the peptides of this invention range from four amino acids to about ten amino acids. The terminal amino acids are typically hydrophobic either because of a hydrophobic side chain or because the terminal amino acids bear one or more hydrophobic protecting groups end amino acids (X^1 and X^4) are hydrophobic either because of a hydrophobic side chain or because the side chain or the C and/or N terminus is blocked with one or more hydrophobic protecting group(s) (*e.g.*, the N-terminus is blocked with Boc-, Fmoc-, Nicotinyl-, *etc.*, and the C-terminus blocked with (*t*Bu)-O*t*Bu, *etc.*). Typically, the central portion of the peptide comprises a basic amino acid and an acidic amino acid (*e.g.*, in a 4 mer) or a basic domain and/or an acidic domain in a longer molecule.

[0081] These four-mers can be represented by Formula I in which X^1 and X^4 are hydrophobic and/or bear hydrophobic protecting group(s) as described herein and X^2 is acidic while X^3 is basic or X^2 is basic while X^3 is acidic. The peptide can be all L- amino acids or include one or more or all D-amino acids.

[0082] Certain preferred of this invention include, but are not limited to the peptides shown in Table 4.

[0083] Table 4. Illustrative examples of small peptides with central acidic and basic amino acids.

X^1	X^2	X^3	X^4	SEQ ID NO
Boc-Lys(εBoc)	Arg	Asp	Ser(*t*Bu)-O*t*Bu	235
Boc-Lys(εBoc)	Arg	Asp	Thr(*t*Bu)-O*t*Bu	236
Boc-Trp	Arg	Asp	Ile-O*t*Bu	237
Boc-Trp	Arg	Asp	Leu-O*t*Bu	238
Boc-Phe	Arg	Asp	Leu-O*t*Bu	239
Boc-Phe	Arg	Asp	Ile-O*t*Bu	240
Boc-Phe	Arg	Asp	norLeu-O*t*Bu	241
Boc-Phe	Arg	Glu	norLeu-O*t*Bu	242
Boc-Phe	Arg	Glu	Ile-*Ot*Bu	243
Boc-Phe	Asp	Arg	Ile-O*t*Bu	244
Boc-Phe	Glu	Arg	Ile-O*t*Bu	245
Boc-Phe	Asp	Arg	Leu-O*t*Bu	246
Boc-Phe	Arg	Glu	Leu-O*t*Bu	247
Boc-Phe	Glu	Arg	Leu-O*t*Bu	248
Boc-Phe	Asp	Arg	norLeu-O*t*Bu	249
Boc-Phe	Glu	Arg	norLeu-O*t*Bu	250
Boc-Lys(εBoc)	Glu	Arg	Ser(*t*Bu)-O*t*Bu	251
Boc-Lys(εBoc)	Glu	Arg	Thr(*t*Bu)-O*t*Bu	252
Boc-Lys(εBoc)	Asp	Arg	Ser(*t*Bu)-O*t*Bu	253
Boc-Lys(εBoc)	Asp	Arg	Thr(*t*Bu)-O*t*Bu	254
Boc-Lys(εBoc)	Arg	Glu	Ser(*t*Bu)-O*t*Bu	255
Boc-Lys(εBoc)	Arg	Glu	Thr(*t*Bu)-O*t*Bu	256
Boc-Leu	Glu	Arg	Ser(*t*Bu)-O*t*Bu	257
Boc-Leu	Glu	Arg	Thr(*t*Bu)-O*t*Bu	258
Fmoc-Trp	Arg	Asp	Ser(*t*Bu)-O*t*Bu	259
Fmoc-Trp	Asp	Arg	Ser(*t*Bu)-O*t*Bu	260
Fmoc-Trp	Glu	Arg	Ser(*t*Bu)-O*t*Bu	261

Fmoc-Trp	Arg	Glu	Ser(*t*Bu)-O*t*Bu	262
Boc-Lys(εBoc)	Glu	Arg	Leu-*Ot*Bu	263
Fmoc-Leu	Arg	Asp	Ser(*t*Bu)-O*t*Bu	264
Fmoc-Leu	Asp	Arg	Ser(*t*Bu)-O*t*Bu	265
Fmoc-Leu	Glu	Arg	Ser(*t*Bu)-O*t*Bu	266
Fmoc-Leu	Arg	Glu	Ser(*t*Bu)-O*t*Bu	267
Fmoc-Leu	Arg	Asp	Thr(*t*Bu)-O*t*Bu	268
Boc-Glu	Asp	Arg	Tyr(*t*Bu)-O*t*Bu	269
Fmoc-Lys(εFmoc)	Arg	Asp	Ser(*t*Bu)-O*t*Bu	270
Fmoc-Trp	Arg	Asp	Ile-O*t*Bu	271
Fmoc-Trp	Arg	Asp	Leu-O*t*Bu	272
Fmoc-Phe	Arg	Asp	Ile-O*t*Bu	273
Fmoc-Phe	Arg	Asp	Leu-O*t*Bu	274
Boc-Trp	Arg	Asp	Phe-O*t*Bu	275
Boc-Trp	Arg	Asp	Tyr-O*t*Bu	276
Fmoc-Trp	Arg	Asp	Phe-O*t*Bu	277
Fmoc-Trp	Arg	Asp	Tyr-O*t*Bu	278
Boc-Orn(δBoc)	Arg	Glu	Ser(*t*Bu)-O*t*Bu	279
Nicotinyl Lys(εBoc)	Arg	Asp	Ser(*t*Bu)-O*t*Bu	280
Nicotinyl Lys(εBoc)	Arg	Asp	Thr(*t*Bu)-O*t*Bu	281
Fmoc-Leu	Asp	Arg	Thr(*t*Bu)-OtBu	282
Fmoc-Leu	Glu	Arg	Thr(*t*Bu)-OtBu	283
Fmoc-Leu	Arg	Glu	Thr(*t*Bu)-OtBu	284
Fmoc-norLeu	Arg	Asp	Ser(*t*Bu)-OtBu	285
Fmoc-norLeu	Asp	Arg	Ser(*t*Bu)-OtBu	286
Fmoc-norLeu	Glu	Arg	Ser(*t*Bu)-OtBu	287
Fmoc-norLeu	Arg	Glu	Ser(*t*Bu)-OtBu	288
Fmoc-Lys(εBoc)	Arg	Asp	Ser(*t*Bu)-OtBu	289
Fmoc-Lys(εBoc)	Arg	Asp	Thr(*t*Bu)-OtBu	290
Fmoc-Lys(εBoc)	Glu	Arg	Ser(*t*Bu)-OtBu	291
Fmoc-Lys(εBoc)	Glu	Arg	Thr(*t*Bu)-OtBu	292
Fmoc-Lys(εBoc)	Asp	Arg	Ser(*t*Bu)-OtBu	293
Fmoc-Lys(εBoc)	Asp	Arg	Thr(*t*Bu)-OtBu	294
Fmoc-Lys(εBoc)	Arg	Glu	Ser(*t*Bu)-OtBu	295
Fmoc-Lys(εBoc)	Arg	Glu	Thr(*t*Bu)-OtBu	296
Fmoc-Lys(εBoc)	Glu	Arg	Leu-*OtBu*	297
Fmoc-Lys(εBoc)	Arg	Glu	Leu-*OtBu*	298
Fmoc-Lys(εFmoc)	Arg	Asp	Thr(*t*Bu)-OtBu	299

Fmoc- Lys(εFmoc)	Glu	Arg	Ser(*t*Bu)-OtBu	300
Fmoc- Lys(εFmoc)	Glu	Arg	Thr(*t*Bu)-OtBu	301
Fmoc- Lys(εFmoc)	Asp	Arg	Ser(*t*Bu)-OtBu	302
Fmoc- Lys(εFmoc)	Asp	Arg	Thr(*t*Bu)-OtBu	303
Fmoc- Lys(εFmoc)	Arg	Glu	Ser(*t*Bu)-OtBu	304
Fmoc- Lys(εFmoc)	Arg	Glu	Thr(*t*Bu)-OtBu	305
Fmoc- Lys(εFmoc))	Glu	Arg	Leu-*OtBu*	306
Boc-Lys(εFmoc)	Arg	Asp	Ser(*t*Bu)-OtBu	307
Boc-Lys(εFmoc)	Arg	Asp	Thr(*t*Bu)-OtBu	308
Boc-Lys(εFmoc)	Glu	Arg	Ser(*t*Bu)-OtBu	309
Boc-Lys(εFmoc)	Glu	Arg	Thr(*t*Bu)-OtBu	310
Boc-Lys(εFmoc)	Asp	Arg	Ser(*t*Bu)-OtBu	311
Boc-Lys(εFmoc)	Asp	Arg	Thr(*t*Bu)-OtBu	312
Boc-Lys(εFmoc)	Arg	Glu	Ser(*t*Bu)-OtBu	313
Boc-Lys(εFmoc)	Arg	Glu	Thr(*t*Bu)-OtBu	314
Boc-Lys(εFmoc)	Glu	Arg	Leu-*OtBu*	315
Boc-Orn(δFmoc)	Arg	Glu	Ser(*t*Bu)-OtBu	316
Boc-Orn(δFmoc)	Glu	Arg	Ser(*t*Bu)-OtBu	317
Boc-Orn(δFmoc)	Arg	Asp	Ser(*t*Bu)-OtBu	318
Boc-Orn(δFmoc)	Asp	Arg	Ser(*t*Bu)-OtBu	319
Boc-Orn(δFmoc)	Asp	Arg	Thr(*t*Bu)-OtBu	320
Boc-Orn(δFmoc)	Arg	Asp	Thr(*t*Bu)-OtBu	321
Boc-Orn(δFmoc)	Glu	Arg	Thr(*t*Bu)-OtBu	322
Boc-Orn(δFmoc)	Arg	Glu	Thr(*t*Bu)-OtBu	323
Fmoc-Trp	Asp	Arg	Ile-OtBu	324
Fmoc-Trp	Arg	Glu	Ile-OtBu	325
Fmoc-Trp	Glu	Arg	Ile-OtBu	326
Fmoc-Trp	Asp	Arg	Leu-OtBu	327
Fmoc-Trp	Arg	Glu	Leu-OtBu	328
Fmoc-Trp	Glu	Arg	Leu-OtBu	329
Fmoc-Phe	Asp	Arg	Ile-OtBu	330
Fmoc-Phe	Arg	Glu	Ile-OtBu	331
Fmoc-Phe	Glu	Arg	Ile-OtBu	332
Fmoc-Phe	Asp	Arg	Leu-OtBu	333
Fmoc-Phe	Arg	Glu	Leu-OtBu	334
Fmoc-Phe	Glu	Arg	Leu-OtBu	335
Fmoc-Trp	Arg	Asp	Phe-OtBu	336
Fmoc-Trp	Arg	Glu	Phe-OtBu	337

Fmoc-Trp	Glu	Arg	Phe-OtBu	338
Fmoc-Trp	Asp	Arg	Tyr-OtBu	339
Fmoc-Trp	Arg	Glu	Tyr-OtBu	340
Fmoc-Trp	Glu	Arg	Tyr-OtBu	341
Fmoc-Trp	Arg	Asp	Thr(*t*Bu)-OtBu	342
Fmoc-Trp	Asp	Arg	Thr(*t*Bu)-OtBu	343
Fmoc-Trp	Arg	Glu	Thr(*t*Bu)-OtBu	344
Fmoc-Trp	Glu	Arg	Thr(*t*Bu)-OtBu	345
Fmoc-Phe	Arg	Asp	norLeu-OtBu	346
Fmoc-Phe	Arg	Glu	norLeu-OtBu	347
Boc-Phe	Lys	Asp	Leu-OtBu	348
Boc-Phe	Asp	Lys	Leu-OtBu	349
Boc-Phe	Lys	Glu	Leu-OtBu	350
Boc-Phe	Glu	Lys	Leu-OtBu	351
Boc-Phe	Lys	Asp	Ile-OtBu	352
Boc-Phe	Asp	Lys	Ile-OtBu	353
Boc-Phe	Lys	Glu	Ile-OtBu	354
Boc-Phe	Glu	Lys	Ile-OtBu	355
Boc-Phe	Lys	Asp	norLeu-OtBu	356
Boc-Phe	Asp	Lys	norLeu-OtBu	357
Boc-Phe	Lys	Glu	norLeu-OtBu	358
Boc-Phe	Glu	Lys	norLeu-OtBu	359
Boc-Phe	His	Asp	Leu-OtBu	360
Boc-Phe	Asp	His	Leu-OtBu	361
Boc-Phe	His	Glu	Leu-OtBu	362
Boc-Phe	Glu	His	Leu-OtBu	363
Boc-Phe	His	Asp	Ile-OtBu	364
Boc-Phe	Asp	His	Ile-OtBu	365
Boc-Phe	His	Glu	Ile-OtBu	366
Boc-Phe	Glu	His	Ile-OtBu	367
Boc-Phe	His	Asp	norLeu-OtBu	368
Boc-Phe	Asp	His	norLeu-OtBu	369
Boc-Phe	His	Glu	norLeu-OtBu	370
Boc-Phe	Glu	His	norLeu-OtBu	371
Boc-Lys(εBoc)	Lys	Asp	Ser(*t*Bu)-OtBu	372
Boc-Lys(εBoc)	Asp	Lys	Ser(*t*Bu)-OtBu	373
Boc-Lys(εBoc)	Lys	Glu	Ser(*t*Bu)-OtBu	374
Boc-Lys(εBoc)	Glu	Lys	Ser(*t*Bu)-OtBu	375
Boc-Lys(εBoc)	His	Asp	Ser(*t*Bu)-OtBu	376

Boc-Lys(εBoc)	Asp	His	Ser(*t*Bu)-OtBu	377
Boc-Lys(εBoc)	His	Glu	Ser(*t*Bu)-OtBu	378
Boc-Lys(εBoc)	Glu	His	Ser(*t*Bu)-OtBu	379

[0084] While the peptides of Table 4 are illustrated with particular protecting groups, it is noted that these groups may be substituted with other protecting groups as described herein and/or one or more of the shown protecting group can be eliminated.

4) Small peptides having either an acidic or basic amino acid in the center together with a central aliphatic amino acid.

[0085] In certain embodiments, the peptides of this invention range from four amino acids to about ten amino acids. The terminal amino acids are typically hydrophobic either because of a hydrophobic side chain or because the terminal amino acids bear one or more hydrophobic protecting groups. End amino acids (X^1 and X^4) are hydrophobic either because of a hydrophobic side chain or because the side chain or the C and/or N terminus is blocked with one or more hydrophobic protecting group(s) (*e.g.*, the N-terminus is blocked with Boc-, Fmoc-, Nicotinyl-, *etc.*, and the C-terminus blocked with (*t*Bu)-O*t*Bu, *etc.*). Typically, the central portion of the peptide comprises a basic or acidic amino acid and an aliphatic amino acid (*e.g.*, in a 4 mer) or a basic domain or an acidic domain and an aliphatic domain in a longer molecule.

[0086] These four-mers can be represented by Formula I in which X^1 and X^4 are hydrophobic and/or bear hydrophobic protecting group(s) as described herein and X^2 is acidic or basic while X^3 is aliphatic or X^2 is aliphatic while X^3 is acidic or basic. The peptide can be all L- amino acids or include one, or more, or all D-amino acids.

[0087] Certain preferred peptides of this invention include, but are not limited to the peptides shown in Table 5.

[0088] Table 5. Examples of certain preferred peptides having either an acidic or basic amino acid in the center together with a central aliphatic amino acid.

X^1	X^2	X^3	X^4	SEQ ID NO

Fmoc-Lys(εBoc)	Leu	Arg	Ser(*t*Bu)-OtBu	380
Fmoc-Lys(εBoc)	Arg	Leu	Ser(*t*Bu)-OtBu	381
Fmoc-Lys(εBoc)	Leu	Arg	Thr(*t*Bu)-OtBu	382
Fmoc-Lys(εBoc)	Arg	Leu	Thr(*t*Bu)-OtBu	383
Fmoc-Lys(εBoc)	Glu	Leu	Ser(*t*Bu)-OtBu	384
Fmoc-Lys(εBoc)	Leu	Glu	Ser(*t*Bu)-OtBu	385
Fmoc-Lys(εBoc)	Glu	Leu	Thr(*t*Bu)-OtBu	386
Fmoc- Lys(εFmoc)	Leu	Arg	Ser(*t*Bu)-OtBu	387
Fmoc- Lys(εFmoc)	Leu	Arg	Thr(*t*Bu)-OtBu	388
Fmoc- Lys(εFmoc)	Glu	Leu	Ser(*t*Bu)-OtBu	389
Fmoc- Lys(εFmoc)	Glu	Leu	Thr(*t*Bu)-OtBu	390
Boc-Lys(Fmoc)	Glu	Ile	Thr(*t*Bu)-OtBu	391
Boc-Lys(εFmoc)	Leu	Arg	Ser(*t*Bu)-OtBu	392
Boc-Lys(εFmoc)	Leu	Arg	Thr(*t*Bu)-OtBu	393
Boc-Lys(εFmoc)	Glu	Leu	Ser(*t*Bu)-OtBu	394
Boc-Lys(εFmoc)	Glu	Leu	Thr(*t*Bu)-OtBu	395
Boc-Lys(εBoc)	Leu	Arg	Ser(*t*Bu)-OtBu	396
Boc-Lys(εBoc)	Arg	Phe	Thr(*t*Bu)-OtBu	397
Boc-Lys(εBoc)	Leu	Arg	Thr(*t*Bu)-OtBu	398
Boc-Lys(εBoc)	Glu	Ile	Thr(*t*Bu)	399
Boc-Lys(εBoc)	Glu	Val	Thr(*t*Bu)	400
Boc-Lys(εBoc)	Glu	Ala	Thr(*t*Bu)	401
Boc-Lys(εBoc)	Glu	Gly	Thr(*t*Bu)	402
Boc--Lys(εBoc)	Glu	Leu	Ser(*t*Bu)-OtBu	403
Boc-Lys(εBoc)	Glu	Leu	Thr(*t*Bu)-OtBu	404

[0089] While the pepides of Table 5 are illustrated with particular protecting groups, it is noted that these groups may be substituted with other protecting groups as described herein and/or one or more of the shown protecting group can be eliminated.

5) Small peptides having either an acidic or basic amino acid in the center together with a central aromatic amino acid.

[0090] In certain embodiments, the "small" peptides of this invention range from four amino acids to about ten amino acids. The terminal amino acids are typically hydrophobic either because of a hydrophobic side chain or because the terminal amino acids bear one or more hydrophobic protecting groups end amino acids (X^1 and X^4) are hydrophobic either because of a hydrophobic side chain or because the side chain or the C

and/or N terminus is blocked with one or more hydrophobic protecting group(s) (*e.g.*, the N-terminus is blocked with Boc-, Fmoc-, Nicotinyl-, *etc.*, and the C-terminus blocked with (*t*Bu)-O*t*Bu, *etc.*). Typically, the central portion of the peptide comprises a basic or acidic amino acid and an aromatic amino acid (*e.g.*, in a 4 mer) or a basic domain or an acidic domain and an aromatic domain in a longer molecule.

[0091] These four-mers can be represented by Formula I in which X^1 and X^4 are hydrophobic and/or bear hydrophobic protecting group(s) as described herein and X^2 is acidic or basic while X^3 is aromatic or X^2 is aromatic while X^3 is acidic or basic. The peptide can be all L- amino acids or include one, or more, or all D-amino acids. Five-mers can be represented by a minor modification of Formula I in which X^5 is inserted as shown in Table 6 and in which X^5 is typically an aromatic amino acid.

[0092] Certain preferred peptides of this invention include, but are not limited to the peptides shown in Table 6.

[0093] Table 6. Examples of certain preferred peptides having either an acidic or basic amino acid in the center together with a central aromatic amino acid.

X^1	X^2	X^3	X^5	X^4	SEQ ID NO
Fmoc-Lys(εBoc)	Arg	Trp		Tyr(*t*Bu)-OtBu	405
Fmoc-Lys(εBoc)	Trp	Arg		Tyr(*t*Bu)-OtBu	406
Fmoc-Lys(εBoc)	Arg	Tyr		Trp-OtBu	407
Fmoc-Lys(εBoc)	Tyr	Arg		Trp-OtBu	408
Fmoc-Lys(εBoc)	Arg	Tyr	Trp	Thr(*t*Bu)-OtBu	409
Fmoc-Lys(εBoc)	Arg	Tyr		Thr(*t*Bu)-OtBu	410
Fmoc-Lys(εBoc)	Arg	Trp		Thr(*t*Bu)-OtBu	411
Fmoc- Lys(εFmoc)	Arg	Trp		Tyr(*t*Bu)-OtBu	412
Fmoc- Lys(εFmoc)	Arg	Tyr		Trp-OtBu	413
Fmoc- Lys(εFmoc)	Arg	Tyr	Trp	Thr(*t*Bu)-OtBu	414
Fmoc- Lys(εFmoc)	Arg	Tyr		Thr(*t*Bu)-OtBu	415
Fmoc- Lys(εFmoc)	Arg	Trp		Thr(*t*Bu)-OtBu	416
Boc-Lys(εFmoc)	Arg	Trp		Tyr(*t*Bu)-OtBu	417
Boc-Lys(εFmoc)	Arg	Tyr		Trp-OtBu	418
Boc-Lys(εFmoc)	Arg	Tyr	Trp	Thr(*t*Bu)-OtBu	419
Boc-Lys(εFmoc)	Arg	Tyr		Thr(*t*Bu)-OtBu	420
Boc-Lys(εFmoc)	Arg	Trp		Thr(*t*Bu)-OtBu	421
Boc-Glu	Lys(εFmoc)	Arg		Tyr(*t*Bu)-OtBu	422

Boc-Lys(εBoc)	Arg	Trp		Tyr(*t*Bu)-OtBu	423
Boc-Lys(εBoc)	Arg	Tyr		Trp-OtBu	424
Boc-Lys(εBoc)	Arg	Tyr	Trp	Thr(*t*Bu)-OtBu	425
Boc-Lys(εBoc)	Arg	Tyr		Thr(*t*Bu)-OtBu	426
Boc-Lys(εBoc)	Arg	Phe		Thr(*t*Bu)-OtBu	427
Boc-Lys(εBoc)	Arg	Trp		Thr(*t*Bu)-OtBu	428

[0094] While the peptides of Table 6 are illustrated with particular protecting groups, it is noted that these groups may be substituted with other protecting groups as described herein and/or one or more of the shown protecting group can be eliminated.

6) Small peptides having aromatic amino acids or aromatic amino acids separated by histidine(s) at the center.

[0095] In certain embodiments, the peptides of this invention are characterized by π electrons that are exposed in the center of the molecule which allow hydration of the particle and that allow the peptide particles to trap pro-inflammatory oxidized lipids such as fatty acid hydroperoxides and phospholipids that contain an oxidation product of arachidonic acid at the sn-2 position.

[0096] In certain embodiments, these peptides consist of a minimum of 4 amino acids and a maximum of about 10 amino acids, preferentially (but not necessarily) with one or more of the amino acids being the D-sterioisomer of the amino acid, with the end amino acids being hydrophobic either because of a hydrophobic side chain or because the terminal amino acid(s) bear one or more hydrophobic blocking group(s), (*e.g.*, an N-terminus blocked with Boc-, Fmoc-, Nicotinyl-, and the like, and a C-terminus blocked with (*t*Bu)-O*t*Bu groups and the like). Instead of having an acidic or basic amino acid in the center, these peptides generally have an aromatic amino acid at the center or have aromatic amino acids separated by histidine in the center of the peptide.

[0097] Certain preferred peptides of this invention include, but are not limited to the peptides shown in Table 7.

[0098] Table 7. Examples of peptides having aromatic amino acids in the center or aromatic amino acids or aromatic domains separated by one or more histidines.

X^1	X^2	X^3	X^4	X^5	SEQ ID NO
Boc-Lys(εBoc)	Phe	Trp	Phe	Ser(*t*Bu)-OtBu	429
Boc-Lys(εBoc)	Phe	Trp	Phe	Thr(*t*Bu)-OtBu	430
Boc-Lys(εBoc)	Phe	Tyr	Phe	Ser(*t*Bu)-OtBu	431
Boc-Lys(εBoc)	Phe	Tyr	Phe	Thr(*t*Bu)-OtBu	432
Boc-Lys(εBoc)	Phe	His	Phe	Ser(*t*Bu)-OtBu	433
Boc-Lys(εBoc)	Phe	His	Phe	Thr(*t*Bu)-OtBu	434
Boc-Lys(εBoc)	Val	Phe	Phe-Tyr	Ser(*t*Bu)-OtBu	435
Nicotinyl-Lys(εBoc)	Phe	Trp	Phe	Ser(*t*Bu)-OtBu	436
Nicotinyl-Lys(εBoc)	Phe	Trp	Phe	Thr(*t*Bu)-OtBu	437
Nicotinyl-Lys(εBoc)	Phe	Tyr	Phe	Ser(*t*Bu)-OtBu	438
Nicotinyl-Lys(εBoc)	Phe	Tyr	Phe	Thr(*t*Bu)-OtBu	439
Nicotinyl-Lys(εBoc)	Phe	His	Phe	Ser(*t*Bu)-OtBu	440
Nicotinyl-Lys(εBoc)	Phe	His	Phe	Thr(*t*Bu)-OtBu	441
Boc-Leu	Phe	Trp	Phe	Thr(*t*Bu)-OtBu	442
Boc-Leu	Phe	Trp	Phe	Ser(*t*Bu)-OtBu	443

[0099] While the peptides of Table 7 are illustrated with particular protecting groups, it is noted that these groups may be substituted with other protecting groups as described herein and/or one or more of the shown protecting group can be eliminated.

7) Summary of tripeptides and tetrapeptides.

[0100] For the sake of clarity, a number of tripeptides and tetrapeptides of this invention are generally summarized below in Table 8.

[0101] Table 8. General structure of certain peptides of this invention.

X^1	X^2	X^3	X^4
hydrophobic side chain or hydrophobic protecting group(s)	Acidic or Basic	----	hydrophobic side chain or hydrophobic protecting group(s)
hydrophobic side chain or hydrophobic protecting group(s)	Basic	Acidic	hydrophobic side chain or hydrophobic protecting group(s)
hydrophobic side chain or hydrophobic protecting group(s)	Acidic	Basic	hydrophobic side chain or hydrophobic protecting group(s)
hydrophobic side chain	Acidic or Basic	Aliphatic	hydrophobic side

or hydrophobic protecting group(s)				chain or hydrophobic protecting group(s)
hydrophobic side chain or hydrophobic protecting group(s)	Aliphatic	Acidic or Basic		hydrophobic side chain or hydrophobic protecting group(s)
hydrophobic side chain or hydrophobic protecting group(s)	Acidic or Basic	Aromatic		hydrophobic side chain or hydrophobic protecting group(s)
hydrophobic side chain or hydrophobic protecting group(s)	Aromatic	Acidic or Basic		hydrophobic side chain or hydrophobic protecting group(s)
hydrophobic side chain or hydrophobic protecting group(s)	Aromatic	His	Aromatic	hydrophobic side chain or hydrophobic protecting group(s)

[0102] Where longer peptides are desired, X^2 and X^3 can represent domains (*e.g.*, regions of two or more amino acids of the specified type) rather than individual amino acids. Table 8 is intended to be illustrative and not limiting. Using the teaching provided herein, other suitable peptides can readily be identified.

8) Paired amino acids and dipeptides.

[0103] In certain embodiments, this invention pertains to the discovery that certain pairs of amino acids, administered in conjunction with each other or linked to form a dipeptide have one or more of the properties described herein. Thus, without being bound to a particular theory, it is believed that when the pairs of amino acids are administered in conjunction with each other, as described herein, they are capable participating in or inducing the formation of micelles *in vivo*.

[0104] Similar to the other small peptides described herein, it is believed that the pairs of peptides will associate *in vivo*, and demonstrate physical properties including high solubility in ethyl acetate (*e.g.*, greater than about 4 mg/mL), solubility in aqueous buffer at pH 7.0. Upon contacting phospholipids such as 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), in an aqueous environment, it is believed the pairs of amino acids induce or participate in the formation of particles with a diameter of approximately 7.5 nm

(± 0.1 nm), and/or induce or participate in the formation of stacked bilayers with a bilayer dimension on the order of 3.4 to 4.1 nm with spacing between the bilayers in the stack of approximately 2 nm, and/or also induce or participate in the formation of vesicular structures of approximately 38 nm).

[0105] Moreover, it is further believed that the pairs of amino acids can display one or more of the following physiologically relevant properties:

[0106] 1. They convert pro-inflammatory HDL to anti-inflammatory HDL or make anti-inflammatory HDL more anti-inflammatory;

[0107] 2. They decrease LDL-induced monocyte chemotactic activity generated by artery wall cells;

[0108] 3. They stimulate the formation and cycling of pre-β HDL;

[0109] 4. They raise HDL cholesterol; and/or

[0110] 5. They increase HDL paraoxonase activity.

[0111] The pairs of amino acids can be administered as separate amino acids (administered sequentially or simultaneously, *e.g.* in a combined formulation) or they can be covalently coupled directly or through a linker (*e.g.* a PEG linker, a carbon linker, a branched linker, a straight chain linker, a heterocyclic linker, a linker formed of derivatized lipid, *etc.*). In certain embodiments, the pairs of amino acids are covalently linked through a peptide bond to form a dipeptide. In various embodiments while the dipeptides will typically comprise two amino acids each bearing an attached protecting group, this invention also contemplates dipeptides wherein only one of the amino acids bears one or more protecting groups.

[0112] The pairs of amino acids typically comprise amino acids where each amino acid is attached to at least one protecting group (*e.g.*, a hydrophobic protecting group as described herein). The amino acids can be in the D or the L form. In certain embodiments, where the amino acids comprising the pairs are not attached to each other, each amino acid bears two protecting groups (*e.g.*, such as molecules 1 and 2 in Table 9).

[0113] Table 9. Illustrative amino acid pairs of this invention.

	Amino Acid Pair/dipeptide
1.	Boc-Arg-OtBu*
2.	Boc-Glu-OtBu*
3.	Boc-Phe-Arg-OtBu**
4.	Boc-Glu-Leu-OtBu**
5.	Boc-Arg-Glu-OtBu***

*This would typically be administered in conjunciton with a second amino acid.

**In certain embodiments, these dipeptides would be administered in conjunction with each other.

*** In certain embodiments, this peptide would be administered either alone or in combination with one of the other peptides described herein..

[0114] Suitable pairs of amino acids can readily be identified by providing the pair of protected amino acids and/or a dipeptide and then screening the pair of amino acids/dipeptide for one or more of the physical and/or physiological properties described above. In certain embodiments, this invention excludes pairs of amino acids and/or dipeptides comprising aspartic acid and phenylalanine. In certain embodiments, this invention excludes pairs of amino acids and/or dipeptides in which one amino acid is (-)-N-[(trans-4-isopropylcyclohexane)carbonyl]-D-phenylalanine (nateglinide).

[0115] In certain embodiments, the amino acids comprising the pair are independently selected from the group consisting of an acidic amino acid (*e.g.*, aspartic acid, glutamic acid, *etc.*), a basic amino acid (*e.g.*, lysine, arginine, histidine, *etc.*), and a non-polar amino acid (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, *etc.*). In certain embodiments, where the first amino acid is acidic or basic, the second amino acid is non-polar and where the second amino acid is acidic or basic, the first amino acid is non-polar. In certain embodiments, where the first amino acid is acidic, the second amino acid is basic, and vice versa. (*see, e.g.*, Table 10).

[0116] Similar combinations can be obtained by administering pairs of dipeptides. Thus, for example in certain embodiments, molecules 3 and 4 in Table 9 would be administered in conjunction with each other.

Table 10. Certain generalized amino acid pairs/dipeptides.

	First Amino acid	Second Amino acid
1.	Acidic	Basic
2.	Basic	Acidic
3.	Acidic	Non-polar
4.	Non-polar	Acidic
5.	Basic	Non-polar
6.	Non-polar	Basic

[0117] It is noted that these amino acid pairs/dipeptides are intended to be illustrative and not limiting. Using the teaching provided herein other suitable amino acid pairs/dipeptides can readily be determined.

D) Apo-J (G* peptides).

[0118] In certain It was a discovery of this invention that peptides that mimicking the amphipathic helical domains of apo J are capable of mitigating one or more symptoms of atherosclerosis and/or other pathologies described herein. Apolipoprotein J possesses a wide nonpolar face termed globular protein-like, or G* amphipathic helical domains. The class G amphipathic helix is found in globular proteins, and thus, the name class G. This class of amphipathic helix is characterized by a random distribution of positively charged and negatively charged residues on the polar face with a narrow nonpolar face. Because of the narrow nonpolar face this class does not readily associate with phospholipids. The G* of amphipathic helix possesses similar, but not identical, characteristics to the G amphipathic helix. Similar to the class G amphipathic helix, the G* class peptides possesses a random distribution of positively and negatively charged residues on the polar face. However, in contrast to the class G amphipathic helix which has a narrow nonpolar face, this class has a wide nonpolar face that allows this class to readily bind phospholipid and the class is termed G* to differentiate it from the G class of amphipathic helix.

[0119] A number of suitable G* amphipathic peptides are described in copending applications USSN 10/120,508, filed April 5, 2002, USSN 10/520,207, filed April 1, 2003, and PCT Application PCT/US03/09988, filed April 1, 2003. In addition, a variety of

suitable peptides of this invention that are related to G* amphipathic helical domains of apo J are illustrated in Table 11.

[0120] Table 11. Preferred peptides for use in this invention related to g* amphipathic helical domains of apo J.

Amino Acid Sequence	SEQ ID NO
LLEQLNEQFNWVSRLANLTQGE	444
LLEQLNEQFNWVSRLANL	445
NELQEMSNQGSKYVNKEIQNAVNGV	446
IQNAVNGVKQIKTLIEKTNEE	447
RKTLLSNLEEAKKKKEDALNETRESETKLKEL	448
PGVCNETMMALWEECK	449
PCLKQTCMKFYARVCR	450
ECKPCLKQTCMKFYARVCR	451
LVGRQLEEFL	452
MNGDRIDSLLEN	453
QQTHMLDVMQD	454
FSRASSIIDELFQD	455
PFLEMIHEAQQAMDI	456
PTEFIREGDDD	457
RMKDQCDKCREILSV	458
PSQAKLRRELDESLQVAERLTRKYNELLKSYQ	459
LLEQLNEQFNWVSRLANLTEGE	460
DQYYLRVTTVA	461
PSGVTEVVVKLFDS	462
PKFMETVAEKALQEYRKKHRE	463

[0121] The peptides of this invention, however, are not limited to G* variants of apo J. Generally speaking G* domains from essentially any other protein preferably apo proteins are also suitable. The particular suitability of such proteins can readily be determined using assays for protective activity (*e.g.*, protecting LDL from oxidation, and the like), *e.g.* as illustrated herein in the Examples. Some particularly preferred proteins include G* amphipathic helical domains or variants thereof (*e.g.*, conservative substitutions, and the like) of proteins including, but not limited to apo AI, apo AIV, apo E, apo CII, apo CIII, and the like.

[0122] Certain preferred peptides for related to G* amphipathic helical domains related to apoproteins other than apo J are illustrated in Table 12.

[0123] **Table 12.** Peptides for use in this invention related to G* amphipathic helical domains related to apoproteins other than apo J.

Amino Acid Sequence	SEQ ID NO
WDRVKDLATVYVDVLKDSGRDYVSQF (Related to the 8 to 33 region of apo AI)	464
VATVMWDYFSQLSNNAKEAVEHLQK (Related to the 7 to 31 region of apo AIV)	465
RWELALGRFWDYLRWVQTLSEQVQEEL (Related to the 25 to 51 region of apo E)	466
LSSQVTQELRALMDETMKELKELKAYKSELEEQLT (Related to the 52 to 83 region of apo E)	467
ARLSKELQAAQARLGADMEDVCGRLV (Related to the 91 to 116 region of apo E)	468
VRLASHLRKLRKRLLRDADDLQKRLA (Related to the135 to 160 region of apo E)	469
PLVEDMQRQWAGLVEKVQA (267 to 285 of apo E.27)	470
MSTYTGIFTDQVLSVLK (Related to the 60 to 76 region of apo CII)	471
LLSFMQGYMKHATKTAKDALSS (Related to the 8 to 29 region of apo CIII)	472

[0124] Additional illustrative G* peptides are shown in Table 13.

[0125] Table 13. Additional illustrative G* peptides.

Peptide	SEQ ID NO
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	473
Ac-Lys-Trp-Phe-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	474
Ac-Lys-Trp-Leu-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	475
Ac-Lys-Trp-Val-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	476

Ac-Lys-Tyr-Ile-Trp-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	477
Ac-Lys-Trp-Ile-Tyr-His-Phe-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	478
Ac-Lys-Trp-Phe-Tyr-His-Ile-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	479
Ac-Lys-Trp-Leu-Tyr-His-Val-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	480
Ac-Lys-Trp-Val-Tyr-His-Tyr-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	481
Ac-Lys-Tyr-Ile-Trp-His-Phe-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	482
Ac-Lys-Tyr-Ile-Trp-His-Ile-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	483
Ac-Lys-Tyr-Ile-Trp-His-Val-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	484
Ac-Lys-Tyr-Ile-Trp-His-Tyr-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	485
Ac-Lys-Phe-Ile-Trp-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	486
Ac-Lys-Leu-Ile-Trp-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	487
Ac-Lys-Ile-Ile-Trp-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	488
Ac-Lys-Tyr-Ile-Trp-Phe-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	489
Ac-Lys-Trp-Ile-Tyr-Phe-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	490
Ac-Lys-Trp-Ile-Tyr-Leu-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	491
Ac-Lys-Trp-Ile-Tyr-His-Phe-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	492
Ac-Lys-Trp-Ile-Tyr-His-Tyr-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	493
Ac-Lys-Trp-Ile-Tyr-His-Ile-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	494
Ac-Lys-Trp-Ile-Tyr-His-Leu-Ser-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	495
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Asp-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	496
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Thr-Ser-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	497
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Glu-Leu-Arg-Thr-Glu-Gly-NH_2	498
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Phe-Arg-Thr-Glu-Gly-NH_2	499
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Tyr-Arg-Thr-Glu-Gly-NH_2	500
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Arg-Thr-Glu-Gly-NH_2	501
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Val-Arg-Thr-Glu-Gly-NH_2	502

Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Lys-Thr-Glu-Gly-NH_2	503
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Ser-Glu-Gly-NH_2	504
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Asp-Gly-NH_2	505
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Lys-Thr-Glu-Gly-NH_2	506
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Arg-Ser-Glu-Gly-NH_2	507
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Lys-Ser-Glu-Gly-NH_2	508
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Lys-Ser-Asp-Gly-NH_2	509
Ac-Arg-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	510
Ac-Arg-Tyr-Ile-Trp-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Arg-Thr-Glu-Gly-NH_2	511
Ac-Arg-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Arg-Thr-Asp-Gly-NH_2	512
Ac-Arg-Trp-Ile-Phe-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Arg-Thr-Glu-Gly-NH_2	513
Ac-Arg-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Lys-Thr-Glu-Gly-NH_2	514
Ac-Arg-Trp-Ile-Tyr-His-Leu-Thr-Asp-Gly-Ser-Thr-Asp-Ile-Arg-Thr-Glu-Gly-NH_2	515
Ac-Arg-Trp-Ile-Tyr-His-Leu-Thr-Asp-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	516
Ac-Arg-Trp-Ile-Tyr-Phe-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Arg-Thr-Glu-Gly-NH_2	517
Ac-Arg-Trp-Ile-Tyr-Phe-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	518
Ac-Lys-Trp-Phe-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Phe-Arg-Thr-Glu-Gly-NH_2	519
Ac-Arg-Trp-Phe-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	520
Ac-Lys-Trp-Ile-Phe-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Arg-Thr-Asp-Gly-NH_2	521
Ac-Arg-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Arg-Thr-Asp-Gly-NH_2	522
Ac-Arg-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Asp-Gly-NH_2	523
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Lys-Thr-Glu-Gly-NH_2	524
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Lys-Thr-Asp-Gly-NH_2	525
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Phe-Lys-Thr-Glu-Gly-NH_2	526
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Tyr-Lys-Thr-Glu-Gly-NH_2	527
Ac-Lys-Trp-Ile-Tyr-His-Leu-Thr-Glu-Gly-Ser-Thr-Asp-Ile-Arg-Thr-Glu-Gly-NH_2	528

Ac-Lys-Trp-Phe-Tyr-His-Phe-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	529
Ac-Arg-Trp-Phe-Tyr-His-Phe-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	530
Ac-Lys-Trp-Phe-Tyr-His-Phe-Thr-Glu-Gly-Ser-Thr-Asp-Phe-Arg-Thr-Glu-Gly-NH_2	531
Ac-Lys-Trp-Phe-Tyr-His-Phe-Thr-Asp-Gly-Ser-Thr-Asp-Ile-Arg-Thr-Glu-Gly-NH_2	532
Ac-Arg-Trp-Phe-Tyr-His-Phe-Thr-Glu-Gly-Ser-Thr-Asp-Leu-Arg-Thr-Glu-Gly-NH_2	533
Ac-Arg-Trp-Phe-Tyr-His-Phe-Thr-Glu-Gly-Ser-Thr-Asp-Phe-Arg-Thr-Glu-Gly-NH_2	534
Ac-Arg-Trp-Phe-Tyr-His-Phe-Thr-Glu-Gly-Ser-Thr-Asp-Phe-Arg-Thr-Asp-Gly-NH_2	535
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Leu-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	536
Ac-Asp-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Leu-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	537
Ac-Glu-Lys-Cys-Val-Asp-Glu-Phe-Lys-Ser-Leu-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	538
Ac-Glu-Lys-Cys-Val-Glu-Asp-Phe-Lys-Ser-Leu-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	539
Ac-Glu-Arg-Cys-Val-Glu-Glu-Phe-Lys-Ser-Leu-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	540
Ac-Asp-Lys-Cys-Val-Asp-Asp-Phe-Lys-Ser-Leu-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	541
Ac-Asp-Arg-Cys-Val-Glu-Glu-Phe-Lys-Ser-Leu-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	542
Ac-Glu-Arg-Cys-Val-Asp-Asp-Phe-Lys-Ser-Leu-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	543
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	544
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Ile-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	545
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Val-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	546
Ac-Glu-Arg-Cys-Val-Glu-Glu-Phe-Lys-Ser-Tyr-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	547
Ac-Glu-Arg-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	548
Ac-Glu-Arg-Cys-Val-Glu-Glu-Phe-Lys-Ser-Ile-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	549
Ac-Glu-Arg-Cys-Val-Glu-Glu-Phe-Lys-Ser-Val-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	550
Ac-Glu-Arg-Cys-Val-Glu-Glu-Phe-Lys-Ser-Tyr-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	551
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Thr-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	552
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Ile-Ser-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	553
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Val-Ser-Thr-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	554

Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Tyr-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	555
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Thr-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	556
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Ser-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	557
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	558
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	559
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	560
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Asp-Ser-Lys-Ala-Phe-NH_2	561
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Glu-Ser-Lys-Ala-Phe-NH_2	562
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Glu-Ser-Lys-Ala-Phe-NH_2	563
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Ile-Asp-Ser-Lys-Ala-Phe-NH_2	564
Ac-Glu-Lys-Cys-Val-Glu-Glu-Leu-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Asp-Ser-Lys-Ala-Phe-NH_2	565
Ac-Asp-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Asp-Ser-Lys-Ala-Phe-NH_2	566
Ac-Asp-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Glu-Ser-Lys-Ala-Phe-NH_2	567
Ac-Glu-Arg-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Asp-Ser-Lys-Ala-Phe-NH_2	568
Ac-Glu-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Asp-Ser-Lys-Ala-Phe-NH_2	569
Ac-Glu-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Glu-Ser-Lys-Ala-Phe-NH_2	570
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Ser-Ser-Cys-Phe-Glu-Ser-Lys-Ala-Phe-NH_2	571
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Gln-Ser-Cys-Phe-Asp-Ser-Lys-Ala-Phe-NH_2	572
Ac-Glu-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Gln-Ser-Cys-Phe-Asp-Ser-Lys-Ala-Phe-NH_2	573
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Gln-Phe-Thr-Ser-Cys-Phe-Asp-Ser-Lys-Ala-Phe-NH_2	574
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Gln-Leu-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	575
Ac-Glu-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Gln-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	576
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Gln-Phe-Thr-Ser-Cys-Phe-Asp-Ser-Lys-Ala-Phe-NH_2	577
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Glu-Ser-Lys-Ala-Phe-NH_2	578
Ac-Glu-Arg-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Asp-Ser-Lys-Ala-Phe-NH_2	579
Ac-Asp-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Asp-Ser-Lys-Ala-Phe-NH_2	580

Ac-Glu-Arg-Cys-Val-Glu-Glu-Phe-Lys-Ser-Leu-Thr-Ser-Cys-Leu-Glu-Ser-Lys-Ala-Phe-NH_2	581
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Leu-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Phe-Phe-NH_2	582
Ac-Glu-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Asp-Ser-Lys-Phe-Phe-NH_2	583
Ac-Asp-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Phe-Phe-NH_2	584
Ac-Asp-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Glu-Ser-Lys-Phe-Phe-NH_2	585
Ac-Asp-Lys-Cys-Phe-Glu-Glu-Leu-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Phe-Phe-NH_2	586
Ac-Glu-Arg-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Phe-Phe-NH_2	587
Ac-Glu-Lys-Ala-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	588
Ac-Asp-Lys-Ala-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Phe-Phe-NH_2	589
Ac-Glu-Lys-Ala-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Ala-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	590
Ac-Asp-Lys-Ala-Val-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Ala-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	591
Ac-Asp-Arg-Ala-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Phe-Phe-NH_2	592
Ac-Asp-Arg-Ala-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Ala-Leu-Asp-Ser-Lys-Phe-Phe-NH_2	593
Ac-Asp-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Phe-Glu-Ser-Lys-Phe-Phe-NH_2	594
Ac-Glu-Lys-Cys-Tyr-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Phe-Phe-NH_2	595
Ac-Asp-Lys-Cys-Trp-Glu-Glu-Phe-Lys-Ser-Phe-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Phe-Phe-NH_2	596
Ac-Glu-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Tyr-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Phe-Phe-NH_2	597
Ac-Glu-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Trp-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Phe-Phe-NH_2	598
Ac-Glu-Lys-Cys-Val-Glu-Glu-Phe-Lys-Ser-Trp-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	599
Ac-Asp-Lys-Cys-Phe-Glu-Glu-Phe-Lys-Ser-Trp-Thr-Ser-Cys-Leu-Asp-Ser-Lys-Ala-Phe-NH_2	600

[0126] Other suitable peptides include, but are not limited to the peptides of Table 14.

[0127] Table 14. Illustrative peptides having an improved hydrophobic phase.

Name	Sequence	SEQ ID NO
V2W3A5F1017-D-4F	Ac-Asp-Val-Trp-Lys-Ala-Ala-Tyr-Asp-Lys-Phe-Ala-Glu-Lys-Phe-Lys-Glu-Phe-Phe-NH2	601

V2W3F10-D-4F	Ac-Asp-Val-Trp-Lys-Ala-Phe-Tyr-Asp-Lys-Phe-Ala-Glu-Lys-Phe-Lys-Glu-Ala-Phe-NH2	602
W3-D-4F	Ac-Asp-Phe-Trp-Lys-Ala-Phe-Tyr-Asp-Lys-Val-Ala-Glu-Lys-Phe-Lys-Glu-Ala-Phe-NH2	603

[0128] The peptides described here (V2W3A5F10,17-D-4F; V2W3F10-D-4F; W3-D-4F) may be more potent than the original D-4F.

[0129] Still other suitable peptides include, but are not limited to: P^1-Dimethyltyrosine-D-Arg-Phe-Lys-P^2 (SEQ ID NO:604) and P^1-Dimethyltyrosine-Arg-Glu-Leu-P^2 where P1 and P2 are protecting groups as described herein. In certain embodiments, these peptides include, but are not limited to BocDimethyltyrosine-D-Arg-Phe-Lys(OtBu) and BocDimethyltyrosine-Arg-Glu-Leu(OtBu).

[0130] In certain embodiments, the peptides of this invention include peptides comprising or consisting of the amino acid sequence LAEYHAK (SEQ ID NO:605) comprising at least one D amino acid and/or at least one or two terminal protecting groups. In certain embodiments, this invention includes a A peptide that ameliorates one or more symptoms of an inflammatory condition, wherein the peptide: ranges in length from about 3 to about 10 amino acids; comprises an amino acid sequence where the sequence comprises acidic or basic amino acids alternating with aromatic or hydrophobic amino acids; comprises hydrophobic terminal amino acids or terminal amino acids bearing a hydrophobic protecting group; is not the sequence LAEYHAK (SEQ ID NO:606) comprising all L amino acids; where the peptide converts pro-inflammatory HDL to anti-inflammatory HDL and/or makes anti-inflammatory HDL more anti-inflammatory.

[0131] It is also noted that the peptides listed in the Tables herein are not fully inclusive. Using the teaching provided herein, other suitable peptides can routinely be produced (*e.g.* by conservative or semi-conservative substitutions (*e.g.* D replaced by E), extensions, deletions, and the like). Thus, for example, one embodiment utilizes truncations of any one or more of peptides identified by SEQ ID Nos:444-472.

[0132] Longer peptides are also suitable. Such longer peptides may entirely form a class G or G* amphipathic helix, or the G amphipathic helix (helices) can form one or more domains of the peptide. In addition, this invention contemplates multimeric versions of the peptides. Thus, for example, the peptides illustrated in the tables herein can be coupled

together (directly or through a linker (*e.g.* a carbon linker, or one or more amino acids) with one or more intervening amino acids). Suitable linkers include, but are not limited to Proline (-Pro-), Gly_4Ser_3 (SEQ ID NO: 607), $(Gly_4Ser)_3$ (SEQ ID NO: 608) and the like. Thus, one illustrative multimeric peptide according to this invention is (D-J336)-P-(D-J336) (*i.e.* Ac-L-L-E-Q-L-N-E-Q-F-N-W-V-S-R-L-A-N-L-T- Q-G-E-**P**-L-L-E-Q-L-N-E-Q-F-N-W-V-S-R-L-A-N-L-T-Q-G-E-NH_2, SEQ ID NO: 609).

[0133] This invention also contemplates the use of "hybrid" peptides comprising a one or more G or G* amphipathic helical domains and one or more class A amphipathic helices. Suitable class A amphipathic helical peptides are described in PCT publication WO 02/15923. Thus, by way of illustration, one such "hybrid" peptide is (D-J336)-Pro-(4F) (*i.e.* Ac-L-L-E-Q-L-N-E-Q-F-N-W-V-S-R-L-A-N-L-T-Q-G-E-**P**-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2, SEQ ID NO: 610), and the like.

[0134] Using the teaching provided herein, one of skill can routinely modify the illustrated amphipathic helical peptides to produce other suitable apo J variants and/or amphipathic G and/or A helical peptides of this invention. For example, routine conservative or semi-conservative substitutions (*e.g.*, E for D) can be made of the existing amino acids. The effect of various substitutions on lipid affinity of the resulting peptide can be predicted using the computational method described by Palgunachari *et al.* (1996) *Arteriosclerosis, Thrombosis, & Vascular Biology* 16: 328-338. The peptides can be lengthened or shortened as long as the class helix structure(s) are preserved. In addition, substitutions can be made to render the resulting peptide more similar to peptide(s) endogenously produced by the subject species.

[0135] While, in preferred embodiments, the peptides of this invention utilize naturally-occurring amino acids or D forms of naturally occurring amino acids, substitutions with non-naturally occurring amino acids (*e.g.*, methionine sulfoxide, methionine methylsulfonium, norleucine, episilon-aminocaproic acid, 4-aminobutanoic acid, tetrahydroisoquinoline-3-carboxylic acid, 8-aminocaprylic acid, 4-aminobutyric acid, Lys(N(epsilon)-trifluoroacetyl), α-aminoisobutyric acid, and the like) are also contemplated.

[0136] New peptides can be designed and/or evaluated using computational methods. Computer programs to identify and classify amphipathic helical domains are well known to those of skill in the art and many have been described by Jones *et al.*(1992) *J.*

Lipid Res. 33: 287-296). Such programs include, but are not limited to the helical wheel program (WHEEL or WHEEL/SNORKEL), helical net program (HELNET, HELNET/SNORKEL, HELNET/Angle), program for addition of helical wheels (COMBO or COMBO/SNORKEL), program for addition of helical nets (COMNET, COMNET/SNORKEL, COMBO/SELECT, COMBO/NET), consensus wheel program (CONSENSUS, CONSENSUS/SNORKEL), and the like.

E) Blocking groups and D residues.

[0137] While the various peptides and/or amino acid pairs described herein may be be shown with no protecting groups, in certain embodiments (*e.g.* particularly for oral administration), they can bear one, two, three, four, or more protecting groups. The protecting groups can be coupled to the C- and/or N-terminus of the peptide(s) and/or to one or more internal residues comprising the peptide(s) (*e.g.*, one or more R-groups on the constituent amino acids can be blocked). Thus, for example, in certain embodiments, any of the peptides described herein can bear, *e.g.* an acetyl group protecting the amino terminus and/or an amide group protecting the carboxyl terminus. One example of such a "dual protected peptide is Ac-L-L-E-Q-L-N-E-Q-F-N-W-V-S-R-L-A-N-L-T-Q-G-E-NH_2 (SEQ ID NO:444 with blocking groups), either or both of these protecting groups can be eliminated and/or substituted with another protecting group as described herein.

[0138] Without being bound by a particular theory, it was a discovery of this invention that blockage, particularly of the amino and/or carboxyl termini of the subject peptides of this invention greatly improves oral delivery and significantly increases serum half-life.

[0139] A wide number of protecting groups are suitable for this purpose. Such groups include, but are not limited to acetyl, amide, and alkyl groups with acetyl and alkyl groups being particularly preferred for N-terminal protection and amide groups being preferred for carboxyl terminal protection. In certain particularly preferred embodiments, the protecting groups include, but are not limited to alkyl chains as in fatty acids, propeonyl, formyl, and others. Particularly preferred carboxyl protecting groups include amides, esters, and ether-forming protecting groups. In one preferred embodiment, an acetyl group is used to protect the amino terminus and an amide group is used to protect the carboxyl terminus. These blocking groups enhance the helix-forming tendencies of the peptides. Certain

particularly preferred blocking groups include alkyl groups of various lengths, *e.g.* groups having the formula: CH_3-$(CH_2)_n$-CO- where n ranges from about 1 to about 20, preferably from about 1 to about 16 or 18, more preferably from about 3 to about 13, and most preferably from about 3 to about 10.

[0140] In certain particularly preferred embodiments, the protecting groups include, but are not limited to alkyl chains as in fatty acids, propeonyl, formyl, and others. Particularly preferred carboxyl protecting groups include amides, esters, and ether-forming protecting groups. In one preferred embodiment, an acetyl group is used to protect the amino terminus and an amide group is used to protect the carboxyl terminus. These blocking groups enhance the helix-forming tendencies of the peptides. Certain particularly preferred blocking groups include alkyl groups of various lengths, *e.g.* groups having the formula: CH_3-$(CH_2)_n$-CO- where n ranges from about 3 to about 20, preferably from about 3 to about 16, more preferably from about 3 to about 13, and most preferably from about 3 to about 10.

[0141] Other protecting groups include, but are not limited to Fmoc, t-butoxycarbonyl (*t*-BOC), 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

[0142] Protecting/blocking groups are well known to those of skill as are methods of coupling such groups to the appropriate residue(s) comprising the peptides of this invention (*see, e.g.,* Greene *et al.,* (1991) *Protective Groups in Organic Synthesis, 2nd ed.,* John Wiley & Sons, Inc. Somerset, N.J.). In one preferred embodiment, for example, acetylation is accomplished during the synthesis when the peptide is on the resin using acetic

anhydride. Amide protection can be achieved by the selection of a proper resin for the synthesis. During the synthesis of the peptides described herein in the examples, rink amide resin was used. After the completion of the synthesis, the semipermanent protecting groups on acidic bifunctional amino acids such as Asp and Glu and basic amino acid Lys, hydroxyl of Tyr are all simultaneously removed. The peptides released from such a resin using acidic treatment comes out with the n-terminal protected as acetyl and the carboxyl protected as NH_2 and with the simultaneous removal of all of the other protecting groups.

[0143] In certain particularly preferred embodiments, the peptides comprise one or more D-form (dextro rather than levo) amino acids as described herein. In certain embodiments at least two enantiomeric amino acids, more preferably at least 4 enantiomeric amino acids and most preferably at least 8 or 10 enantiomeric amino acids are "D" form amino acids. In certain embodiments every other, ore even every amino acid (*e.g.* every enantiomeric amino acid) of the peptides described herein is a D-form amino acid.

[0144] In certain embodiments at least 50% of the enantiomeric amino acids are "D" form, more preferably at least 80% of the enantiomeric amino acids are "D" form, and most preferably at least 90% or even all of the enantiomeric amino acids are "D" form amino acids.

F) Peptide Mimetics.

[0145] In addition to the peptides described herein, peptidomimetics are also contemplated. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics" (Fauchere (1986) *Adv. Drug Res.* 15: 29; Veber and Freidinger (1985) *TINS* p.392; and Evans *et al.* (1987) *J. Med. Chem.* 30: 1229) and are usually developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect.

[0146] Generally, peptidomimetics are structurally similar to a paradigm polypeptide (*e.g.* SEQ ID NO:5 shown in Table 1), but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: $-CH_2NH-$, $-CH_2S-$, $-CH_2-CH_2-$, -CH=CH- (cis and trans), $-COCH_2-$, $-CH(OH)CH_2-$, $-CH_2SO-$, *etc.* by methods

known in the art and further described in the following references: Spatola (1983) p. 267 in *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*, B. Weinstein, eds., Marcel Dekker, New York,; Spatola (1983) *Vega Data* 1(3) *Peptide Backbone Modifications*. (general review); Morley (1980) *Trends Pharm Sci* pp. 463-468 (general review); Hudson *et al.* (1979) *Int J Pept Prot Res* 14:177-185 (-CH_2NH-, CH_2CH_2-); Spatola et al. (1986) *Life Sci* 38:1243-1249 (-CH_2-S); Hann, (1982) *J Chem Soc Perkin Trans* I 307-314 (-CH-CH-, cis and trans); Almquist *et al.* (1980) *J Med Chem.* 23:1392-1398 (-$COCH_2$-); Jennings-White *et al.*(1982) *Tetrahedron Lett.* 23:2533 (-$COCH_2$-); Szelke et al., European Appln. EP 45665 (1982) CA: 97:39405 (1982) (-CH(OH)CH2-); Holladay *et al.* (1983) *Tetrahedron Lett* 24:4401-4404 (-$C(OH)CH_2$-); and Hruby (1982) *Life Sci.*, 31:189-199 (-CH_2-S-)).

[0147] One particularly preferred non-peptide linkage is -CH_2NH-. Such peptide mimetics may have significant advantages over polypeptide embodiments, including, for example: more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, *etc.*), reduced antigenicity, and others.

[0148] In addition, circularly permutations of the peptides described herein or constrained peptides (including cyclized peptides) comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch (1992) *Ann. Rev. Biochem.* 61: 387); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

G) Small organic molecules.

[0149] In certain embodiments, the active agents of this invention include small organic molecules, *e.g.* as described in copending application USSN 60/600,925, filed August 11, 2004. In various embodiments the small organic molecules are similar to, and in certain cases, mimetics of the tetra- and penta-peptides described in copending application USSN 10/649,378, filed on August 26, 2003 and USSN 60/494,449, filed on August 11.

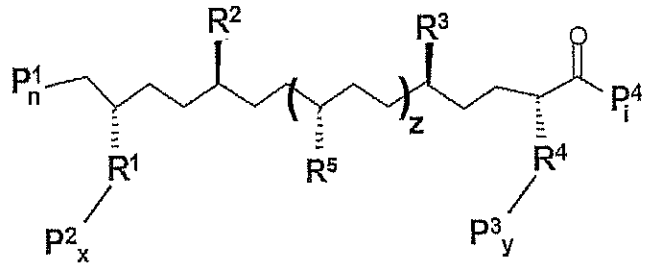
[0150] The small organic molecules of this invention typically have molecular weights less than about 900 Daltons. Typically the molecules are are highly soluble in ethyl

acetate (*e.g.*, at concentrations equal to or greater than 4 mg/mL), and also are soluble in aqueous buffer at pH 7.0.

[0151] Contacting phospholipids such as 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), with the small organic molecules of this invention in an aqueous environment typically results in the formation of particles with a diameter of approximately 7.5 nm (± 0.1 nm). In addition, stacked bilayers are often formed with a bilayer dimension on the order of 3.4 to 4.1 nm with spacing between the bilayers in the stack of approximately 2 nm. Vesicular structures of approximately 38 nm are also often formed. Moreover, when the molecules of this invention are administered to a mammal they render HDL more anti-inflammatory and mitigate one or more symptoms of atherosclerosis and/or other conditions characterized by an inflammatory response.

[0152] Thus, in certain embodiments, the small organic molecule is one that ameliorates one or more symptoms of a pathology characterized by an inflammatory response in a mammal (*e.g.* atherosclerosis), where the small molecule is soluble in in ethyl acetate at a concentration greater than 4mg/mL, is soluble in aqueous buffer at pH 7.0, and, when contacted with a phospholipid in an aqueous environment, forms particles with a diameter of approximately 7.5 nm and forms stacked bilayers with a bilayer dimension on the order of 3.4 to 4.1 nm with spacing between the bilayers in the stack of approximately 2 nm, and has a molecular weight les than 900 daltons.

[0153] In certain embodiment, the molecule has the formula:



I.

where P^1, P^2, P^3, and P^4 are independently selected hydrophobic protecting groups; R^1 and $R^4$are independently selected amino acid R groups; n, i, x, y, and z are independently zero or 1 such that when n and x are both zero, R^1 is a hydrophobic group and when y and i are

both zero, R^4 is a hydrophobic group; R^2 and R^3 are acidic or basic groups at pH 7.0 such that when R^2 is acidic, R^3 is basic and when R^2 is basic, R^3 is acidic; and R^5, when present is selected from the group consisting of an aromatic group, an aliphatic group, a postively charged group, or a negatively charged group. In certain embodiments, R^2 or R^3 is -(CH_2)j-COOH where j=1, 2, 3, or 4 and/or -(CH_2)j-NH_2 where j = 1, 2, 3, 4, or 5, or -(CH_2)j-NH-C(=NH)-NH_2 where n= 1, 2, 3 or 4. In certain embodiments, R^2, R^3, and R^5, when present, are amino acid R groups. Thus, for example, In various embodiments R^2 and R^3 are independently an aspartic acid R group, a glutamic acid R group, a lysine R group, a histidine R group, or an arginine R group (*e.g.*, as illustrated in Table 1).

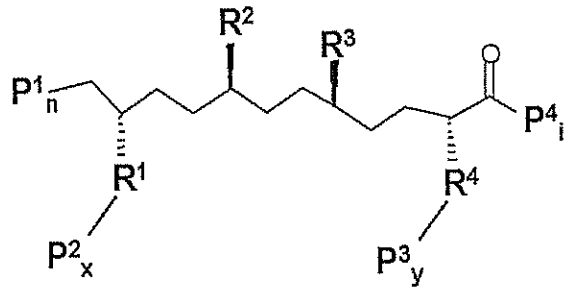
[0154] In certain embodiments, R^1 is selected from the group consisting of a Lys R group, a Trp R group, a Phe R group, a Leu R group, an Orn R group, pr a norLeu R group. In certain embodiments, R^4 is selected from the group consisting of a Ser R group, a Thr R group, an Ile R group, a Leu R group, a norLeu R group, a Phe R group, or a Tyr R group.

[0155] In various embodiments x is 1, and R^5 is an aromatic group (*e.g.*, a Trp R group).

[0156] In various embodiments at least one of n, x, y, and i is 1 and P^1, P^2, P^3, and P^4 when present, are independently selected from the group consisting of polyethylene glycol (PEG), an acetyl, amide, a 3 to 20 carbon alkyl group, fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-fluorenecarboxylic, 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts),-4,4-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), benzyloxy (BzlO), benzyl (Bzl), benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), a propyl group, a butyl group, a pentyl group, a hexyl group, and trifluoroacetyl (TFA). In certain embodiments, P^1 when present and/or P^2 when present are independently selected from the group consisting of Boc-, Fmoc-, and Nicotinyl- and/or P^3 when present and/or P^4 when present are independently selected from the group consisting of *t*Bu, and O*t*Bu.

[0157] While a number of protecting groups (P^1, P^2, P^3, P^4) are illustrated above, this list is intended to be illustrative and not limiting. In view of the teachings provided herein, a number of other protecting/blocking groups will also be known to one of skill in the art. Such blocking groups can be selected to minimize digestion (*e.g.*, for oral pharmaceutical delivery), and/or to increase uptake/bioavailability (*e.g.*, through mucosal surfaces in nasal delivery, inhalation therapy, rectal administration), and/or to increase serum/plasma half-life. In certain embodiments, the protecting groups can be provided as an excipient or as a component of an excipient.

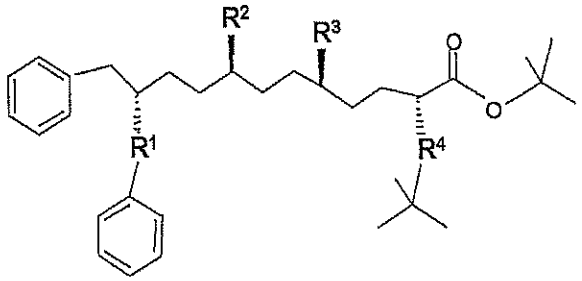
[0158] In certain embodiments, z is zero and the molecule has the formula:



II.

where P^1, P^2, P^3, P^4, R^1, R^2, R^3, R^4, n, x, y, and i are as described above.

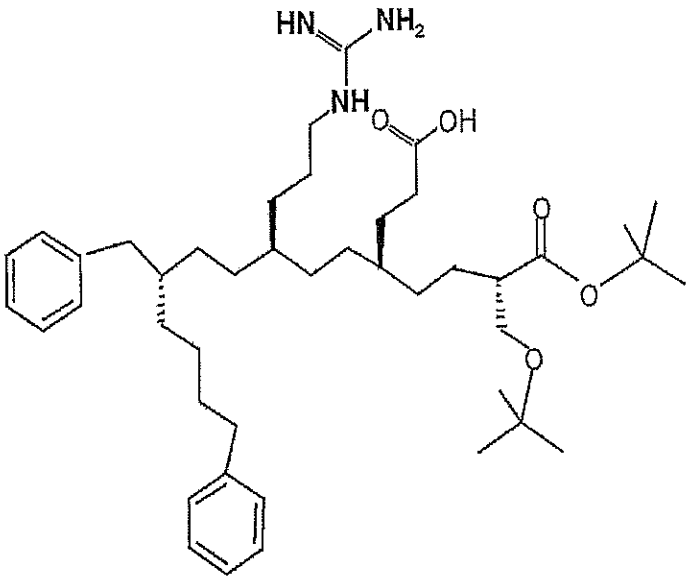
[0159] In certain embodiments, z is zero and the molecule has the formula:



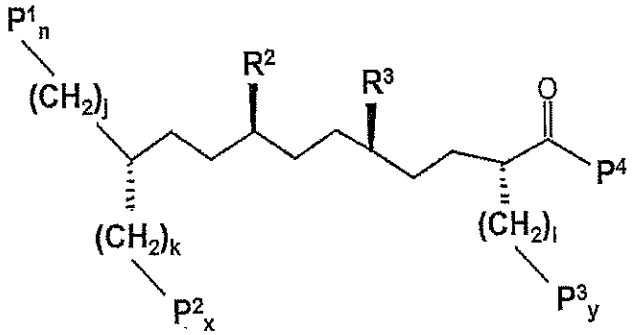
III.

where R^1, R^2, R^3, and R^4 are as described above .

[0160] In one embodiment, the molecule has the formula:



[0161] In certain embodiments, this invention contemplates small molecules having one or more of the physical and/or functional properties described herein and having the formula:



where P^1, P^2, P^3, and P^4 are independently selected hydrophobic protecting groups as described above, n, x, and y are independently zero or 1; j, k, and l are independently zero, 1, 2, 3, 4, or 5; and R^2 and R^3 are acidic or basic groups at pH 7.0 such that when R^2 is acidic, R^3 is basic and when R^2 is basic, R^3 is acidic. In certain preferred embodiments, the small molecule is soluble in water; and the small molecule has a molecular weight less than about 900 Daltons. In certain embodiments, n, x, y, j, and l are 1; and k is 4.

[0162] In certain embodiments, P^1 and/or P^2 are aromatic protecting groups. In certain embodiments, R^2 and R^3 are amino acid R groups, *e.g.*, as described above. In various embodiments least one of n, x, and y, is 1 and P^1, P^2, P^3 and P^4 when present, are independently protecting groups, *e.g.* as described above.selected from the group consisting of polyethylene glycol (PEG), an acetyl, amide, 3 to 20 carbon alkyl groups, Fmoc, 9-

fluoreneacetyl group, 1-fluorenecarboxylic group, 9-fluorenecarboxylic, 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts),-4,4-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-penta

II. Functional assays of active agents.

[0163] Certain active agents for use in the methods of this invention are described herein by various formulas (*e.g.*, Formula I, above) and/or by particular sequences. In certain embodiments, preferred active agents of this invention are characterized by one or more of the following functional properties:

1. They convert pro-inflammatory HDL to anti-inflammatory HDL or make anti-inflammatory HDL more anti-inflammatory;
2. They decrease LDL-induced monocyte chemotactic activity generated by artery wall cells;
3. They stimulate the formation and cycling of pre-β HDL;
4. They raise HDL cholesterol; and/or
5. They increase HDL paraoxonase activity.

[0164] The specific agents disclosed herein, and/or agents corresponding to the various formulas described herein can readily be tested for one or more of these activities as desired.

[0165] Methods of screening for each of these functional properties are well known to those of skill in the art. In particular, it is noted that assays for monocyte chemotactic activity, HDL cholesterol, and HDL HDL paraoxonase activity are illustrated in PCT/US01/26497 (WO 2002/15923).

III. Peptide preparation.

[0166] The peptides used in this invention can be chemically synthesized using standard chemical peptide synthesis techniques or, particularly where the peptide does not comprise "D" amino acid residues, can be recombinantly expressed. In certain embodiments, even peptides comprising "D" amino acid residues are recombinantly

expressed. Where the polypeptides are recombinantly expressed, a host organism (*e.g.* bacteria, plant, fungal cells, *etc.*) in cultured in an environment where one or more of the amino acids is provided to the organism exclusively in a D form. Recombinantly expressed peptides in such a system then incorporate those D amino acids.

[0167] In preferred embodiments the peptides are chemically synthesized by any of a number of fluid or solid phase peptide synthesis techniques known to those of skill in the art. Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence is a preferred method for the chemical synthesis of the polypeptides of this invention. Techniques for solid phase synthesis are well known to those of skill in the art and are described, for example, by Barany and Merrifield (1963) *Solid-Phase Peptide Synthesis;* pp. 3-284 *in The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in PeptideSynthesis, Part A.*; Merrifield *et al.* (1963) *J. Am. Chem. Soc.*, 85: 2149-2156, and Stewart et al. (1984) *Solid Phase Peptide Synthesis,* 2nd ed. Pierce Chem. Co., Rockford, Ill.

[0168] In certain embodiments, the peptides are synthesized by the solid phase peptide synthesis procedure using a benzhyderylamine resin (Beckman Bioproducts, 0.59 mmol of NH_2/g of resin) as the solid support. The COOH terminal amino acid (*e.g.*, *t*-butylcarbonyl-Phe) is attached to the solid support through a 4-(oxymethyl)phenacetyl group. This is a more stable linkage than the conventional benzyl ester linkage, yet the finished peptide can still be cleaved by hydrogenation. Transfer hydrogenation using formic acid as the hydrogen donor is used for this purpose. Detailed protocols used for peptide synthesis and analysis of synthesized peptides are described in a miniprint supplement accompanying Anantharamaiah *et al.* (1985) *J. Biol. Chem.*, 260(16): 10248-10255.

[0169] It is noted that in the chemical synthesis of peptides, particularly peptides comprising D amino acids, the synthesis usually produces a number of truncated peptides in addition to the desired full-length product. The purification process (*e.g.* HPLC) typically results in the loss of a significant amount of the full-length product.

[0170] It was a discovery of this invention that, in the synthesis of a D peptide (*e.g.* D-4), in order to prevent loss in purifying the longest form one can dialyze and use the

mixture and thereby eliminate the last HPLC purification. Such a mixture loses about 50% of the potency of the highly purified product (*e.g.* per wt of protein product), but the mixture contains about 6 times more peptide and thus greater total activity.

IV. Pharmaceutical formulations.

[0171] In order to carry out the methods of the invention, one or more agents (*e.g.* peptides, peptide mimetics, lipids) of this invention are administered, *e.g.* to an individual diagnosed as having impaired arteriole function or as being at risk of impaired arteriole function (*e.g.* in the brain or kidney). The agents (*e.g.* peptides, peptide mimetics, lipids) can be administered in the "native" form or, if desired, in the form of salts, esters, amides, prodrugs, derivatives, and the like, provided the salt, ester, amide, prodrug or derivative is suitable pharmacologically, *i.e.*, effective in the present method. Salts, esters, amides, prodrugs and other derivatives of the active agents may be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry and described, for example, by March (1992) *Advanced Organic Chemistry; Reactions, Mechanisms and Structure*, 4th Ed. N.Y. Wiley-Interscience.

[0172] For example, acid addition salts are prepared from the free base using conventional methodology, that typically involves reaction with a suitable acid. Generally, the base form of the drug is dissolved in a polar organic solvent such as methanol or ethanol and the acid is added thereto. The resulting salt either precipitates or may be brought out of solution by addition of a less polar solvent. Suitable acids for preparing acid addition salts include both organic acids, *e.g.*, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like, as well as inorganic acids, *e.g.*, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. An acid addition salt may be reconverted to the free base by treatment with a suitable base. Particularly preferred acid addition salts of the active agents herein are halide salts, such as may be prepared using hydrochloric or hydrobromic acids. Conversely, preparation of basic salts of the agents (*e.g.* peptides, peptide mimetics) are prepared in a similar manner using a pharmaceutically acceptable base such as sodium hydroxide, potassium hydroxide,

ammonium hydroxide, calcium hydroxide, trimethylamine, or the like. Particularly preferred basic salts include alkali metal salts, *e.g.*, the sodium salt, and copper salts.

[0173] Preparation of esters typically involves functionalization of hydroxyl and/or carboxyl groups which may be present within the molecular structure of the drug. The esters are typically acyl-substituted derivatives of free alcohol groups, *i.e.*, moieties that are derived from carboxylic acids of the formula RCOOH where R is alky, and preferably is lower alkyl. Esters can be reconverted to the free acids, if desired, by using conventional hydrogenolysis or hydrolysis procedures.

[0174] Amides and prodrugs may also be prepared using techniques known to those skilled in the art or described in the pertinent literature. For example, amides may be prepared from esters, using suitable amine reactants, or they may be prepared from an anhydride or an acid chloride by reaction with ammonia or a lower alkyl amine. Prodrugs are typically prepared by covalent attachment of a moiety that results in a compound that is therapeutically inactive until modified by an individual's metabolic system.

[0175] The agents (*e.g.* peptides, peptide mimetics, lipids) identified herein are useful for parenteral, topical, oral, nasal (or otherwise inhaled), rectal, or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment of atherosclerosis and/or symptoms thereof. The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. Suitable unit dosage forms, include, but are not limited to powders, tablets, pills, capsules, lozenges, suppositories, patches, nasal sprays, injectibles, implantable sustained-release formulations, lipid complexes, *etc.*

[0176] The agents (*e.g.* peptides, peptide mimetics, and/or lipids) of this invention are typically combined with a pharmaceutically acceptable carrier (excipient) to form a pharmacological composition. Pharmaceutically acceptable carriers can contain one or more physiologically acceptable compound(s) that act, for example, to stabilize the composition or to increase or decrease the absorption of the active agent(s). Physiologically acceptable compounds can include, for example, carbohydrates, such as glucose, sucrose, or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins, protection and uptake enhancers such as lipids, compositions that reduce

the clearance or hydrolysis of the active agents, or excipients or other stabilizers and/or buffers.

[0177] Other physiologically acceptable compounds include wetting agents, emulsifying agents, dispersing agents or preservatives that are particularly useful for preventing the growth or action of microorganisms. Various preservatives are well known and include, for example, phenol and ascorbic acid. One skilled in the art would appreciate that the choice of pharmaceutically acceptable carrier(s), including a physiologically acceptable compound depends, for example, on the route of administration of the active agent(s) and on the particular physio-chemical characteristics of the active agent(s).

[0178] The excipients are preferably sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well-known sterilization techniques.

[0179] In therapeutic applications, the compositions of this invention are administered to a patient suffering from one or more symptoms of atherosclerosis or at risk for atherosclerosis in an amount sufficient to cure or at least partially prevent or arrest the disease and/or its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's health. Single or multiple administrations of the compositions may be administered depending on the dosage and frequency as required and tolerated by the patient. In any event, the composition should provide a sufficient quantity of the active agents of the formulations of this invention to effectively treat (ameliorate one or more symptoms) the patient.

[0180] The concentration of agent can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs. Concentrations, however, will typically be selected to provide dosages ranging from about 0.1 or 1 mg/kg/day to about 50 mg/kg/day and sometimes higher. Typical dosages range from about 3 mg/kg/day to about 3.5 mg/kg/day, preferably from about 3.5 mg/kg/day to about 7.2 mg/kg/day, more preferably from about 7.2 mg/kg/day to about 11.0 mg/kg/day, and most preferably from about 11.0 mg/kg/day to about 15.0 mg/kg/day. In certain preferred embodiments, dosages range from about 10 mg/kg/day to about 50 mg/kg/day. In certain embodiments, dosages range from about 20 mg to about 50 mg given orally twice daily. It

will be appreciated that such dosages may be varied to optimize a therapeutic regimen in a particular subject or group of subjects.

[0181] In certain preferred embodiments, the (*e.g.* peptides, peptide mimetics, and/or lipids) of this invention are administered orally (*e.g.* via a tablet) or as an injectable in accordance with standard methods well known to those of skill in the art. In other preferred embodiments, the agent(s), may also be delivered through the skin using conventional transdermal drug delivery systems, *i.e.*, transdermal "patches" wherein the active agent(s) are typically contained within a laminated structure that serves as a drug delivery device to be affixed to the skin. In such a structure, the drug composition is typically contained in a layer, or "reservoir," underlying an upper backing layer. It will be appreciated that the term "reservoir" in this context refers to a quantity of "active ingredient(s)" that is ultimately available for delivery to the surface of the skin. Thus, for example, the "reservoir" may include the active ingredient(s) in an adhesive on a backing layer of the patch, or in any of a variety of different matrix formulations known to those of skill in the art. The patch may contain a single reservoir, or it may contain multiple reservoirs.

[0182] In one embodiment, the reservoir comprises a polymeric matrix of a pharmaceutically acceptable contact adhesive material that serves to affix the system to the skin during drug delivery. Examples of suitable skin contact adhesive materials include, but are not limited to, polyethylenes, polysiloxanes, polyisobutylenes, polyacrylates, polyurethanes, and the like. Alternatively, the drug-containing reservoir and skin contact adhesive are present as separate and distinct layers, with the adhesive underlying the reservoir which, in this case, may be either a polymeric matrix as described above, or it may be a liquid or hydrogel reservoir, or may take some other form. The backing layer in these laminates, which serves as the upper surface of the device, preferably functions as a primary structural element of the "patch" and provides the device with much of its flexibility. The material selected for the backing layer is preferably substantially impermeable to the active agent(s) and any other materials that are present.

[0183] Other preferred formulations for topical drug delivery include, but are not limited to, ointments and creams. Ointments are semisolid preparations which are typically based on petrolatum or other petroleum derivatives. Creams containing the selected active

agent, are typically viscous liquid or semisolid emulsions, often either oil-in-water or water-in-oil. Cream bases are typically water-washable, and contain an oil phase, an emulsifier and an aqueous phase. The oil phase, also sometimes called the "internal" phase, is generally comprised of petrolatum and a fatty alcohol such as cetyl or stearyl alcohol; the aqueous phase usually, although not necessarily, exceeds the oil phase in volume, and generally contains a humectant. The emulsifier in a cream formulation is generally a nonionic, anionic, cationic or amphoteric surfactant. The specific ointment or cream base to be used, as will be appreciated by those skilled in the art, is one that will provide for optimum drug delivery. As with other carriers or vehicles, an ointment base should be inert, stable, nonirritating and nonsensitizing.

[0184] Unlike typical peptide formulations, the peptides of this invention comprising D-form amino acids can be administered, even orally, without protection against proteolysis by stomach acid, *etc.* Nevertheless, in certain embodiments, peptide delivery can be enhanced by the use of protective excipients. This is typically accomplished either by complexing the polypeptide with a composition to render it resistant to acidic and enzymatic hydrolysis or by packaging the polypeptide in an appropriately resistant carrier such as a liposome. Means of protecting polypeptides for oral delivery are well known in the art (see, *e.g.*, U.S. Patent 5,391,377 describing lipid compositions for oral delivery of therapeutic agents).

[0185] Elevated serum half-life can be maintained by the use of sustained-release protein "packaging" systems. Such sustained release systems are well known to those of skill in the art. In one preferred embodiment, the ProLease biodegradable microsphere delivery system for proteins and peptides (Tracy (1998) *Biotechnol. Prog.* 14: 108; Johnson *et al.* (1996), *Nature Med.* 2: 795; Herbert *et al.* (1998), *Pharmaceut. Res.* 15, 357) a dry powder composed of biodegradable polymeric microspheres containing the protein in a polymer matrix that can be compounded as a dry formulation with or without other agents.

[0186] The ProLease microsphere fabrication process was specifically designed to achieve a high protein encapsulation efficiency while maintaining protein integrity. The process consists of (i) preparation of freeze-dried protein particles from bulk protein by spray freeze-drying the drug solution with stabilizing excipients, (ii) preparation of a drug-polymer suspension followed by sonication or homogenization to reduce the drug particle

size, (iii) production of frozen drug-polymer microspheres by atomization into liquid nitrogen, (iv) extraction of the polymer solvent with ethanol, and (v) filtration and vacuum drying to produce the final dry-powder product. The resulting powder contains the solid form of the protein, which is homogeneously and rigidly dispersed within porous polymer particles. The polymer most commonly used in the process, poly(lactide-co-glycolide) (PLG), is both biocompatible and biodegradable.

[0187] Encapsulation can be achieved at low temperatures (*e.g.*, -40°C). During encapsulation, the protein is maintained in the solid state in the absence of water, thus minimizing water-induced conformational mobility of the protein, preventing protein degradation reactions that include water as a reactant, and avoiding organic-aqueous interfaces where proteins may undergo denaturation. A preferred process uses solvents in which most proteins are insoluble, thus yielding high encapsulation efficiencies (*e.g.*, greater than 95%).

[0188] In another embodiment, one or more components of the solution can be provided as a "concentrate", *e.g.*, in a storage container (*e.g.*, in a premeasured volume) ready for dilution, or in a soluble capsule ready for addition to a volume of water.

[0189] The foregoing formulations and administration methods are intended to be illustrative and not limiting. It will be appreciated that, using the teaching provided herein, other suitable formulations and modes of administration can be readily devised.

V. Kits for the treatment of conditions characterized by impaired arteriole structure and/or function..

[0190] In another embodiment this invention provides kits for amelioration of one or more symptoms of a pathology characterized by impaired arteriole structure and/or function or for the prophylactic treatment of a subject (human or animal) at risk for such a condition. The kit(s) preferably comprise a container containing one or more of the active agents described herein.. The active agent(s) can be provided in a unit dosage formulation (*e.g.* suppository, tablet, caplet, patch, *etc.*) and/or may be optionally combined with one or more pharmaceutically acceptable excipients.

[0191] The kit(s) can, optionally, further comprise one or more other agents used in the treatment of the condition/pathology of interest. Such agents include, but are not limited

to, beta blockers, vasodilators, aspirin, statins, ace inhibitors or ace receptor inhibitors (ARBs) and the like, *e.g.* as described above.

[0192] In addition, the kits optionally include labeling and/or instructional materials providing directions (*i.e.*, protocols) for the practice of the methods or use of the "therapeutics" or "prophylactics" of this invention. Preferred instructional materials describe the use of one or more active agent(s) of this invention to mitigate one or more associated with a condition characterized by impaired arteriole structure and/or function and/or to prevent the onset or increase of one or more of such symptoms in an individual at risk for such a condition. The instructional materials may also, optionally, teach preferred dosages/therapeutic regiment, counter indications and the like.

[0193] While the instructional materials typically comprise written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to electronic storage media (*e.g.*, magnetic discs, tapes, cartridges, chips), optical media (*e.g.*, CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

EXAMPLES

[0194] The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1

[0195] As shown in Figures 1A, 1B, and 1C, mice with an absence of LDL receptors (LDLR-/-) have thickened brain arterioles compared to wild-type mice (WT). On a low fat chow diet the LDL receptor null mice have twice the level of plasma LDL of wild-type mice. However, they have very little atherosclerosis on a chow diet but as shown in the figure below even though they have minimal atherosclerosis, their brain arterioles are significantly thickened. Also as shown in the following figures when placed on a high-fat, high-cholesterol (Western) diet, these mice develop additional thickening of their brain arterioles. On the Western diet, these mice also develop extensive atherosclerosis.

[0196] It was recently reported that LDLR-/- mice have impaired spatial memory associated with a decreased synaptic density in the hippocampus (Mulder *et al.* (2004) *Neurobiology of Disease* 16: 212-219, *see, e.g.*, Figure 2 therein).

[0197] As shown in Figure 2, treatment of LDLR -/- mice on a Western diet for six weeks with D-4F (added to the drinking water at 300 μg/mL) significantly improved the number of spontaneous alterations in the T-maze test while adding the same concentration of the control peptide (scrambled D-4F) did not.

[0198] The results shown in Figure 2 for the mice receiving D-4F compared to the control peptide are remarkably similar to those shown in Figure 2 from Mulder et al. (*supra.*) where LDLR-/- mice were compared to wild-type mice (LDLR+/+) suggesting that oral D-4F reversed the abnormality in the LDLR-/- mice. The data in the figure from Mulder *et al.* are shown as "percentage alternation". The data in Figure 2, herein, are shown for "Number of Spontaneous Alternations". As shown in Figure 3 below the data with D-4F compared to scrambled D-4F are similar when presented as "Percentage Alternation

[0199] Further evidence of the improvement in the T-maze test with D-4F treatment compared to the control peptide, scrambled D-4F (Sc D-4F) comes from the data in Figure 4.

[0200] It was previously reported that injection of D-4F improved vasoreactivity of facial arteries (Ou Z, Ou J, Ackerman AW et al. L-4F, an apolipoprotein A-I mimetic, restores nitric oxide and superoxide anion balance in low-density lipoprotein-treated endothelial cells. Ou *et al.* (2003) *Circulation*; 107: 1520-1524; Ou *et al.* (2003) *Circulation* 107: 2337-2341). In these published studies the mouse facial artery was used. This artery has an internal diameter of about 250 μM.

[0201] Another example of the application of this invention comes from the data shown below in Figure 5 which show that administering DMPC orally to LDL receptor null mice on a Western diet improved the vasoreactivity of their facial arteries significantly better than administering soy lecithin.

[0202] Eight week old female LDL receptor null mice were maintained on a Western diet and given drinking water alone (Control, n=6) or were maintained on a

Western diet and given drinking water supplemented with 1 mg/mL of either soy lecithin (n=6), or DMPC (n=6). After six weeks the submandibular segment of the facial artery was dissected out and the percent relaxation of the preconstricted 2mm arterial rings was determined in response to the addition of acetylcholine (an endothelium-dependent relaxant) in concentrations ranging from 0.01 to 10 μM. The specificity of the relaxation was confirmed by addition of 300 μM L-NAME (a nitric oxide synthase inhibitor) and sodium nitroprusside (an endothelium-independent nitric oxide donor). There was no difference between groups with addition of L-NAME (which inhibited the vasorelaxation elicited by acetylcholine) and sodium nitroprusside or papaverine (which maximally vasodilated). In the absence of these additions there was a marked inhibition in acetylcholine vasorelaxation in the Control group. There was a trend toward improved relaxation in the soy lecithin group but this did not reach statistical significance. There was a significant improvement in vasorelaxation in the mice that received DMPC ($p<0.01$ at 1 μM acetylcholine; $p<0.001$ at 10 μM acetylcholine). The Log of the acetylcholine concentration producing 50% vasorelaxation (Log EC50 in mM) was 0.473 for the Control group, 0.057 for the group receiving soy lecithin, and 0.006 for the group receiving DMPC.

[0203] We have previously published that administering DMPC to apoE null mice caused an increase in plasma apoA-I levels and HDL-cholesterol, resulting in sequestration/removal/destruction of inflammatory lipids and conversion of HDL from pro-inflammatory to anti-inflammatory with both prevention and regression of atherosclerosis in this mouse model (Navab M, Hama S, Hough G et al. Oral synthetic phospholipids (DMPC) raises high-density lipoprotein cholesterol levels, improves high-density lipoprotein function, and markedly reduces atherosclerosis in apolipoprotein E-null mice. Circulation 2003;108:1735-1739).

[0204] Thus, we have shown that two different agents that sequester/remove/destroy inflammatory lipids (D-4F and DMPC) one an oral peptide and one a oral phospholipids that increases apoA-I and HDL cholesterol both improve arterial function as measured in a small artery, the facial artery, The novel findings of this invention relate to a method for improving the structure and function of vessels smaller than even small arteries, i.e. arterioles. These arterioles are ultimately responsible for the perfusion of tissues as diverse as brain and kidney. Based on data shown herein and unpublished data, we believe this

invention provides a general method to improve the structure and function of arterioles by administering agents that sequester/remove/destroy inflammatory lipids and convert pro-inflammatory high density lipoproteins (HDL) to anti-inflammatory or render anti-inflammatory HDL more anti-inflammatory. These agents include peptides containing a class A amphipathic helix, peptides containing a G* amphipathic helix, short peptides and non-peptides with a molecular weight of less than 900 daltons that have a solubility in ethyl acetate of at least 4 mg/mL, and which are soluble in aqueous buffer at pH 7.0 and when contacted with a phospholipid in an aqueous environment, form particles with a diameter of approximately 7.5 nm and form stacked bilayers with a bilayer dimension on the order of 3.4 to 4.1 nm with spacing between the bilayers in the stack of approximately 2 nm; and oral synthetic phospholipids in which the sn-1 and sn-2 positions are identical and contain at least 3 carbons.

Example 2

ApoA-I Mimetic Peptide D-4F Reduces Brain Arteriolar Wall Thickening and Improves Spatial Memory in LDL Receptor Null Mice Fed a Western Diet

Summary

[0205] The wall thickness of brain arterioles 10 – 100 μm in diameter was determined in wild-type and LDL-receptor null C57BL/6 mice on chow or a Western diet administered alone or with D-4F or scrambled D-4F. Spatial memory was determined by use of the T-maze continuous alternation task. On chow, brain arteriolar wall thickness in LDL receptor null mice was increased compared to wild-type and further increased on the Western diet ($p<0.001$). The increased brain arteriolar wall thickness was in part due to an increase in smooth muscle α-actin content and was decreased by treatment with D - 4F but not scrambled D-4F. Adding the Western diet significantly impaired performance in the T-maze ($p<0.05$) and was improved with D-4F compared to scrambled D-4F ($p<0.05$). The changes in performance and in arteriolar wall thickness were independent of plasma lipids and arteriolar lumen diameter.

[0206] Treatment of LDL-receptor null mice fed a Western Diet with D-4F reduces brain arteriolar wall thickness independent of plasma lipids and arteriolar lumen diameter and improves spatial memory.

Materials and Methods

Materials

[0207] D-4F and scrambled D-4F (a peptide with the same D-amino acids as in D-4F but arranged in a sequence which prevents the peptide from achieving the helical conformation needed for lipid binding) were synthesized as previously described (Navab *et al.* (2002) *Circulation*, 105: 290-292; Navab *et al.* (2004) *Circulation*, 109:r120-r125). All other reagents were from sources previously reported (Navab *et al.* (2005) *Arterioscler Thromb Vasc Biol.*, 25: 1-7).

Mice and histopathology

[0208] Female wild-type and LDL receptor null C57BL/6 mice were from Jackson Laboratories (Bar Harbour, ME). The mice were maintained on a chow diet (Ralston Purina) prior to administration of a Western diet (Teklad/Harlan, Madison WI, diet No. 88137; 42% fat, 0.15% cholesterol, w/w). For studies of brain arterioles the mice were anesthetized with intramuscular ketamine (100 mg/kg) and acepromazine (2.5 mg/kg) and the heart was perfused via the left ventricle with 25 mL phosphate buffered saline (PBS) containing heparin (10 U/mL) followed by 100 mL of 4% paraformaldehyde (PFA) in PBS at pH 7.4 as described by Fernagut et al. (Fernagut *et al.* (2002) *Neuroscience*, 114: 1005-1017; Fernagut *et al.* (2004) *Exp Neurol.*, 185: 47-62). Brains were quickly removed and stored for 24 hrs in 4% PFA at 4°C and then transferred to 10% sucrose in PBS (pH 7.4) and left until they sank to the bottom of the solution. The right half of the brains were embedded in OCT (Tissue-Tek; Miles Laboratories Ltd, Elkhart IN) and frozen in isopentane at -40°C and stored at -80°C until sectioned in a cryostat at -20°C . The frozen brain was cut into 8 μm sections coronally to include the underlying white matter and stained with hematoxylin-eosin (H&E) and for smooth muscle α-actin (Serotec, Raleigh, NC). The left half of each brain was embedded in paraffin, cut coronally into 6 μm thick sections and stained with H&E. The UCLA Animal Research Committee approved all studies.

Morphometry and associated statistical methods

[0209] Morphometry was performed to determine vascular wall thickness for all arterioles that were distended and perpendicularly cross-sectioned. Using a 40 x

microscope objective, the sectioned vessels were photographed using SPOT Image software and three measurements of the internal and external diameters were taken for each and averaged. The range of vessels sizes were between 10 and 160 μm and the comparison of wall to lumen ratios was made separately for arteries with internal diameter values of 10-20 μm, 2 1-50 μm, 5 1-100 μm and >100 μm. A minimum of 10 arteries from each diameter group was examined in the cortical area and the deep white matter regions from each brain, and the wall thickness and wall to lumen ratios determined. The ratio of immunoreactive media thickness to the internal diameter of each vessel was assessed in sections immunostained for smooth muscle α-actin. All measurements were performed on a single focal plane using an Olympus BH-2 microscope equipped with a 40 x lens by one investigator and repeated by two observers blinded to treatment. Inter-observer variation was determined by having the three investigators measure the same 20 arterioles for wall thickness and lumen diameter and calculate the wall to lumen ratio. The coefficient of variation was found to be 14 ± 1%. All data were computed using InStat and Prism software (Graphpad, San Diego, CA, U.S.A.). Statistical significance of difference between means of different groups was performed using unpaired student *t*-test or one-way ANOVA. Multiple comparisons of the different groups were performed using Tukey-Kramer multiple comparisons test. A probability level of 5% ($p<0.05$) was considered significant.

Behavioral studies and associated statistical methods

[0210] T-maze continuous alternation task (T-CAT) testing took place daily between 9 A.M. and 4 P.M. and was performed by one investigator unaware of the treatment groups. The mice were delivered to the testing room two hours prior to behavioral studies to allow familiarization with the extra-maze visual cues of the room. The T-maze apparatus used in our studies is identical to the one described by Gerlai et al. (Gerlai (1998) *Behavioural Brain Research*, 95: 91-101) and was made of transparent acrylic walls with a black acrylic bottom. The dimensions of start and goal arms were: length 75 cm, width 12 cm and height 20 cm. The maze was equipped with three removable guillotine doors that could be operated by manual remote control. The testing room was illuminated by ceiling and floor lights and a fan provided a constant background noise. The T-maze was separated from the investigator by a black curtain and the movement of the mice in the maze was observed on a TV monitor and videotaped. After each individual mouse the T-

maze was carefully cleaned with Windex spray and dried with paper towels. The T-maze continuous alternation task (T-CAT) limits the handling of mice and permits their exploratory behavior to be carried out undisturbed. T he procedure used in this study is identical to that described by Gerlai et al. (*Id.*) and consisted of one forced and 14 free choice trials. Consecutive choices made by the mice were measured and the alternation rate during the 14 free choice trials was calculated (0%-no alternation, 100%-alternation at each trial, 50%-random choice). The time (in seconds) needed to complete the 15 trials was recorded and analyzed. The T-CAT testing was continuously registered by a video tracking system (SD Instruments Inc., San Diego, CA) and stored on a computer. Statistics were performed using StatView software (SAS Institute, Cary, NC.)

Other Procedures

[0211] Plasma lipoprotein and lipid levels were determined as described previously (Navab *et al.* (2004) *Circulation*, 109:r120-r125; Navab *et al.* (2005) *Arterioscler Thromb Vasc Biol.*, 25: 1-7).

Results

Brain arteriolar wall thickness is increased in LDL receptor null mice and is further increased with addition of the Western diet

[0212] As shown in Figure 6 on a chow diet arteriolar wall thickness was greater in LDL receptor null mice compared to wild-type mice and after a Western diet for six weeks arteriolar wall thickness was further increased in the LDL receptor null mice.

Brain arteriolar wall thickness is reduced by treatment with D-4F but not with scrambled D-4F

[0213] Figures 7A-7C demonstrate that adding 300 μg/mL of D-4F to the drinking water of LDL receptor null mice on a Western diet for 6 weeks resulted in reduced brain arteriolar wall thickness compared to adding the same concentration of scrambled D-4F to the drinking water. Figure 7D demonstrates that there was no difference in the lumen diameters of the brain arterioles between mice receiving D-4F or scrambled D-4F. Some investigators have argued that the most reliable measurement of arteriole wall thickness is

obtained by dividing the wall thickness for each arteriole by the lumen diameter for that arteriole (Mulvany (1999) *Cardiovascular Research*, 41: 9-13). Figures 7E – 7G demonstrate that the ratio of wall to lumen diameter was significantly less in mice receiving D-4F compared to scrambled D-4F. There was no significant difference in the concentrations of plasma total cholesterol, LDL-cholesterol, HDL-cholesterol, or triglycerides when the mice were administered D-4F compared to scrambled D-4F. The total cholesterol concentrations were 1,076 ± 75 (Mean ± SEM) for mice receiving D-4F compared to 970 ± 61 mg/dL for mice receiving scrambled D-4F. LDL and HDL cholesterol concentrations were 924 ± 76 and 86 ± 6 mg/dL, respectively, for mice receiving D-4F compared to 834 ± 63 and 79 ± 5 mg/dL, respectively, for the mice receiving scrambled D-4F. Triglycerides were 330 ± 25 compared to 288± 25 mg/dL for mice receiving D-4F or scrambled D-4F, respectively.

Brain arteriolar wall thickening is in part due to an increase in smooth muscle α-actin content

[0214] As shown in Figure 8A administration of the Western diet to LDL receptor null mice resulted in a significant increase in the amount of smooth muscle α-actin in the walls of the brain arterioles of LDL receptor null mice fed a Western diet. Figure 8B shows representative brain arterioles stained for smooth muscle cell α-actin from mice that were treated with D-4F or scrambled D-4F. Figures 8C – 8E demonstrate quantitatively that treatment of the mice with D-4F significantly reduced brain arteriolar wall smooth muscle á-actin content compared to mice treated with scrambled D-4F.

Feeding LDL receptor null mice a Western diet results in impaired spatial memory, which is significantly improved by treatment with D-4F but not scrambled D-4F.

[0215] Figures 9A – 9D demonstrate that when the LDL receptor null mice described in Figure 3A were placed on a Western diet they had impaired spatial memory as measured with the T-CAT. Figures 9E – 9G demonstrate that treatment with oral D-4F (but not scrambled D-4F) of the mice described in Figures 7 and 8B – 8E significantly improved performance as measured with the T-CAT. Although the mice receiving scrambled D-4F required more time than the mice that received D-4F to complete the 15 trials (579 ± 23

seconds vs. 548 ± 37 seconds, respectively) this difference did not reach statistical significance. Nonetheless, the data in Figures 9E – 9G clearly demonstrate improvement with D-4F treatment.

Discussion

[0216] The data presented in this example together with that previously published[8-10] suggest that LDL levels affect all branches of the arterial tree in mice. On a chow diet the LDL receptor null mice had significantly increased arteriole wall thickness in brain arterioles with lumens of 15 – 40 μm in diameter (Figure 6A). After only six weeks on a Western diet there was a significant increase in the wall thickness of brain arterioles in these LDL receptor null mice compared to wild-type mice (Figure 6A – 6C).

[0217] Heistad and colleagues (Heistad *et al.* (1995) *Hypertension*, 26: 509-513) emphasized the differences and similarities in atherosclerotic and hypertensive vessels. These authors made the observation that "Changes in vascular structure in both atherosclerosis and hypertension are characterized by thickening of the vessel wall and vascular 'remodeling'." Remodeling tends to preserve the size of the lumen in atherosclerotic vessels and results in a smaller lumen in hypertensive vessels." As shown in Figure 7D it appears that the thickening of brain arterioles in LDL receptor null mice induced by the Western diet (fed for six weeks) can be independent of changes in lumen diameter. We did not measure blood pressure in these mice and we do not know if the lumen diameters would be altered by a longer period of exposure. However, it is clear that within a period of only six weeks the wall to lumen ratio of brain arterioles as determined by smooth muscle cell α-actin content was significantly increased with feeding of the Western diet (Figure 8A).

[0218] It has long been known that LDL enriched in reactive oxygen species can stimulate vascular smooth muscle cell growth (Gorog (1997) Atherosclerosis, 129: 1-7). It has also been reported that mice with increased oxidative stress because of a deficiency in cystathionine α-synthase have cerebral vascular hypertrophy with increased smooth muscle content in their brain arterioles (Baumbach et al. (2002) *Circ Res*, 91: 931-937). It is tempting to speculate that treatment with oral D-4F, which is known to decrease LDL lipid hydroperoxides in mice without changing plasma lipid levels (Navab *et al.* (2004)

Circulation, 109:r120-r125)[1], might have ameliorated the increase in brain arteriolar smooth muscle α-actin (Figures 8B – 8E) by reducing lipoprotein lipid hydroperoxides without changing plasma lipids.

[0219] Mulder et al. (Mulder *et al.* (2004) *Neurobiology of Disease*, 16: 212-219) first reported that LDL receptor null mice on a chow diet compared to wild-type mice on a chow diet have impaired spatial memory. These authors concluded that the abnormality was similar to that reported in apoE null mice (Krugers *et al.* (1997) *Neruo Report,* 8: 2505-2510; Oitzl *et al.* (1997) *Brain Res.*, 752: 189-196; Zhou *et al.* (1998) *Brain Res.*, 788:151-159; Veinbergs *et al.* (1999) *Neuroscience* 91:401-403; Krzywkowski *et al.* (1999) *Neuroscience* 92:1273-1286; Raber *et al.* (2000) *Nature* 404:352-354) and was due to a primary abnormality in brain cells induced by a failure to provide lipoprotein constituents to the brain cells. The data reported here in Figure 9 together with that in Figures 6-8, suggest an alternative hypothesis. The primary abnormality may be due in part or entirely to the "Sick Vessel Syndrome" described by Heistad *et al.* (1995) *Hypertension*, 26: 509-513, and not to the failure to deliver lipoprotein constituents to brain cells. In favor of this hypothesis is the worsening of the functional defect with the worsening of the hyperlipidemia (Figures 9A – 9D). If the primary defect were due to a lack of lipoprotein constituents delivered to brain cells because of an absence of LDL receptors, one would have expected improvement in function with increased plasma lipoprotein levels since delivery of lipoproteins into the brain cells by non-receptor-mediated pathways would likely increase with increasing hyperlipidemia. Further support for a vascular basis for the functional abnormalities noted in Figure 9 is the correlation between the functional abnormalities and the structural changes in arterioles which were worsened by the Western diet (Figures 6 and 8A) and improved by oral D-4F (but not scrambled D-4F) (Figures 7 and 8B – 8E). We did not measure brain arteriole vasoreactivity in these mice. However, Pritchard and colleagues found that vasoreactivity in the facial artery (approximately 240 μm in diameter) of LDL receptor null mice was severely impaired by a Western diet and was dramatically improved with 4F treatment (Ou *et al.* (2003) *Circulation*, 107: 2337-2341; Ou *et al.* (2005) *Circulation Research* 97: 1190-1197. Heistad and colleagues (Heistad *et al.* (1980) *Am. J. Physiol.* 239 (Heart Circ. Physiol. 8):H539-H544) reported that in monkeys made hypercholesterolemic by feeding an atherogenic diet maximal cerebral vasodilator responses to hypercapnia were impaired, but during a less pronounced vasodilator stimulus

autoregulatory responses to hypotension were preserved. Interestingly, when the monkeys were put on a regression diet and subjected to maximal vasodilation, the responsiveness of the cerebral arterial bed was significantly improved (Armstrong *et al.*(1983) *J Clin. Invest.*, 71: 104-113).

[0220] It is interesting to note that it has been observed in humans suffering from the angiopathy of subcortical arteriosclerotic encephalopathy (Binswanger's disease) that there is an increase in smooth muscle α-actin in brain vessels smaller than 100 μm in diameter (Lin *et al.* (2000) *Stroke*, 31:1838-1842). It is also tempting to speculate that the "Sick Vessel Syndrome" may play a broader role in human dementias than has been previously recognized and that the use of apoA-I mimetic peptides such as D-4F may have beneficial effects in such diseases.

[0221] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

CLAIMS

What is claimed is:

1. The use of peptide comprising the amino acid sequence D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F (SEQ ID NO:5) for the manufacture of a medicament for the prophylaxis or treatment of arterioles having impaired structure or function.

2. The use of claim 1, wherein said peptide comprises all D amino acids.

3. The use of claim 1, wherein said peptide comprises all L amino acids.

4. The use according to anyone of claims 1-3, wherein said peptide comprises a carboxyl and/or an amino protecting group.

5. The use according to claim 4, wherein said peptide comprises a carboxyl terminal protecting group and an amino terminal protecting group.

6. The use according to claim 5, wherein said carboxyl terminal protecting group is NH_2.

7. The use according to claim 5, wherein said amino terminal protecting group is acetyl.

8. The use according to claim 5, wherein:

   said carboxyl terminal protecting group is NH_2; and

   said amino terminal protecting group is acetyl.

9. The use according to any of claims 1-8, wherein said peptide is provided in combination with a pharmaceutically acceptable excipient.

10. The use of claim 9, wherein said peptide is formulated in a unit dosage formulation.

11. The use of claim 9, wherein said peptide is formulated for administration by a route selected from the group consisting of oral administration, nasal administration, rectal administration, intraperitoneal injection, and intravascular injection, subcutaneous injection, transcutaneous administration, and intramuscular injection.