protein by preventing its degradation, which ensures high-levels of cellular cholesterol export and HDL assembly.

[0004] The clinical importance of HDL has sparked interest in the development of strategies to manipulate RCT for therapeutic purposes. Peptides have been identified that can stimulate cholesterol efflux *in vivo* (see, *e.g.,* WO 2008/115303 and WO 2009/155366). These peptides are characterized by alpha helices having a polar and non-polar surface and an alignment of acidic amino acids residues. However, in some contexts, these peptides have exhibited toxicity when administered at very high pharmacological doses. Accordingly, there is a need to provide improved peptides that have reduced toxicity. The present invention fulfills this need.

SUMMARY OF THE INVENTION

[0005] The present disclosure relates to peptides that have cholesterol efflux activity and that have superior properties in terms of cytotoxicity profile.

[0006] In one aspect, the invention provides an isolated polypeptide having cholesterol efflux activity, the polypeptide comprising an amphipathic α--helix that has a non-polar surface and a polar surface, wherein the polar surface comprises charged and uncharged amino acid residues at the lipid-water interface, wherein the α-helix amino acid sequence: has at least 60% identity to SEQ ID NO:1; has an acidic amino acid residue at positions 1, 7, 8, 15, 18 and 19 as determined with reference to SEQ ID NO:1; has an aliphatic amino acid independently selected from V, L, or I, or an uncharged amino acid analog thereof, at positions 10, 13, 16 and 20, as determined with reference to SEQ ID NO:1; wherein the uncharged amino acid analog has an aliphatic side chain and has a hydrophobicity of V or greater than V; and has an uncharged polar amino acid residue at at least two of positions 3, 14, and 23 as determined with reference to SEQ ID NO:1. Disclosed is a family of peptides having cholesterol efflux activity that parallels that of full-length apolipoproteins (*e.g.*, Apo AI and Apo E); and having high selectivity for ABCA1 that parallels that of full-length apolipoproteins. Moreover, the family of peptides has a desirable cytotoxicity profile in that they display little or no cytotoxicity when administered at high pharmacological doses. The polypeptides of the present invention also stimulate cholesterol efflux from macrophage foam cells *in vivo*, promote a sustained increase in fecal sterol secretion, and reduce the severity of established atherosclerosis in the presence of hypercholesterolemia and a high-fat dietary insult in an apolipoprotein E-deficient mouse model of disease.

[0007] The peptides of the present disclosure can be used therapeutically to promote ABCA1-stabilization as well as ABCA1–lipid efflux activity, and can be used alone or, alternatively, in combination with other known pharmacological agents, for the treatment of cardiovascular disease to reduce atherosclerosis. In addition, the polypeptides of the present disclosure can be used alone or, alternatively, in combination with other known pharmacological agents, for the treatment of acute coronary syndrome to reduce plaque lipid content and to stabilize vulnerable plaques. Further, the peptides of the present disclosure can be used alone or, alternatively, in combination with other known pharmacological agents, for the treatment of dyslipidemia, hypercholesterolemia and inflammation to raise plasma HDL concentrations and/or to promote reverse cholesterol transport. Further, the peptides of the present disclosure

can be used therapeutically, alone or with other pharmacological agents, to lower glucose in patients having diseases of abnormal glucose metabolism. In some examples, the peptides of the present disclosure can be used therapeutically to treat or prevent one or more symptoms of Alzheimer's Disease or frontotemporal dementia. In various examples, the disclosure includes, but is not limited to, the following examples.

[0008] The peptides of the disclosure comprise certain features that together define the pharmacokinetic and pharmacodynamic properties of the peptides. In some examples, the peptides comprise a core sequence of 24 amino acid residues that selectively bind to HDL in plasma and target the ABCA1 transporter in cells. Features of the peptides include an amphipathic α-helix structure, with alignment of acidic residues down the center of the polar surface and positively charged amino acids at the lipid-water interface. Further a peptide of the disclosure has one or more uncharged residues at the polar surface of the lipid water interface, *e.g.*, at position 3, 14, and/or 23 of the 24 amino acid sequence, as numbered with reference to SEQ ID NO:1. In some examples the one or more uncharged residues is a polar uncharged residue. In some examples, the peptide comprises one or more uncharged hydrophobic amino acids at the polar surface, *e.g.,* at one or two of positions 3, 14, and 23. In some examples, the peptide comprises one or more uncharged aliphatic amino acids at the polar surface, *e.g.,* at one or two of positions 3, 14, and 23. In typical examples, a peptide of the disclosure has a net negative charge. In some examples, aliphatic amino acids are preferred at the non-polar surface. In some examples , alanine can be used to reduce hydrophobicity.

[0009] The peptides also lack substantial stereo-specific effect, *e.g.,* peptides that comprise L and D amino acids and inverted forms work equally well.

[0010] In one examples, the disclosure provides an isolated peptide having cholesterol efflux activity and little or no toxicity at high pharmacological doses, where the peptide comprises an amino acid sequence that is an amphipathic α-helix having a non-polar surface and a polar surface, where the polar surface comprises charged and uncharged amino acid residues at the lipid-water interface. In some embodiments, the peptide comprises the amino acid sequence EVcitSKLEEWLAALcitELAEELLARL (SEQ ID NO:1), wherein "cit" represents citrulline. In some embodiments, the peptide comprises a variant of SEQ ID NO:1 that has at least 50% identity, or at least 60% identity, or at least 70%, at least 80%, at least 85%, at least 90% identity, or at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98, or 99% identity to SEQ ID

NO:1). In some embodiments, the peptide comprises the amino acid sequence EVcitSKLEEWIAAIcitEIAEEILARL (SEQ ID NO:2), wherein "cit" represents citrulline. In some embodiments, the peptide comprises a variant of SEQ ID NO:2 that has at least 50% identity, or at least 60% identity, or at least 70%, at least 80%, at least 85%, at least 90% identity, or at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98, or 99% identity to SEQ ID NO:2. The amino acid positions described here are determined with reference to SEQ ID NO:1 or SEQ ID NO:2. Peptide variants of SEQ ID NO:1 or SEQ ID NO:2 typically have an acidic amino acid residue at position 1, 7, 8, 15, 18, and 19 as numbered with reference to SEQ ID NO:1. In some embodiments, a variant has an uncharged residue at the polar surface, *e.g.,* at at least one of positions 3, 14, or 23 as numbered with reference to SEQ ID NO:1. In some embodiments, the variant has citrulline at two of positions 3, 14, or 23 as numbered with reference to SEQ ID NO:1. In some embodiments, a variant has citrulline at positions 3 and 14, positions 3 and 23, or positions 14 and 23. In some embodiments, a variant has citrulline at positions 3 and 14 and an R or K at position 23; citrulline at positions 3 and 23 and an R or K at position 14; or citrulline at positions 14 and 23 and an R or K at position 3. In some embodiments, a variant has no more than two R residues. For example, in some embodiments, a variant may have an R at position 5 and an R at position 23. In some embodiments, the amino acid sequence comprises an uncharged residue at one or two of positions 3, 14, and 23 where the residue is a hydrophobic amino acid, such as Q, N, Y, W, A, I, L, V. In some embodiments, the uncharged residue at one or two of positions 3, 14, and 23 is an aliphatic amino acid, such as A, I, L, or V. In embodiments when there is a hydrophobic or aliphatic amino acid at one or two of positions 3, 14, and 23, the third positions is an R, K, or citrulline. In some embodiments, a variant comprises a hydrophobic amino acid at position 2, 6, 9, 10, 13, 16, 17, 20, 21, 22, and 24 as determined with reference to SEQ ID NO:1. In some embodiments, a variant comprises an aliphatic amino acid at at least one, or at least two, three, four, five, six, seven, eight, or nine of positions 2, 6, 10, 13, 16, 17, 20, 21, 22, and 24. In some embodiments, a variant comprises an aliphatic residue at each of positions 2, 6, 10, 13, 16, 20, 21, and 24. In some embodiments, the aliphatic amino acid is L, V, or I. In some embodiment, the aliphatic amino acid residue at position 2, 6, 10, 13, 16, 20, and 21 is L. In some embodiment, the aliphatic amino acid residue at position 2, 6, 10, 13, 16, 20, and 21 is I. In some embodiments, the aliphatic amino acid residue at position 2 is V. In some embodiments, the aliphatic amino acid residue at position 2 is V and the aliphatic amino acid residue at position 6, 10, 13, 16, 20, and 21 is L. In some

embodiments, the aliphatic amino acid at each of positions 10, 13, 16, and 20 is the same amino acid. In some embodiments, the aliphatic amino acid is a branched chain amino acid. In some embodiments, the aliphatic amino acid at each of positions 10, 13, 16, and 20 is the same amino acid selected from the group consisting of L, I, or V. In some embodiments, the aliphatic amino acid at each of positions 10, 13, 16, and 20 is I. In some embodiments, the aliphatic amino acid at each of positions 10, 13, 16, and 20 is L. In some embodiments, the aliphatic amino acid at position 2 is V, the aliphatic amino acid at position 6, 21, and 24 is L, and the aliphatic amino acid residue at position 10, 13, 16, and 20 is I or L. In some embodiments, a variant comprises A at positions 11 and 12. In some embodiments, a peptide of the invention has glucose-lowering activity and/or treats or prevents one or more symptoms of Alzheimer's Disease or frontotemporal dementia. In some embodiments, a peptide of the invention can sensitize a subject to insulin. For example, in some embodiments, the peptide has the same aliphatic residue at X_{10}, X_{13}, X_{16} and X_{21} wherein the residue is a branched chain aliphatic amino acid residue. In some embodiments, X_{10}, X_{13}, X_{16} and X_{21} is L or X_{10}, X_{13}, X_{16} and X_{21} is I. In some embodiments, the polypeptide comprises SEQ ID NO:1 or SEQ ID NO:2.

[0011] In some examples, a peptide of the disclosure, e.g., SEQ ID NO:1 or a variant as described herein, further comprises amino acids at positions 25 and 26, as numbered with reference to SEQ ID NO:1. In someexamples, the amino acid residue at position 25 is K or N and the amino acid residue at positions 26 is S or Y. In some examples, the amino acid at position 25 is K and the amino acid at position 26 is S. In some examples, the peptide comprises the amino acid sequence EVcitSKLEEWLAALcitELAEELLARLKS (SEQ ID NO:3). In some examples, the polypeptide comprises EVcitSKLEEWIAAIcitEIAEEILARLKS (SEQ ID NO:4).

[0012] In some examples, the disclosure provides an isolated polypeptide having cholesterol efflux activity and little or no cytotoxicity, where the polypeptide comprises an amino acid sequence that has the following features: an amphipathic α-helix structure, with alignment of acidic residues down the center of the polar surface and positively charged amino acids at the lipid-water interface. Further a peptide of the disclosure has one or more uncharged residues at the polar surface of the lipid water interface, *e.g.*, at positions 3, 14, or 23. Thus, in some examples, the disclosure provides a peptide that comprises the following 24-amino acid sequence:

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_{22}X_{23}X_{24}$ (sequence 1)

wherein X_1, X_7, X_8, X_{15}, X_{18} and X_{19} are independently selected from the group consisting of E and D; X_2, X_6, X_9, X_{10}, X_{12}, X_{13}, X_{16}, X_{17}, X_{20}, X_{21} and X_{24} are hydrophobic amino acid residues; X_3, X_{14} and X_{23} are uncharged amino acids; X_4, X_{11}, and X_{22} are amino acids independently selected from the group consisting of S, T, G, A and Y; and X_5 is R or K. In examples in which X_5 is R, the peptide typically has no more than two R residues. In some examples, X_2, X_6, X_{10}, X_{12}, X_{13}, X_{16}, X_{17}, X_{20}, X_{21} and X_{24} are aliphatic amino acids. In some examples, two of positions X_3, X_{14}, and X_{23} are citrulline and the third position is R or K. In some examples, the 24-amino acid sequence comprises no more than two aromatic amino acids. In some examples, a peptide of the disclosure has glucose-lowering activity and/or treats or prevents one or more symptoms of Alzheimer's Disease or frontotemporal dementia. In some examples, a peptide of the disclosure can sensitize a subject to insulin. For example, in some examples, the peptide has the same aliphatic residue at X_{10}, X_{13}, X_{16} and X_{21} wherein the residue is a branched chain aliphatic amino acid residue. In some examples, X_{10}, X_{13}, X_{16} and X_{21} is L or X_{10}, X_{13}, X_{16} and X_{21} is I.

[0013] The disclosure additionally provides an isolated peptide having cholesterol efflux activity that has little or no cytotoxicity that comprises the following 24-amino acid sequence:

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_{22}X_{23}X_{24}$ (sequence 2)

wherein X_1, X_7, X_8, X_{15}, X_{18} and X_{19} are acid amino acids; and X_2, X_6, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{16}, X_{17}, X_{20}, X_{21}, X_{22}, and X_{24} are hydrophobic amino acids, *e.g.,* aliphatic amino acids. In some examples, X_3, X_{14} and X_{23} are uncharged amino acids. In some examples, two of positions X_3, X_{14} and X_{23} are citrulline and the third position is R or K. In some examples, X_{11} and X_{22} are A. In some examples, X_4 is a polar amino acid, preferably a polar uncharged amino acid. In some examples, X_4 is S, T, G, or Y. In some examples, X_4 is S. In some examples, X_5 is a positively charged amino acid. In some examples, X_5 is R or K. In some examples, X_5 is R. In some examples, X_3 and X_{14} are citrulline. In some examples, X_{10}, X_{13}, X_{16}, and X_{20} are I. In some examples, the 24-amino acid sequence comprises no more than two aromatic amino acids. In some examples, where X_5 is R, the peptide has no more than two R residues. In some examples, X_1, X_7, X_8, X_{15}, X_{18} and X_{19} are independently selected from D and E;

X_{10}, X_{13}, X_{16}, and X_{20} are I; X_2, X_6, X_9, X_{11}, X_{12}, X_{17}, X_{21}, X_{22}, and X_{24} are hydrophobic amino acids; and two of positions X_3, X_{14} and X_{23} are citrulline and the third position is R or K. In some examples, X_1, X_7, X_8, X_{15}, X_{18} and X_{19} are independently selected from D and E; X_{10}, X_{13}, X_{16}, and X_{20} are L; X_2, X_6, X_{11}, X_{12}, X_{17}, X_{21}, X_{22}, and X_{24} are aliphatic amino acids; and two of positions X_3, X_{14} and X_{23} are citrulline and the third position is R or K. In some examples, X_1, X_7, X_8, X_{15}, X_{18} and X_{19} are independently selected from D and E; X_{10}, X_{13}, X_{16}, and X_{20} are I; X_2, X_6, X_9, X_{11}, X_{12}, X_{17}, X_{21}, X_{22}, and X_{24} are hydrophobic amino acids, *e.g.,* aliphatic amino acids; positions X_3, and X_{14} are citrulline, and position X_{23} is R or K. In some examples, X_1, X_7, X_8, X_{15}, X_{18} and X_{19} are independently selected from D and E; X_{10}, X_{13}, X_{16}, and X_{20} are L; X_2, X_6, X_{11}, X_{12}, X_{17}, X_{21}, X_{22}, and X_{24} are aliphatic amino acids; positions X_3, and X_{14} are citrulline, and position X_{23} is R or K. In some examples, a peptide of the invention has glucose-lowering activity and/or treats or prevents one or more symptoms of Alzheimer's Disease or frontotemporal dementia. In some examples, a peptide of the invention can sensitize a subject to insulin.

[0014] In some examples, the disclosure provides an isolated peptide having cholesterol efflux activity that has little or no cytotoxicity and comprises the following 24-amino acid sequence:

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_{22}X_{23}$ X_{24} (sequence 3)

wherein X_1, X_7, X_8, X_{15}, X_{18} and X_{19} are amino acids independently selected from the group consisting of E and D; X_2, X_6, X_{10}, X_{12}, X_{13}, X_{16}, X_{17}, X_{20}, X_{21} and X_{24} are amino acids independently selected from the group consisting of A, V, L, or I; X_9 is selected from the group consisting of A, V, L, I and W; at least two of X_3, X_{14} and X_{23} are citrulline; X_5 is K or R, and X_4, X_{11}, and X_{22} are amino acids independently selected from the group consisting of S, T, G, A and Y. In some examples, X_3 and X_{14} are citrulline. In some examples, X_3 and X_{14} are citrulline and X_{23} is R or K. In some examples, X_3 and X_{23} are citrulline and X_{14} is R or K. In some examples, X_{14} and X_{23} are citrulline and X_3 is R or K. In some examples, *e.g.,* in which X_5 is R, the peptide has no more than two R residues. In some examples, the 24-amino acid sequence comprises no more than two aromatic amino acids. In some examples, a peptide of the disclosure has glucose-lowering activity and/or treats or prevents one or more symptoms of Alzheimer's Disease or frontotemporal dementia. In some examples, a peptide of the disclosure can sensitize a subject to insulin. For

example, in some examples, X_{10}, X_{13}, X_{16}, and X_{20} are the same aliphatic amino acid residue, *e.g.,* I or L.

[0015] In some examples, the disclosure provides an isolated peptide having cholesterol efflux activity that has little or no cytotoxicity and comprises the following 24-amino acid sequence:

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_{22}X_{23}$ X_{24} (sequence 4)

wherein X_1, X_7, X_8, X_{15}, X_{18} and X_{19} are D or E; X_2, X_6, X_{10}, X_{12}, X_{13}, X_{16}, X_{17}, X_{20}, X_{21} and X_{24} are amino acids independently selected from the group consisting of A, V, L, or I; at least two of X_3, X_{14} and X_{23} are citrulline; X_9 is W; X_5 is K, and X_4, X_{11}, and X_{22} are independently selected from the group consisting of S and A. In some examples, X_3 and X_{14} are citrulline. In some examples, X_3 and X_{14} are citrulline and X_{23} is R or K. In some examples, the 24-amino acid sequence comprises no more than two aromatic amino acids. In some examples, a peptide of the invention has glucose-lowering activity and/or treats or prevents one or more symptoms of Alzheimer's Disease or frontotemporal dementia. In some examples, a peptide of the invention can sensitize a subject to insulin. For example, in some examples, X_{10}, X_{13}, X_{16}, and X_{20} are the same aliphatic amino acid residue, *e.g.,* I or L.

[0016] In some examples, the disclosure provides an isolated peptide having cholesterol efflux activity that has little or no cytotoxicity and comprises the following 24-amino acid sequence:

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_{22}X_{23}$ X_{24} (sequence 5)

wherein X_1, X_7, X_8, X_{15}, X_{18} and X_{19} are E; X_2, X_6, X_{10}, X_{12}, X_{13}, X_{16}, X_{17}, X_{20}, X_{21} and X_{24} are amino acids independently selected from the group consisting of L and I; at least two of X_3, X_{14} and X_{23} are citrulline; X_9 is W; X_5 is K, X_4, is S; and X_{11} and X_{22} are A. In some examples, X_3 and X_{14} are citrulline. In some examples, X_3 and X_{14} are citrulline amd X_{23} is R or K. In some examples, a peptide of the disclosure has glucose-lowering activity and/or treats or prevents one or more symptoms of Alzheimer's Disease or frontotemporal dementia. In some examples, a peptide of the invention can sensitize a subject to insulin. For example in some examples, X_{10}, X_{13}, X_{16}, and X_{20} are the same aliphatic amino acid residue, *e.g.,* I or L.

[0017] The disclosure further provides an isolated polypeptide having cholesterol efflux activity that has little or no cytotoxicity, where the peptide comprises an amino acid sequence that is an amphipathic α-helix having a non-polar surface and a polar surface, wherein the peptide has at least 60% identity, or at least 70%, at least 80%, or at least 90% identity to SEQ ID NO:1 or has at least 60% identity, or at least 70%, at least 80%, or at least 90% identity to SEQ ID NO:2, wherein the peptide comprises at least one chemical staple at position 3, 14, or 23. The positions described here are determined with reference to SEQ ID NO:1 or SEQ ID NO:2. Thus, for example, the peptide may comprise a residue at position 14 that forms a staple with a residue at position 21. Preferably, the peptide has acidic amino acid residues at positions 1, 7, 8, 15, 18, and 19. In some examples, a variant comprises a hydrophobic residue at positions 2, 6, 9, 10, 13, 16, 17, 20, 21, 22, and 24 as determined with reference to SEQ ID NO:1. In some examples, the variant comprises an aliphatic amino acid at at least one, or at least two, three, four, five, six, seven, eight, or nine of positions 2, 6, 10, 13, 16, 17, 20, 21, 22, and 24 as determined with reference to SEQ ID NO:1. In some examples a variant comprises an aliphatic residue at each of positions 2, 6, 10, 13, 16, 20, 21, and 24. In some examples, the aliphatic amino acid is L, V, or I. In some examples, the aliphatic amino acid residue at position 2, 6, 10, 13, 16, 20, and 21 is L. In some examples, the aliphatic amino acid residue at position 2, 6, 10, 13, 16, 20, and 21 is I. In some examples, the aliphatic residue at each of positions 10, 13, 16, and 20 is I. In some examples, the aliphatic residue at each of positions 10, 13, 16, and 20 is L. In some examples, the aliphatic amino acid residue at position 2 is V. In some examples, the aliphatic amino acid residue at position 2 is V and the aliphatic amino acid residue at position 10, 13, 16, and 20 is L. In some examples, the aliphatic amino acid at position 2 is V, the aliphatic amino acid at position 6, 21, and 24 is L, and the aliphatic amino acid residue at position 10, 13, 16, and 20 is I. In some examples, a variant comprises A at positions 11 and 12. In some examples, a peptide of the disclosure has glucose-lowering activity and/or treats or prevents one or more symptoms of Alzheimer's Disease or frontotemporal dementia. In some examples, a peptide of the disclosure can sensitize a subject to insulin. For example, in some examples, X_{10}, X_{13}, X_{16}, and X_{20} are the same aliphatic amino acid residue, *e.g.,* I or L.

[0018] As understood in the art, a variant peptide of any of the foregoing examples can comprise one or more non-naturally occurring amino acid residues. In some examples, at least one citrulline in any one of the foregoing examplesis replaced with a citulline

amino acid analog. Thus, in some examples, one or more of positions X_3, X_{14} and X_{23} may be an analog of citrulline. In some examples, one or more hydrophobic amino acids is replaced with a non-nautrally occurring analog amino acid that has long aliphatic carbon changes, *e.g.,* long carbon (C_{5-8}) alkenyl or alkanyl side chains.

[0019] In one example, the polypeptides of the present disclosure further comprise a protecting group. For instance, the polypeptides can be modified so that the R-groups on the constituent amino acids and/or the terminal amino acids are blocked, *i.e.*, protected, by a protecting group. It has been found that blockage, particularly of the amino and/or carboxy termini, can greatly improve oral delivery and significantly increases serum half-life. Thus, in one example, the polypeptides of the present disclosure further comprise a protecting group coupled to the amino or carboxy terminus. In one example, the polypeptides further comprise a first protecting group coupled to the amino terminus and a second protecting group coupled to the carboxyl terminus.

[0020] Suitable protecting groups include, but are not limited to, acetyl (Ac), amide, 3 to 20 carbon alkyl groups, Fmoc, t-butoxycarbonyl (Tboc), 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-fluorenecarboxylic 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-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), cyclohexyloxy (cHxO), t-butoxymethyl (Bum), t-butoxy (tBuO), t-butyl (tBu), and trifluoroacetyl (TFA).

[0021] In a preferred example, the polypeptides comprise a first protecting group coupled to the amino terminus, the first protecting group including, but not limited to, acetyl, propionyl, and a 3 to 20 carbon alkyl. In a preferred example, the first protecting group is an acetyl. In another preferred example, the polypeptides comprise a second protecting group coupled to the carboxyl terminus, the second protecting being an amide.

[0022] The polypeptides of the present disclosure can comprise all "L" amino acids, all "D" amino acids or a mixture of "L" and "D" amino acids.

[0023] A polypeptide of the present disclosure has cholesterol efflux activity and/or has ABCA1 stabilizing activity. In yet another example, a polypeptide of the present disclosure protects a phospholipids from oxidation by an oxidizing agent (*i.e.*, the polypeptide has anti-oxidant activity). In still another example, a polypeptide of the present disclosure has anti-inflammatory activity, including inhibition of adhesion molecules. In another example, administration of a polypeptide of the disclosure lowers LDL and/or has favorable effects on glucose control, i.e., glucose-lowering effects. In some examples, a peptide of the invention treats or prevents symptoms of Alzheimer's disease or frontotemporal dementia. In some examples, a polypeptide of the present disclosure comprises each of these activities.

[0024] Also disclosed are pharmaceutical compositions comprising at least one peptide of the disclosure as described herein and a pharmaceutically acceptable carrier or excipient. In some examples, the pharmaceutical compositions comprise an additional therapeutic agent (*e.g.*, a statin such as atorvastatin, lovastatin, pravastatin, simvastatin, fluvastatin, or rosuvastatin; a bile acid binder such as cholestyramine or colestipol; a Nieman-Pick C1-Like 1 sterol transporter channel inhibitor such as Ezetimibe; a platelet clumping inhibitor such as aspirin, ticlopidine, or clopidogrel, niacin/nicotinamide, a PPAR activator, Vitamin E, or combinations thereof, for treating a disease or disorder associated with cholesterol efflux (*e.g.*, cardiovascular disease).

[0025] Also disclosed are peptidomimetics of the polypeptides disclosed herein, wherein the peptidomimetic is an analog peptide, *e.g.,* a retro-inverso analog or retro-enantio analog; or surrogate peptide having a non-amide backbone. In yet another example, the analog is a trans-olefin surrogate peptide or derivative. In some examples, a peptide of the disclosure can comprise other back-bone modifications. Such peptide analogs or surrogates can further comprise a protecting group as described herein and, preferably, a protecting group at both the amino and carboxyl termini.

[0025A] In another aspect, there is provided a retro-inverso or a retro-enantio analog of a peptide as described herein.

[0025B] In yet another aspect, there is provided a composition comprising a polypeptide as described herein and a pharmaceutically acceptable carrier.

[0025C] In yet another aspect, there is provided a composition comprising a polypeptide as described herein complexed with a lipid.

[0026] Also disclosed are a composition comprising a polypeptide of the present disclosure as described herein, *e.g.,* a polypeptide comprising SEQ ID NO:1 or SEQ ID NO:2, or variants thereof, or a peptidomimetic thereof complexed with a lipid. In one example, the lipid is a phospholipid. In another example, the phospholipids is 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphatidylcholine ("POPC"). In yet

another example, the composition further comprises a pharmaceutically acceptable carrier.

[0027] Also disclosed are methods of mediating cholesterol efflux in a mammalian subject (*e.g.*, a primate such as a human or chimpanzee, or a rodent such as a rat or mouse) by administering at least one polypeptide or peptidomimetic described herein to the subject. Those of skill in the art will appreciate that a nucleic acid encoding such a polypeptide (or peptidomimetic) can be administered to the subject in lieu of administering the polypeptide (or peptidomimetic). The present disclosure provides such nucleic acids. Based on their cholesterol efflux activity, the polypeptides and peptidomimetics of the present disclosure can be advantageously used to treat, ameliorate or prevent a disease or condition associated with dyslipidemia, hypercholesterolemia, abnormal glucose metabolism, Alzheimer's Disease, frontotemporal dementia, and inflammation.

[0028] Also disclosed are methods for treating or preventing a symptom of atherosclerosis in a mammal by administering at least one polypeptide or peptidomimetic described herein to the subject. Again, those of skill in the art will appreciate that a nucleic acid encoding such a polypeptide (or peptidomimetic) can be administered to the subject in lieu of administering the polypeptide (or peptidomimetic). Such nucleic acids are provided by the present disclosure. In one example of this method, the mammal is a mammal diagnosed as having one or more symptoms of atherosclerosis. In another example, the mammal is diagnosed as at risk for atherosclerosis. Preferably, the mammal is a human, but can also be a non-human animal. In one example, the polypeptide comprises an amino acid sequence of SEQ ID NO:1 or a variant thereof as described herein. In one example, the polypeptide comprises SEQ ID NO:2, or a variant thereof as described herein.

[0029] In another related example, the methods further comprise administering at least one additional therapeutic agent. Examples of such therapeutic agents include, but are not limited to, agents that treat abnormalities of glucose metabolism, *e.g.,* anti-diabetic agents, agents that treat Alzheimer's disease and/or frontotemporal dementia, an antibody, an enzyme inhibitor, an antibacterial agent, an antiviral agent, a steroid, a non-steroidal anti-inflammatory agent, an anti-metabolite, a cytokine, or a soluble cytokine receptor. The enzyme inhibitor may be a protease inhibitor or a cyclooxygenase inhibitor. The additional agent may be added as a part of a pharmaceutical composition, or may be administered

concomitantly or within a time period when the physiological effect of the additional agent overlaps with the physiological effect of the polypeptide(s) or peptidomimetic(s) of the present disclosure. For example, more specifically, an additional agent may be administered concomitantly one week, several days, 24 hours, 8 hours, or immediately before the administration of the polypeptide(s) or peptidomimetic(s). Alternatively, an additional agent may, for example, be administered one week, several days, 24 hours, 8 hours, or immediately after the administration of the polypeptide(s) or peptidomimetic(s).

[0030] Also disclosed are methods for stabilizing a vulnerable plaque, the method comprising administering to a mammal at least one polypeptide or peptidomimetic described herein. Again, those of skill in the art will appreciate that a nucleic acid encoding such a polypeptide can be administered to the subject in lieu of administering the polypeptide. Such nucleic acids are provided by the present disclosure. In one example of this method, the mammal is a mammal diagnosed as having one or more vulnerable plaques. In another example, the mammal is diagnosed as at risk for having a vulnerable plaque(s). Preferably, the mammal is a human, but can also be a non-human animal. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:1 or is a variant as described herein. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:2, or is a variant as described herein.

[0031] Also disclosed are methods of lowering LDL, the method comprising administering to a mammal at least one polypeptide or peptidomimetic described herein. Again, those of skill in the art will appreciate that a nucleic acid encoding such a polypeptide can be administered to the subject in lieu of administering the polypeptide. Such nucleic acids are provided by the present disclosure. In one example of this method, the mammal is a mammal diagnosed as having elevated LDL. In another example, the mammal is diagnosed as at risk for having elevated LDL. Preferably, the mammal is a human, but can also be a non-human animal. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:1 or is a variant as described herein. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:2 or is variant as described herein.

[0032] In another example of the present disclosure provides methods of lowering glucose levels in a patient having abnormal glucose metabolism, *e.g.,* in a patient having diabetes, *e.g.,* Type II or Type I diabetes, or metabolic syndrome, or pre-diabetes, the method

comprising administering to a mammal at least one polypeptide or peptidomimetic described herein. Again, those of skill in the art will appreciate that a nucleic acid encoding such a polypeptide can be administered to the subject in lieu of administering the polypeptide. Such nucleic acids are provided by the present disclosure. In one example of this method, the mammal is a mammal diagnosed as having abnormal glucose metabolism. In another example, the mammal is diagnosed as at risk for having abnormal glucose metabolism. Preferably, the mammal is a human, but can also be a non-human animal. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:1 or is a variant as described herein. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:2 or is a variant as described herein. In some example, the polypeptide that is administered to lower glucose is a polypeptide as described herein, with the proviso that the polypeptide does not comprise the amino acid sequence of SEQ ID NO:2. In some example, the polypeptide that is administered to lower glucose comprises SEQ ID NO:2.

[0033] Also disclosed are methods of preventing or treating a symptom of frontotemporal dementia, Alzheimer's Disease or Mild Cognitive Impairment, the method comprising administering to a subject at least one polypeptide or peptidomimetic described herein. Again, those of skill in the art will appreciate that a nucleic acid encoding such a polypeptide can be administered to the subject in lieu of administering the polypeptide. Such nucleic acids are provided by the present invention. In one example of this method, the subject is diagnosed as having Alzheimer's Diseae. In another example, the subject is diagnosed as having Mild Cognitive Impairment. In some example, the subject has an ApoE4 allele. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:1 or is a variant as described herein. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:2 or is a variant as described herein.

[0033A] In yet another aspect, the present invention provides a kit for treating a symptom of atherosclerosis, said kit comprising a container containing a polypeptide as described herein.

[0034] The present disclosure also provides kits for treating or preventing a disease or condition associated with dyslipidemia, hypercholesterolemia, abnormal glucose metabolism, inflammation, and/or Alzheimer's Disease or frontotemporal dementia. In one example, the present disclosure provides kits for treating or preventing

a symptom of atherosclerosis, the kit comprising a container containing a polypeptide or peptidomimetic of the present disclosure. In one example, the present disclosure provides kits for treating or preventing a disease associated with abnormal glucose metabolism, *e.g.*, diabetes or metabolic syndrome, the kit comprising a container containing a polypeptide or peptidomimetic of the present

disclosure. In one example, the present diclosure provides kits for treating or preventing a symptom of Alzheimer's Disease, the kit comprising a container containing a polypeptide or peptidomimetic of the present invention. The kit can further comprise a pharmaceutically acceptable carrier. In addition, the kit can further comprise instructional materials teaching the use of the polypeptide or peptidomimetic for treating or preventing the disease or condition. The polypeptides and peptidomimetics provided in the kits of the present example can comprise all L amino acids, all D amino acids or a mixture of L and D amino acids in either natural sequence or (retro)inverted sequence.

[0035] In connection with the above kits, instructional material can include a document or recorded media including a written or audible instruction for the use of a pharmaceutical composition. Instruction material includes, for example, a label on a bottle, a paper inserted in a box, printing on the box or carton, instructions provided by a website at an address given in any of these locations, *etc.*

[0035A] In yet another aspect, the present invention provides an isolated polypeptide having cholesterol efflux activity, wherein the polypeptide comprises an amphipathic α-helix that has a non-polar surface and a polar surface, wherein the α-helix amino acid sequence has at least 60% identity to SEQ ID NO:1, comprises an acidic amino acid residue at positions 1, 7, 8, 15, 18 and 19 as determined with reference to SEQ ID NO:1; comprises at least one chemical staple at positions 3, 14, and 23 and at least two of positions 3, 14, and 23 are polar uncharged amino acids as determined with reference to SEQ ID NO:1; and comprises an aliphatic amino acid independently selected from V, L, or I, or an uncharged amino acid analog thereof, at positions 10, 13, 16, and 20, as determined with reference to SEQ ID NO:1; wherein the uncharged amino acid analog has an aliphatic side chain and has a hydrophobicity of V or greater than V.

[0036] Also disclosed is use of at least one polypeptide or peptidomimetic of the present disclosure in the preparation of a medicament for mediating cholesterol efflux in a mammal, *e.g.,* a human. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:1 or is a variant as described herein. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:2 or is a variant as described herein.

[0037] Also disclosed is use of at least one polypeptide or peptidomimetic of the present invention in the preparation of a medicament for treating a symptom of atherosclerosis in a mammal, *e.g.,* a human. In one examples, the polypeptide has an amino acid of SEQ ID NO:1 or a variant as described herein. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:2 or is a variant as described herein.

[0038] Also disclosed is use of at least one polypeptide or peptidomimetic of the present disclosure in the preparation of a medicament for stabilizing a vulnerable plaque in a mammal, *e.g.,* a human. In one example, the polypeptide has an amino acid of SEQ ID NO:1 or a variant as described herein. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:2 or is a variant as described herein.

[0039] Also disclosed is use of at least one polypeptide or peptidomimetic of the present disclosure in the preparation of a medicament for treating dyslipidemia or hypercholesterolemia in a patient. In one example, the polypeptide has an amino acid of SEQ ID NO:1 or is a variant as described herein. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:2 or is a variant as described herein..

[0040] Also disclosed is use of at least one polypeptide or peptidomimetic of the present disclosure in the preparation of a medicament for decreasing blood glucose levels in a mammal, *e.g.,* a human. In one example, the polypeptide has an amino acid of SEQ ID NO:1 or is a variant as described herein. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:2 or is a variant as described herein..

[0041] Also disclosed is use of at least one polypeptide or peptidomimetic of the present disclosure in the preparation of a medicament for preventing or treating a symptom of frontotemporal dementia, Alzheimer's Disease or Mild Cognitive Impairment in a human. In one example, the polypeptide has an amino acid of SEQ ID NO:1 or is a variant as described herein. In one example, the polypeptide has an amino acid sequence of SEQ ID NO:2 or is a variant as described herein.

[0042] Also disclosed is an isolated nucleic acid encoding a polypeptide of the present disclosure, an expression vector comprising the nucleic acid, and a host cell comprising the expression vector.

[0043] A polypeptide and peptidomimetic of the invention is also useful as a research tool and/or diagnostic tool. For example, such a peptide can be used to identify subjects having reverse cholesterol deficient plasma and those subjects that are responders to reverse cholesterol treatment. Also, a polypeptide of the invention can be used to evaluate the anti-atherosclerotic potential of other compounds (including, *e.g.*, peptidomimetics).

[0044] In addition, a polypeptide or peptidomimetic of the invention can be used for investigating lipoprotein-receptor interactions in animals and animal models, particularly when a polypeptide or peptidomimetic of the present invention is labeled (*e.g.*, radioactive label, fluorescent label, *etc.*).

[0045] A polypeptide or peptidomimetic of the invention can also be used to identify appropriate animal models for elucidation of lipid metabolic pathways. For example, a

WHAT IS CLAIMED IS:

1. An isolated polypeptide having cholesterol efflux activity, the polypeptide comprising an amphipathic α-helix that has a non-polar surface and a polar surface, wherein the polar surface comprises charged and uncharged amino acid residues at the lipid-water interface, wherein the α-helix amino acid sequence:

has at least 60% identity to SEQ ID NO:1;

has an acidic amino acid residue at positions 1, 7, 8, 15, 18 and 19 as determined with reference to SEQ ID NO:1;

has an aliphatic amino acid independently selected from V, L, or I, or an uncharged amino acid analog thereof, at positions 10, 13, 16 and 20, as determined with reference to SEQ ID NO:1; wherein the uncharged amino acid analog has an aliphatic side chain and has a hydrophobicity of V or greater than V; and

has an uncharged polar amino acid residue at at least two of positions 3, 14, and 23 as determined with reference to SEQ ID NO:1.

2. The isolated polypeptide of claim 1, wherein the α-helix amino acid sequence is 24 amino acids in length and further comprises a position 25 and 26, wherein position 25 is K or N and position 26 is S or Y.

3. The isolated polypeptide of claim 1, wherein the amino acid sequence has citrulline or an uncharged analog of citrulline that maintains a salt-bridge configuration in the α-helix at at least one of positions 3, 14, or 23.

4. The isolated polypeptide of claim 1, wherein the amino acid sequence has a citrulline or an uncharged analog of citrulline that maintains a salt-bridge configuration in the α-helix at two of the positions 3, 14, or 23.

5. The isolated polypeptide of claim 4, wherein the α-helix amino acid sequence has an R or K at the third position.

6. The isolated polypeptide of claim 4, wherein the two positions that have a citrulline or uncharged analog of citrulline are positions 3 and 14.

7. The isolated polypeptide of claim 6, wherein the α-helix amino acid sequence has R or K at the third position.

8. The isolated polypeptide of claim 1, wherein the α-helix amino acid sequence has Q, N, L, V, I, or A at position 3, 14, or 23.

9. The isolated polypeptide of claim 8, wherein the α-helix amino acid sequence has Q or N at one position 3, 14, or 23.

10. The isolated polypeptide of claim1, wherein the amino acid residue at at least two of the three positions 3, 14, and 23 is independently selected from Q or N.

11. The isolated polypeptide of claim 10, wherein the amino acid residue at the third position is R, K, or citrulline or an uncharged analog of citrulline that maintains a salt bridge configuration in the α-helix.

12. The isolated polypeptide of claim 1, wherein the α-helix amino acid sequence comprises an aliphatic amino acid at at least one of positions 2, 6, 9, 17 and 24 as determined with reference to SEQ ID NO:1.

13. The isolated polypeptide of claim 12, wherein the aliphatic amino acid at at least one of positions 2, 6, 9, 17, and 24 is independently selected from L, V, A, and I.

14. The isolated polypeptide of claim 12, wherein the aliphatic amino acid at at least one of positions 2, 6, 9, 17, and 24 is L.

15. The isolated polypeptide of claim 1, wherein the aliphatic amino acid at at least two, or at at least three, of positions 10, 13, 16, or 20 is independently selected from V, L, or I.

16. The isolated polypeptide of claim 15, wherein the aliphatic amino acid at at least two, or at at least three, of position 10, 13, 16, or 20 is indenpendently selected from L or I.

17. The isolated polypeptide of claim 16, wherein the α-helix amino acid sequence comprises L at at least two, or at at least three, of positions 10, 13, 16, or 20.

18. The isolated polypeptide of claim 16, wherein the α-helix amino acid sequence comprises I at at least two, or at at least three, of positions 10, 13, 16, or 20.

19. The isolated polypeptide of claim 1, wherein the aliphatic amino acid at each of positions 10, 13, 16, and 20 is independently selected from V, L, or I.

20. The isolated polypeptide of claim 1, wherein the α-helix amino acid sequence comprises L at each of position 6, 10, 13, 16, and 20, or comprises I at each of position 6, 10, 13, 16 and 20.

21. The isolated polypeptide of claim 1, wherein the α-helix amino acid sequence has at least 70% identity to SEQ ID NO:1.

22. The isolated polypeptide of claim 1, wherein the polypeptide further comprises a protecting group.

23. The isolated polypeptide of claim 1, wherein all enantiomeric amino acids are "D" amino acids; or the enantiomeric amino acids are a mixture of "L" amino acids and "D" amino acids.

24. A retro-inverso or a retro-enantio analog of a peptide of claim 1.

25. A composition comprising a polypeptide of claim 1 and a pharmaceutically acceptable carrier.

26. A composition comprising a polypeptide of claim 1 complexed with a lipid.

27. A kit for treating a symptom of atherosclerosis, said kit comprising a container containing a polypeptide of claim 1.

28. The isolated polypeptide of claim 1, wherein the α-helix amino acid sequence has citruline at at least two of the positions 3, 14, or 23; and an R or K at the third position.

29. The isolated polypeptide of claim 28, wherein the α-helix amino acid sequence has citruline at positions 3 and 14 and R or K at position 23.

30. The isolared polypeptide of claim 29, wherein the aliphatic amino acid at at least two, or at least three, of positions 10, 13, 16, and 20 is independently selected from V, L, or I.

31. The isolated polypeptide of claim 29, wherein the α-helix amino acid sequence has an aliphatic amino acid residue at positions 2, 6, 21 and 24.

32. The isolated polypeptide of claim 29, wherein the aliphatic amino acid at each of positions 10, 13, 16, and 20 is independently selected from L, V, or I.

33. The isolated polypeptide of claim 32, wherein the α-helix amino acid sequence comprises L at each of positions 6, 10, 13, 16, and 20.

34. The isolated polypeptide of claim 32, wherein α-helix amino acid sequence comprises I at each of positions 6, 10, 13, 16, and 20.

35. The isolated polypeptide of claim 1, wherein the α-helix amino acid sequence comprises SEQ ID NO:1.

36. The isolated polypeptide of claim1, wherein the α-helix amino acid sequence comprises SEQ ID NO:1 in which I is substitured for L at positions 10, 13, 16, and 20.

37. An isolated polypeptide having cholesterol efflux activity, wherein the polypeptide comprises an amphipathic α-helix that has a non-polar surface and a polar surface, wherein the α-helix amino acid sequence has at least 60% identity to SEQ ID NO:1, comprises an acidic amino acid residue at positions 1, 7, 8, 15, 18 and 19 as determined with reference to SEQ ID NO:1; comprises at least one chemical staple at positions 3, 14, and 23 and at least two of positions 3, 14, and 23 are polar uncharged amino acids as determined with reference to SEQ ID NO:1; and comprises an aliphatic amino acid independently selected from V, L, or I, or an uncharged amino acid analog thereof, at positions 10, 13, 16, and 20, as determined with reference to SEQ ID NO:1; wherein the uncharged amino acid analog has an aliphatic side chain and has a hydrophobicity of V or greater than V.

38. The isolated polypeptide of claim 27, wherein the α-helix amino acid sequence comprises an aliphatic amino acid at positions 2, 6, 21, and 24, as determined with reference to SEQ ID NO:1.

39. The isolated polypeptide of claim 37, wherein the aliphatic amino acid at each of positions 10, 13, 16, or 20 is independently selected from L or I.