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(54) Titre : POLYPEPTIDES DE SYNTHESE IMITANT L'APOLIPOPROTEINE E ET LEURS PROCEDES D'UTILISATION
(54) Title: SYNTHETIC APOLIPOPROTEIN E MIMICKING POLYPEPTIDES AND METHODS OF USE

(57) Abrégé/Abstract:
The present invention provides methods for using synthetic apolipoprotein E (ApoE)-mimicking peptides. Also disclosed are methods for using synthetic apolipoprotein E (ApoE)-mimicking peptides to reduce plasma glucose levels. Methods of using the disclosed apolipoprotein E (ApoE)-mimicking peptides to treat diabetes and diabetic complications are also disclosed.

ABSTRACT

The present invention provides methods for using synthetic apolipoprotein E (ApoE)-mimicking peptides. Also disclosed are methods for using synthetic apolipoprotein E (ApoE)-mimicking peptides to reduce plasma glucose levels. Methods of using the disclosed apolipoprotein E (ApoE)-mimicking peptides to treat diabetes and diabetic complications are also disclosed.

SYNTHETIC APOLIPOPROTEIN E MIMICKING POLYPEPTIDES AND METHODS OF USE

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

This application claims priority to U.S. Provisional Application No. 60/968,362, titled, *Synthetic Apolipoprotein E Mimicking Polypeptides and Methods of Use*, filed on August 28, 2007.

FIELD OF THE INVENTION

This invention relates to the field of molecular biology and protein biology including polypeptides and polypeptide mimics. This application also relates to the field of plasma glucose metabolism, catabolism, and the treatment and management of plasma glucose associated conditions such as diabetes. The present invention also relates generally to the field of medicine. More specifically, the present invention relates to synthetic peptides that can rapidly lower plasma glucose.

BACKGROUND OF THE INVENTION

Diabetes mellitus (DM) is a major cause of morbidity and mortality. Chronically elevated blood glucose leads to debilitating complications: nephropathy, often necessitating dialysis or renal transplant; peripheral neuropathy; retinopathy leading to blindness; ulceration of the legs and feet, leading to amputation; fatty liver disease, sometimes progressing to cirrhosis; vulnerability to coronary artery disease and myocardial infarction, gastroparesis, diseases associated with the autonomic nervous-system, nerve condition abnormalities, i.v. contrast induced nephropathy, small vessel diseases (both within the brain and outside the brain), hypogonadism, and heart faliure.

DM is a group of disorders characterized by high levels of blood glucose. Prevalence of DM is reaching epidemic proportions in the United States and the world. In 2005, approximately 21 million people in the U.S. had DM of which 90% - 95% had type -2 DM (DM-2). Every hour, in the United States, approximately 4100 new cases of DM are diagnosed, and 810 people die from complications of DM. In 2002, DM was the sixth leading cause of death in the U.S. and cost $132 billion. In 2005, DM was responsible for 11.2 million deaths world wide. Contrary to the conventional wisdom, DM affects all socio-economic strata in the world. Cardiovascular complications are the most common causes of morbidity and mortality in DM-2, accounting for up to 70% of the mortality. Interestingly

pre-diabetes, where people have high blood glucose but not sufficient to be classified as DM-2, affects 54 million in the U.S. with age greater than 20 years. These people are at increased risk of DM-2 and cardiovascular disease. Despite significant decline in the coronary heart disease mortality, the effects of such a decline are less significant in diabetics as compared to non-diabetics.

There are two primary types of diabetes. Type I, or insulin-dependent diabetes mellitus (IDDM), is due to autoimmune destruction of insulin-producing beta cells in the pancreatic islets. The onset of this disease is usually in childhood or adolescence. Treatment consists primarily of multiple daily injections of insulin, combined with frequent testing of blood glucose levels to guide adjustment of insulin doses, because excess insulin can cause hypoglycemia and consequent impairment of brain and other functions. Type II diabetes (DM2), or noninsulin-dependent diabetes mellitus (NIDDM), typically develops in adulthood. NIDDM is associated with resistance of glucose-utilizing tissues like adipose tissue, muscle, and liver, to the actions of insulin. Initially, the pancreatic islet beta cells compensate by secreting excess insulin. Eventual islet failure results in decompensation and chronic hyperglycemia. Conversely, moderate islet insufficiency can precede or coincide with peripheral insulin resistance.

Insulin resistance can also occur without marked hyperglycemia, and is generally associated with atherosclerosis, obesity, hyperlipidemia, and essential hypertension. This cluster of abnormalities constitutes the "metabolic syndrome" or "insulin resistance syndrome". Insulin resistance is also associated with fatty liver, which can progress to chronic inflammation (NASH; "nonalcoholic steatohepatitis"), fibrosis, and cirrhosis. Cumulatively, insulin resistance syndromes, including but not limited to diabetes, underlay many of the major causes of morbidity and death of people over age 40.

DM-2, which accounts for 90% - 95% of all DM, is characterized by insulin resistance and relative insulin deficiency. In the early stages, this may manifest as glucose intolerance with relatively non-specific symptoms and may not be diagnosed. However, these patients are at increased risk for continuing progression of the disease with associated clinical complications involving multiple organs. Attempts to delay the onset and progression of DM-2 have met with mixed success. Published in 2002, the Diabetes Prevention Study (DPP) demonstrated that lifestyle modification consisting of moderate exercise regimen and dietary modification can be effective in preventing/delaying the rate

of onset of DM-2. However significant barriers like behavioral modification make the routine implementation of this strategy difficult. Pharmaceutical agents such as metformin have also demonstrated the effectiveness of preventing/delaying the onset of DM-2. Despite advances in the medical and lifestyle therapies, the incidence and prevalence of the DM-2 continues to increase. Even more interesting is the fact that cardiovascular disease in DM-2 is more aggressive with earlier onset. DM-2 demonstrates characteristic lipoprotein changes including lower high density lipoprotein (HDL) and higher triglycerides (TG) concentrations. Low density lipoproteins (LDL) in DM-2 may not be markedly elevated as compared to control cohort. However, small dense LDL is present in greater concentration. This characteristic diabetic dyslipidemia is associated with markedly increased cardiovascular disease mortality (MRFIT) as compared to non-diabetics. Statins are a class of drugs that predominantly lower LDL. These medications are effective in reducing cardiovascular disease risks in both DM and non-DM, however the residual CVD risk in DM despite LDL lowering remains higher than non-diabetics taking placebo. Elevated HDL may provide an additional mechanism of cardiovascular disease risk reduction in both diabetics and non-diabetics. Multiple trials are ongoing to evaluate the efficacy of increasing HDL in decreasing CVD risk in both diabetic and non-diabetic population.

Despite the existence of drugs to treat such disorders, diabetes and other insulin-resistant disorders remain a major and growing public health problem. Late stage complications of diabetes consume a large proportion of national health care resources. There is a need for new active therapeutic agents which effectively address the primary defects of insulin resistance and islet failure with fewer or milder side effects than existing drugs. What is needed in the art are compositions and methods for treating insulin resistance.

Apolipoprotein E is a protein that binds lipid and has two major domains (Mahley, R.W., *et al.* J. Lipid Res. 1999, 40:622-630). The 22 kDa amino terminal domain has been shown by X-ray crystallographic studies to be a 4-helix bundle (Wilson, C., *et al.* Science 1991; 252: 1817-1822) and to contain a positively-charged receptor binding domain. For this region to mediate very low-density lipoprotein (VLDL) binding to its receptors, the apolipoprotein must associate with the lipoprotein surface; this is enabled by the C-terminal amphipathic helical region. If the 4-helix bundle that contains the positively charged receptor-binding domain does not open up on the lipoprotein surface, then the VLDL is

defective in binding to receptors. Thus, the positively charged arginine (Arg)-rich cluster domain of the Apo E and the C-terminal amphipathic helical domain, are both required for the enhanced uptake of atherogenic Apo E-containing lipoproteins.

Apo E is secreted as a 299 amino acid residue protein with a molecular weight of 34,200. Based on thrombin cleavage of apo E into two fragments, a two-domain hypothesis was initially suggested to explain the fact that the C-terminal region of apo E (192-299) is essential for its binding to hypertriglyceridemic VLDL, and the N-terminal 22 kDa domain (1-191) binds to the LDL-R (Bradley, W. A., *et al.*, (1986) J. Lipid Res. 27, 40-48). Additional physical-chemical characterization of the protein and its mutants have extended this concept and have shown that the region 192-211 binds to phospholipid while the amino terminal domain (1-191) is a globular structure that contains the LDL receptor binding domain in the 4-helix bundle (Wilson, C., *et al.*, (1991) Science 252, 1817-1822). Studies with synthetic peptides (Sparrow *et al.*) and monoclonal antibodies pinpointed the LDL receptor binding domain of apo E between residues 129-169, a domain enriched in positively charged amino acids, Arg and Lys (Rall, S. C., Jr., *et al.*, (1982) PNAS USA 79, 4696-4700; Lalazar, A., *et al.*, (1988) J. Biol. Chem. 263, 3542-2545; Dyer, C. A., *et al.*, (1991) J. Biol. Chem. 296, 22803-22806; and Dyer, C. A., *et al.*, (1991) J. Biol. Chem. 266, 15009-15015).

Further studies with synthetic peptides were used to characterize the structural features of the binding domain of apo E that mediates its interaction with the LDL receptor (Dyer, C. A., *et al.*, (1991) J. Biol. Chem. 296, 22803-22806; Dyer, C. A., *et al.*, (1991) J. Biol. Chem. 266, 15009-15015; and Dyer, C. A., *et al.*, (1995) J. Lipid Res. 36, 80-8). Residues 141-155 of apo E, although containing the positively charged residues, did not compete for binding of LDL in a human skin fibroblast assay, but did so only as tandem covalent repeats [*i.e.*, $(141\text{-}155)_2$]. N-acetylation of the $(141\text{-}155)_2$ peptide, on the other hand, enhanced LDL binding to fibroblasts (Nicoulin, I. R., *et al.*, (1998) J. Clin Invest. 101, 223-234). The N-acetylated $(141\text{-}155)_2$ analog selectively associated with cholesterol-rich lipoproteins and mediated their acute clearance *in vivo* (Nicoulin, I. R., *et al.*, (1998) J. Clin Invest. 101, 223-234). Furthermore, these studies indicated that the prerequisite for receptor binding is that the peptides be helical (Dyer, C. A., *et al.*, (1995) J. Lipid Res. 36, 80-88). Enhanced LDL uptake and degradation were also observed (Mims, M. P., *et al.*, (1994) J. Biol. Chem. 269, 20539-20647) using synthetic peptides modified to increase lipid

association by N, N-distearyl derivation of glycine at the N-terminus of the native 129-169 sequence of Apo E (Mims, M. P., *et al.*, (1994) J. Biol. Chem. 269, 20539-20647). Although LDL binding is mediated by the cationic sequence 141-155 of human Apo E, Braddock *et al.* (Braddock. D. T., *et al.*, (1996) Biochemistry 35, 13975-13984) have shown that model peptides of the highly conserved anionic domain (41-60 of human Apo E) also modulate the binding and internalization of LDL to cell surface receptors. However, these peptides do not enhance LDL degradation.

Chylomicron is a lipoprotein found in blood plasma, which carries lipids from the intestines into other body tissues and is made up of a drop of triacylglycerols surrounded by a protein-phospholipid coating. Chylomicron remnants are taken up by the liver (Havel, R.J., 1985, Arteriosclerosis. 5:569-580) after sequestration in the space of Disse, which is enriched with Apo E (Kwiterovich, P.O., Jr., 1998; Deedwania, P.C., 1995; and Watts, G.W., *et al.*, 1998). Apo E is the major mediator of hepatic remnant lipoprotein uptake by the LDL receptor or LRP. Lipolysis of normal VLDL Sf (subfraction) of more than 60 permit binding of the lipolytic remnant to the LDL receptor (Catapano, A.L. *et al.* 1979, J. Biol. Chem. 254:1007-1009; Schonfield, G., et al. 1979. J. Clin. Invest. 64:1288-1297). Lipoprotein lipase (LpL) may facilitate uptake through localization of Apo B-containing lipoproteins to membrane heparan sulphate proteoglycan (HSPG) (Eisenberg, *et al.* 1992. J. Clin. Invest. 90:2013-2021; Hussain, M., *et al.*, J. Biol. Chem. 2000, 275:29324-29330) and/or through binding to the LDL-receptor-related protein (LRP) (Beisiegel, U., et al., 1989, Nature 341:162-164). Cell-surface HSPG may also function as a receptor and has variable binding affinities for specific isoforms of Apo E. In particular, Apo E is synthesized by the liver and also by monocyte/macrophages, where it exerts its effect on cholesterol homeostasis. *In vivo* evidence for the local effect of lack of Apo E comes from the observations of Linton and Fazio, who showed accelerated atherosclerosis in C57BL/6 mice transplanted with bone marrow from Apo E-deficient mice (Linton, M.F. and Fazio, S. Curr. Openi. Lipidol. 1999, 10:97-105). Apo E-dependent LDL cholesteryl ester uptake pathway has been demonstrated in murine adrenocortical cells (Swarnakar, S., et al. J. Biol. Chem. 2001, 276:21121-21126). This appears to involve chondroitin sulphate proteoglycan (CSPG) and a 2-macroglobulin receptor.

U.S. Patent No. 6,506,880 denotes the first effort to synthesize apolipoprotein E-mimicking peptides based on the hypothesis that since lipid binding is essential for surface

localization of the peptide on lipoproteins and for the receptor binding domain of apo E to be appropriately accessible to bind to the LDL receptor, joining a well-characterized, lipid-associating peptide such as the model class A amphipathic helix, 18A, to the 141-150 peptide sequence of apo E should be sufficient to confer biological activity.

The present invention provides novel synthetic ApoE-mimicking peptides wherein the receptor binding domain of ApoE is covalently linked to 18A, the well characterized lipid-associating model class A amphipathic helical peptide as well as possible applications of the synthetic peptides in lowering human plasma glucose levels.

SUMMARY OF THE INVENTION

The present invention provides polypeptides, compositions, and methods of use of said polypeptides and compositions.

Disclosed herein are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases.

Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, wherein the synthetic apolipoprotein E-mimicking peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 11-14, 18-57, 60, 61, and 62-103. Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, wherein the synthetic apolipoprotein E-mimicking peptide comprises a receptor binding domain peptide and a lipid-associating peptide, wherein said lipid binding domain peptide is covalently linked to said receptor binding domain peptide.

Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, wherein the synthetic apolipoprotein E-mimicking peptide comprises a receptor binding domain peptide and a lipid-associating peptide, wherein said lipid binding domain peptide is covalently linked to said receptor binding domain peptide, wherein the receptor binding domain

peptide is from a species selected from the group consisting of human, mouse, rabbit, monkey, rat, bovine, pig, and dog.

Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, wherein the synthetic apolipoprotein E-mimicking peptide comprises a receptor binding domain peptide and a lipid-associating peptide, wherein said lipid binding domain peptide is covalently linked to said receptor binding domain peptide, wherein the receptor binding domain peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 1-2, 3, 5-10, 15, and 58.

Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, wherein the synthetic apolipoprotein E-mimicking peptide comprises a receptor binding domain peptide and a lipid-associating peptide, wherein said lipid binding domain peptide is covalently linked to said receptor binding domain peptide, wherein the receptor binding domain peptide is mutated

Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, wherein the synthetic apolipoprotein E-mimicking peptide comprises a receptor binding domain peptide and a lipid-associating peptide, wherein said lipid binding domain peptide is covalently linked to said receptor binding domain peptide, wherein the receptor binding domain peptide is scrambled.

Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, wherein the synthetic apolipoprotein E-mimicking peptide comprises a receptor binding domain peptide and a lipid-associating peptide, wherein said lipid binding domain peptide is covalently linked to said receptor binding domain peptide, wherein the receptor binding domain peptide is in a reversed orientation.

Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, wherein the synthetic apolipoprotein E-mimicking peptide comprises a receptor binding domain peptide and a lipid-associating peptide, wherein said lipid binding domain peptide is covalently linked to said receptor binding domain peptide, wherein the lipid-associating peptide is model class A amphipathic helical peptide 18A.

Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, wherein the synthetic apolipoprotein E-mimicking peptide comprises a receptor binding domain peptide and a lipid-associating peptide, wherein said lipid binding domain peptide is covalently linked to said receptor binding domain peptide, wherein said lipid-associating peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 4, 16, 17, and 59.

Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, wherein the synthetic apolipoprotein E-mimicking peptide comprises a receptor binding domain peptide and a lipid-associating peptide, wherein said lipid binding domain peptide is covalently linked to said receptor binding domain peptide, wherein the lipid-associating peptide is mutated, scrambled, or is in a domain switched orientation.

Also disclosed are methods for decreasing the concentration of plasma glucose in a subject, comprising: administering a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject, whereby the concentration of plasma glucose in the subject decreases. Also disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject, whereby the concentration of plasma glucose in the subject decreases. Also disclosed are methods of treating a subject with diabetes comprising: selecting a subject with diabetes; administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject; thereby treating diabetes in the subject.

Also disclosed are methods of treating a subject with diabetes comprising: selecting a subject with diabetes; and administering an effective amount of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject; thereby treating diabetes in the subject.

Various embodiments of the claimed invention pertain to use of a synthetic apolipoprotein E-mimicking peptide or a pharmaceutical composition comprising such a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier for decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus. Also claimed is use of such a synthetic apolipoprotein E-mimicking peptide or pharmaceutical composition for the preparation of a medicament for decreasing the concentration of plasma glucose in a subject.

In another aspect it is provided use of a synthetic apolipoprotein E-mimicking peptide for decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

In a further aspect it is provided use of a synthetic apolipoprotein E-mimicking peptide for the preparation of a medicament for decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

In a further aspect it is provided use of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier for decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

In a further aspect it is provided use of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier for the preparation of a medicament for decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

In another aspect it is provided a synthetic apolipoprotein E-mimicking peptide for use in decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

In a further aspect it is provided a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier for decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating

peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

In yet another aspect it is provided use of a synthetic apolipoprotein E-mimicking peptide for reducing β-cell apoptosis in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

In another aspect it is provided use of a synthetic apolipoprotein E-mimicking peptide for the preparation of a medicament for reducing β-cell apoptosis in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

In a further aspect it is provided use of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier for reducing β-cell apoptosis in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

In a further aspect it is provided use of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier for the preparation of a medicament for reducing β-cell apoptosis in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E

and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A

amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention and together with the description, serve to explain the principles of the invention. These are non-limiting examples.

Figure 1 shows Ac-hE18A-NH_2 causes an increase in HDL associated Paraoxonase (PON) ($p < 0.05$) activity and a decrease in lipid hydroperoxides (LOOH) ($p < 0.05$) in the plasma of WHHL rabbits.

Figure 2 shows administration of Ac-hE18A-NH_2 to high fat diet administered rabbits with initial cholesterol values in the range of 600 mg/dl (1 week on 1% cholesterol diet).

Figure 3 shows *in vitro*, in apoE-null mouse plasma, D-4F causes a major redistribution of apoA-I from α-migrating to pre-β migrating particles.

Figure 4 A and B show the glucose and insulin levels, respectively, of 5-6 week old male ZDF (fa/fa) with defective leptin receptor were administered peptides (5 mg/kg i.v.) that mimic the properties of HDL (Ac-hE-18A-NH_2 and L-4F respectively) as compared to the control group (n = 7-8/group).

Figure 5 shows anti-diabetic and anti-atherosclerotic effects of Apo-E mimetic peptides.

Figure 6 shows a pathway of how Apo-E mimetic peptides increase insulin secretion from pancreatic β-cells.

DETAILED DESCRIPTION OF THE INVENTION

It is to be understood that this invention is not limited to specific synthetic methods, or to specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, to specific pharmaceutical carriers, or to particular pharmaceutical formulations or administration regimens, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

1. Definitions and Nomenclature

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

As used in the specification and the appended claims, the singular forms "a," "an" and "the" can include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a compound" includes mixtures of compounds, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 20%. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

As used herein, the term "amino acid sequence" refers to a list of abbreviations, letters, characters or words representing amino acid residues. The amino acid abbreviations used herein are conventional one letter codes for the amino acids and are expressed as follows: A, alanine; C, cysteine; D aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H histidine; I isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine..

"Polypeptide" as used herein refers to any peptide, oligopeptide, polypeptide, gene product, expression product, or protein. A polypeptide is comprised of consecutive amino acids. The term "polypeptide" encompasses naturally occurring or synthetic molecules.

In addition, as used herein, the term "polypeptide" refers to amino acids joined to each other by peptide bonds or modified peptide bonds, *e.g.*, peptide isosteres, *etc.* and may contain modified amino acids other than the 20 gene-encoded amino acids. The polypeptides can be modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. The same type of modification can be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide can have many types of modifications. Modifications include, without limitation, acetylation, acylation, ADP-ribosylation, amidation, covalent cross-linking or cyclization, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of a phosphytidylinositol, disulfide bond formation, demethylation, formation of cysteine or pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristolyation, oxidation, pergylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, and transfer-RNA mediated addition of amino acids to protein such as arginylation. (*See Proteins – Structure and Molecular Properties* 2nd Ed., T.E. Creighton, W.H. Freeman and Company, New York (1993); *Posttranslational Covalent Modification of Proteins*, B.C. Johnson, Ed., Academic Press, New York, pp. 1-12 (1983)).

As used herein, "peptidomimetic" means a mimetic of a function of a protein which includes some alteration of the normal peptide chemistry. Peptidomimetics typically are short sequences of amino acids that in biological properties, mimic the function(s) of a particular protein. Peptide analogs enhance some property of the original peptide, such as increase stability, increased efficacy, enhanced delivery, increased half life, *etc.* Methods of making peptidomimetics based upon a known polypeptide sequence is described, for example, in U.S. Patent Nos. 5,631,280; 5,612,895; and 5,579,250. Use of peptidomimetics can involve the incorporation of a non-amino acid residue with non-amide linkages at a

given position. One embodiment of the present invention is a peptidomimetic wherein the compound has a bond, a peptide backbone or an amino acid component replaced with a suitable mimic. Some non-limiting examples of unnatural L- or D-amino acids which may be suitable amino acid mimics include β-alanine, L-α-amino butyric acid, L-γ-amino butyric acid, L-α-amino isobutyric acid, L-ε-amino caproic acid, 7-amino heptanoic acid, L-aspartic acid, L-glutamic acid, N-ε-Boc-N-α-CBZ-L-lysine, N-ε-Boc-N-α-Fmoc-L-lysine, L-methionine sulfone, L-norleucine, L-norvaline, N-α-Boc-N-δCBZ-L-ornithine, N-δ-Boc-N-α-CBZ-L-ornithine, Boc-p-nitro-L-phenylalanine, Boc-hydroxyproline, and Boc-L-thioproline.

The word "or" as used herein means any one member of a particular list and also includes any combination of members of that list.

The phrase "nucleic acid" as used herein refers to a naturally occurring or synthetic oligonucleotide or polynucleotide, whether DNA or RNA or DNA-RNA hybrid, single-stranded or double-stranded, sense or antisense, which is capable of hybridization to a complementary nucleic acid by Watson-Crick base-pairing. Nucleic acids of the invention can also include nucleotide analogs (*e.g.*, BrdU), and non-phosphodiester internucleoside linkages (*e.g.*, peptide nucleic acid (PNA) or thiodiester linkages). In particular, nucleic acids can include, without limitation, DNA, RNA, cDNA, gDNA, ssDNA, dsDNA or any combination thereof

As used herein, "reverse oriented", "reversed orientation", "reverse analog" or "reverse sequence" refers to a peptide, or a portion of the peptide, has a reverse amino acid sequence as compared to a non-reverse oriented peptide (*i.e.*, the original sequence is read (or written) from right to left). For example, if one peptide has the amino acid sequence ABCDE, its reverse analog or a peptide having its reverse sequence is as follows: EDCBA. In a dual domain peptide for example, Ac-hE-18A-NH_2, either the hE sequence is read from right to left or the 18A sequence is read from right to left. For a reverse analog of, LRKLRKRLLR- DWLKAFYDKVAEKLKEAF can be RLLRKRLKRL-DWLKAFYDKVAEKLKEAF (SEQ ID NO: 64) or LRKLRKRLLR-FAEKLKEAVKDYFAKLWD (SEQ ID NO: 84).

As used herein a "synthetic apolipoprotein E-mimicking peptide" is meant to include a dual-domain ApoE mimicking peptide or a single-domain ApoE mimicking peptide as disclosed herein.

As used herein a "dual-domain peptide", a "dual-domain synthetic peptide", or a "dual-domain ApoE mimicking peptide" is meant to mean a peptide comprising a lipid-associating peptide/domain and a receptor binding peptide/domain.

As used herein a "single-domain peptide", a "single-domain synthetic peptide", or a "single-domain ApoE mimicking peptide" is meant to mean a peptide comprising either a lipid-associating peptide/domain or a receptor binding peptide/domain, or a single domain amphipathic helix with hydrophobic residues on the nonpolar face and arginine residues at the center of the polar face, but not all.

As used herein "domain switched", "switched domain", or "switched" peptide is meant to mean that the lipid-associating peptide is covalently linked to the receptor binding domain of apolipoprotein E such that the lipid-associating peptide is at the N-terminus of the synthetic apolipoprotein E-mimicking peptide. For example, the peptide 18A-hE (SEQ ID NO: 38) is exemplary of a domain switched peptide.

As used herein, "scrambled" "scrambled version", or "scrambled peptide" is meant to mean that the composition of the amino acid sequence is the same as the unscrambled peptide, however the sequence of the amino acids is altered thus rendering the peptide unable to form either an α-amphipathic helix or does not possess lipid associating (or HSPG associating) properties. However, in some cases, as described in this invention, the scrambled peptide remains able to form a different helical structure, such as a π-helix. For example, if one peptide has the amino acid sequence ABCDE, the scrambled version of the peptide could have the amino acid sequence DEABC. Scrambled peptides are often denoted as having an "Sc" prior to the portion of the peptide that is scrambled. For example, Sc-hE-18A denoted that the hE portion of the peptide is scrambled.

An "α-amphipathic helix" is discussed above and has 3.6 amino acid residues per turn of the helix, whereas a "π-helix" has 4.4 amino acid residues per turn.

As used herein, "sample" is meant to mean an animal; a tissue or organ from an animal; a cell (either within a subject, taken directly from a subject, or a cell maintained in culture or from a cultured cell line); a cell lysate (or lysate fraction) or cell extract; or a solution containing one or more molecules derived from a cell or cellular material (e.g. a polypeptide or nucleic acid), which is assayed as described herein. A sample may also be any body fluid or excretion (for example, but not limited to, blood, urine, stool, saliva, tears, bile) that contains cells or cell components.

As used herein, "modulate" is meant to mean to alter, by increasing or decreasing.

As used herein "lipid binding domain E" and "lipid-associating peptide" are used interchangeably. As used herein, both terms can mean the lipid binding domain of Apolipoprotein E.

As used herein, "normal subject" is meant to mean an individual who does not have "Diabetes" or a "Diabetic Complication".

As used herein, "diabetes" or "diabetes mellitus" shall mean a a metabolic disorder characterized by hyperglycemia (high blood sugar) and other signs, as distinct from a single illness or condition. The term "diabetes" or "diabetes mellitus" as used herein is meant to include the three main forms of diabetes recognized by the World Health Organization, namely: type 1, type 2, gestational diabetes (occurring during pregnancy), and/or associated complications such as juvenile onset diabetes, diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy. The term "diabetes" or "diabetes mellitus" as used herein is also meant to mean all forms of diabetes caused by the beta cells of the pancreas being unable to produce sufficient insulin to prevent hyperglycemia. The term "diabetes" or "diabetes mellitus" as used herein is also meant to include glucose intolerance and diabetes glucose-intolerant subjects.

As used herein, "Inflammatory Disorder" is meant to mean when a subject experiences a cascade of reactions initiated by oxidized lipids in which several cytokine levels go up to alter the normal physiological response. Inflammatory disorders include, but are not limited to Inflammatory Bowel Disease (IBD), systemic lupus erythematosus, Hashimoto's disease, rheumatoid arthritis, graft-versus-host disease, Sjögren's syndrome, pernicious anemia, Addison disease, Alzheimer's disease, scleroderma, Goodpasture's syndrome, ulcerative colitis, Crohn's disease, autoimmune hemolytic anemia, sterility, myasthenia gravis, multiple sclerosis, Basedow's disease, thrombopenia purpura, allergy; asthma, atopic disease, cardiomyopathy, glomerular nephritis, hypoplastic anemia, metabolic syndrome X , peripheral vascular disease, chronic obstructive pulmonary disease (COPD), emphysema, asthma, idiopathic pulmonary fibrosis, pulmonary fibrosis, adult respiratory distress syndrome, osteoporosis, Paget's disease, coronary calcification, polyarteritis nodosa, polymyalgia rheumatica, Wegener's granulomatosis, central nervous system vasculitis (CNSV), Sjogren's syndrome, scleroderma, polymyositis, AIDS inflammatory response, influenza, avian flu, viral pneumonia, endotoxic shock syndrome,

sepsis, sepsis syndrome, trauma/wound, corneal ulcer, chronic/non-healing wound, reperfusion injury (prevent and/or treat), ischemic reperfusion injury (prevent and/or treat), spinal cord injuries (mitigating effects), cancers, myeloma/multiple myeloma, ovarian cancer, breast cancer, colon cancer, bone cancer, osteoarthritis, allergic rhinitis, cachexia, Alzheimer's disease, implanted prosthesis, biofilm formation, dermatitis, acute and chronic, eczema, psoriasis, contact dermatitis, erectile dysfunction, macular degeneration, nephropathy, neuropathy, Parkinson's Disease, peripheral vascular disease, and meningitis, cognition and rejection after organ transplantation. Inflammatory diseases can be bacterial, fungal, parasitic and/or viral in nature.

As used herein, a "diabetic complication" is meant to mean complications induced by an increase in plasma glucose levels above normal level. Examples include, but are not limited to nephropathy, often necessitating dialysis or renal transplant; peripheral neuropathy; retinopathy leading to blindness; ulceration of the legs and feet, leading to amputation; fatty liver disease, sometimes progressing to cirrhosis; and vulnerability to coronary artery disease and myocardial infarction, gastroparesis, diseases associate with the autonomic nervous system, nerve condition abnormalities, i.v. contrast induced nephropathy, small vessel diseases (both within the brain and outside the brain), hypogonadism and heart failure.

As used herein, "effective amount" of a compound is meant to mean a sufficient amount of the compound to provide the desired effect. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of disease (or underlying genetic defect) that is being treated, the particular compound used, its mode of administration, and the like. Thus, it is not possible to specify an exact "effective amount." However, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

As used herein, "isolated polypeptide" or "purified polypeptide" is meant to mean a polypeptide (or a fragment thereof) that is substantially free from the materials with which the polypeptide is normally associated in nature. The polypeptides of the invention, or fragments thereof, can be obtained, for example, by extraction from a natural source (for example, a mammalian cell), by expression of a recombinant nucleic acid encoding the polypeptide (for example, in a cell or in a cell-free translation system), or by chemically

synthesizing the polypeptide. In addition, polypeptide fragments may be obtained by any of these methods, or by cleaving full length proteins and/or polypeptides.

As used herein, "isolated nucleic acid" or "purified nucleic acid" is meant to mean DNA that is free of the genes that, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, such as an autonomously replicating plasmid or virus; or incorporated into the genomic DNA of a prokaryote or eukaryote (*e.g.*, a transgene); or which exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR, restriction endonuclease digestion, or chemical or *in vitro* synthesis). It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence. The term "isolated nucleic acid" also refers to RNA, *e.g.*, an mRNA molecule that is encoded by an isolated DNA molecule, or that is chemically synthesized, or that is separated or substantially free from at least some cellular components, for example, other types of RNA molecules or polypeptide molecules.

As used herein, "treat" is meant to mean administer a compound or molecule of the invention to a subject, such as a human or other mammal (for example, an animal model), that has a Lipid Disorder, or that has coronary artery disease, rheumatoid arthritis, and/or systemic lupus, in order to prevent or delay a worsening of the effects of the disease or condition, or to partially or fully reverse the effects of the disease.

As used herein, "prevent" is meant to mean minimize the chance that a subject who has an increased susceptibility for developing diabetes will develop diabetes.

As used herein, "specifically binds" is meant that an antibody recognizes and physically interacts with its cognate antigen (for example, the disclosed synthetic apolipoprotein E-mimicking peptides) and does not significantly recognize and interact with other antigens; such an antibody may be a polyclonal antibody or a monoclonal antibody, which are generated by techniques that are well known in the art.

As used herein, "probe," "primer," or oligonucleotide is meant to mean a single-stranded DNA or RNA molecule of defined sequence that can base-pair to a second DNA or RNA molecule that contains a complementary sequence (the "target"). The stability of the resulting hybrid depends upon the extent of the base-pairing that occurs. The extent of base-pairing is affected by parameters such as the degree of complementarity between the probe and target molecules and the degree of stringency of the hybridization conditions.

The degree of hybridization stringency is affected by parameters such as temperature, salt concentration, and the concentration of organic molecules such as formamide, and is determined by methods known to one skilled in the art. Probes or primers specific for nucleic acids capable of encoding the disclosed synthetic apolipoprotein E-mimicking peptide (for example, genes and/or mRNAs) have at least 80% - 90% sequence complementarity, preferably at least 91% - 95% sequence complementarity, more preferably at least 96% - 99% sequence complementarity, and most preferably 100% sequence complementarity to the region of the nucleic acid capable of encoding the disclosed synthetic apolipoprotein E-mimicking peptide to which they hybridize. Probes, primers, and oligonucleotides may be detectably-labeled, either radioactively, or non-radioactively, by methods well-known to those skilled in the art. Probes, primers, and oligonucleotides are used for methods involving nucleic acid hybridization, such as: nucleic acid sequencing, reverse transcription and/or nucleic acid amplification by the polymerase chain reaction, single stranded conformational polymorphism (SSCP) analysis, restriction fragment polymorphism (RFLP) analysis, Southern hybridization, Northern hybridization, in situ hybridization, and electrophoretic mobility shift assay (EMSA).

As used herein, "specifically hybridizes" is meant to mean that a probe, primer, or oligonucleotide recognizes and physically interacts (that is, base-pairs) with a substantially complementary nucleic acid (for example, a nucleic acid capable of encoding the disclosed synthetic apolipoprotein E-mimicking peptide) under high stringency conditions, and does not substantially base pair with other nucleic acids.

As used herein, "high stringency conditions" is meant to mean conditions that allow hybridization comparable with that resulting from the use of a DNA probe of at least 40 nucleotides in length, in a buffer containing 0.5 M $NaHPO_4$, pH 7.2, 7% SDS, 1 mM EDTA, and 1% BSA (Fraction V), at a temperature of 65 °C, or a buffer containing 48% formamide, 4.8X SSC, 0.2 M Tris-Cl, pH 7.6, 1X Denhardt's solution, 10% dextran sulfate, and 0.1% SDS, at a temperature of 42 °C. Other conditions for high stringency hybridization, such as for PCR, Northern, Southern, or in situ hybridization, DNA sequencing, *etc.*, are well-known by those skilled in the art of molecular biology. (See, for example, F. Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, 1998).

2. Compositions

Disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

Also disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, *etc.* of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein.

Methods of Use

The invention also provides many therapeutic methods of using the nucleic acids, peptides, polypeptides, vectors, antibodies, and compositions disclosed herein. For example, disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases. The Examples section below provides examples of how the nucleic acids, peptides, polypeptides, vectors, and antibodies, and compositions of the invention can be used and

tested. One of skill in the art would be capable of modifying the methods provided in the Examples section to test and use the the nucleic acids, peptides, polypeptides, vectors, antibodies, and compositions disclosed herein. Subjects may be a mammal, such as a human. Additionally, the subject can be an animal which can be a model system used to test human therapeutics. Non-limiting examples of such animals include dog, pig, primate, murine, feline, bovine, or equine animals.

As described above, the synthetic apolipoprotein E-mimicking peptide can be a dual-domain ApoE mimicking peptide or a single-domain ApoE mimicking peptide. For example, the synthetic apolipoprotein E-mimicking peptide can comprise a sequence selected from the group consisting of SEQ ID NOs: 11-14, 18-57, 60, 61, and 62-103. Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, wherein the synthetic apolipoprotein E-mimicking peptide is administered in a composition comprising a pharmaceutically acceptable carrier.

Also disclosed are methods of decreasing the concentration of plasma glucose in a subject, comprising: administering a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject, whereby the concentration of plasma glucose in the subject decreases.

In the methods described herein, the synthetic apolipoprotein E-mimicking peptides can be administered as a composition comprising the synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier. Subjects for the disclosed methods can have type 1, type 2, gestational diabetes (occurring during pregnancy), juvenile onset diabetes, diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy.

Insulin Resistance

Insulin resistance is prevalent in 20-25% of the population, and the condition is a chief component of Type 2 Diabetes Mellitus and a risk factor for cardiovascular disease and certain forms of cancer (Reaven GM, Panminerva Med. 2005, 47: 201-210). Obesity predisposes individuals to the development of insulin resistance, and several mechanisms have been proposed to explain how increased adiposity antagonizes insulin-stimulation of nutrient uptake and storage. In some obese individuals, increased adipose tissue mass may trigger the synthesis and/or secretion of glucocorticoids (Hermanowski-Vosatka, J Exp

Med. 2005 Aug 15;202: 517-527) or inflammatory cytokines (*e.g.*, tumor necrosis factor alpha) (Hotamisligl GS, Exp Clin Endocrinol Diabetes. 1999;107(2):119-25), which inhibit insulin action in peripheral tissues. Additionally, excess lipids may be delivered to non-adipose tissues which are not suited for fat storage (*i.e.*, skeletal muscle and the liver), thus leading to the formation of specific metabolites that directly antagonize insulin signaling and action (Schmitz-Peiffer C,Cell Signal. 2000 Oct;12(9-10):583-94; McGarry JD, Diabetes. 2002 Jan;51(1):7-18).

The disclosed peptides can also be used to modulate insulin resistance. For example, disclosed herein are methods of modulating insulin resistance in a subject, comprising: administering to the subject one or more of the disclosed dual-domain peptides, thereby modulating insulin resistance in the subject.

Also disclosed herein are methods of modulating insulin resistance in a cell, comprising identifying a cell in need of modulated insulin resistance, and administering to the cell one or more of the disclosed dual-domain peptides, thereby modulating insulin resistance in a cell.

As described elsewhere herein, the cell can be *in vitro*, *in vivo*, or *ex vivo*. When the cell is in a subject, the subject can have any one or more of the following diseases and disorders: metabolic syndrome, obesity, diabetes (such as Type II), or Cushing's disease. The subject can also have inflammation. The subject can also have Gaucher disease. These diseases and disorders, as well as others, are disclosed in more detail elsewhere herein.

As described above, insulin resistance can be manifested in several ways, including Type 2 Diabetes. Type 2 diabetes is the condition most obviously linked to insulin resistance. Compensatory hyperinsulinemia helps maintain normal glucose levels--often for decades--before overt diabetes develops. Eventually the beta cells of the pancreas are unable to overcome insulin resistance through hypersecretion. Glucose levels rise, and a diagnosis of diabetes can be made. Patients with type 2 diabetes remain hyperinsulinemic until they are in an advanced stage of disease.

Insulin resistance can also include hypertension. One half of patients with essential hypertension are insulin resistant and hyperinsulinemic. There is evidence that blood pressure is linked to the degree of insulin resistance.

Hyperlipidemia is also associated with insulin resistance. The lipid profile of patients with type 2 diabetes includes decreased high-density lipoprotein cholesterol levels

(a significant risk factor for heart disease), increased serum very-low-density lipoprotein cholesterol and triglyceride levels and increased small dense low-density lipoprotein cholesterol level. Insulin resistance has been found in persons with low levels of high-density lipoprotein. Insulin levels have also been linked to very-low-density lipoprotein synthesis and plasma triglyceride levels.

Atherosclerotic heart disease is also associated with insulin resistance, as is obesity. Many persons with one or more of the conditions listed above are obese. Obesity is a component of the syndrome, but it promotes insulin resistance rather than resulting from it. Other abnormalities linked to insulin resistance include hyperuricemia, elevated levels of plasminogen activator inhibitor 1 and a preponderance of small-size, low-density lipoprotein particles. Higher plasminogen activator inhibitor 1 levels and decreased low-density lipoprotein particle diameter are thought to increase the risk of coronary heart disease.

Metabolic Syndrome (also known as Syndrome X) is characterized by having at least three of the following symptoms: insulin resistance; abdominal fat – in men this is defined as a 40 inch waist or larger, in women 35 inches or larger; high blood sugar levels – at least 110 milligrams per deciliter (mg/dL) after fasting; high triglycerides – at least 150 mg/dL in the blood stream; low HDL - less than 40 mg/dL; pro-thrombotic state (*e.g.*, high fibrinogen or plasminogen activator inhibitor in the blood); or blood pressure of 130/85 mmHg or higher. A connection has been found between Metabolic Syndrome and other conditions such as obesity, high blood pressure and high levels of LDL "bad" cholesterol, all of which are risk factors for Cardiovascular Disease. For example, an increased link between Metabolic Syndrome and atherosclerosis has been shown. People with Metabolic Syndrome are also more prone to developing Type 2 Diabetes, as well as PCOS (Polycystic Ovarian Syndrome) in women and prostate cancer in men.

Disclosed herein are methods of treating a subject with Syndrome X, comprising identifying a subject with Syndrome X, and administering to the subject one or more of the disclosed dual-domain peptides, thereby treating the subject.

Delivery of Compositions

For delivery of the compositions of the invention to a cell, either *in vitro* or *in vivo*, a number of direct delivery systems can be used. These include liposome fusion, gene gun injection, endocytosis, electroporation, lipofection, calcium phosphate precipitation,

plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991). If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (*e.g.*, in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells into a subject. Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modified to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

Therapeutic Uses

In general, when used for treatment, the therapeutic compositions may be administered orally, parenterally (*e.g.*, intravenously or subcutaneous administration), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, by intracavity administration, transdermally, or topically or the like, including topical intranasal administration or administration by inhalant. The topical administration can be ophthalmically, vaginally, rectally, or intranasally. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (*e.g.*, lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the disorder being treated, the particular nucleic acid or vector used,

its mode of administration and the like. An appropriate amount for a particular composition and a particular subject can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. Parenteral administration includes use of a slow release, a time release or a sustained release system such that a constant dosage is maintained.

Effective dosages and schedules for administering the compositions may be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms of the disorder are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counter-indications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. For example, disclosed are methods comprising administering one or more of the disclosed synthetic apolipoprotein E-mimicking peptides to a subject, whereby the concentration of plasma glucose in the subject decreases, thereby treating diabetes in the subject, wherein said synthetic apolipoprotein E-mimicking peptide is administered in an amount of about 0.001 mg/kg to about 5 mg/kg.

Following administration of a disclosed composition, such as a synthetic apolipoprotein E-mimicking peptide, for treating, inhibiting, or preventing diabetes, the efficacy of the therapeutic peptide can be assessed in various ways well known to the skilled practitioner. For instance, one of ordinary skill in the art will understand that a composition, such as a peptide, disclosed herein is efficacious in treating or inhibiting diabetes in a subject by observing that the composition reduces plasm glucose levels or reduces the amount of glucose present in an assay, as disclosed herein. The compositions that inhibit

increased plasm glucose levels or increases insulin levels, as disclosed herein may be administered prophylactically to patients or subjects who are at risk for diabetes.

The peptides, polypeptides, nucleic acids, antibodies, vectors and therapeutic compositions of the invention can be combined with other well-known therapies and prophylactic vaccines already in use. The compositions of the invention can also be used in combination with drugs used to treat diabetic patients/treat low insulin levels/increase insulin levels. Such drugs include ACE-I, ARB-I. ASA, TZD's fibrates, statins, niclosamide, PPAR-α, PPAR-δ, PPAR γ, niacin, insulin, sulfonylurea, metformin, glyburide, Ezetimibe. As such, the peptides, polypeptides, nucleic acids, antibodies, vectors and therapeutic compositions of the invention can be combined with other well-known therapies and prophylactic vaccines already in use and/or in combination with drugs used to treat diabetic patients/treat low insulin levels/increase insulin levels in any of the methods disclosed herein.

The disclosed peptides, when used in combination with other drugs used to treat diabetic patients/treat low insulin levels/increase insulin levels can also help reduce the side-effects known to be associated with other drugs used to treat diabetic patients/treat low insulin levels/increase insulin levels. For example, the disclosed peptides can be used in combination with statins, such that the dosage of the statins administered to a subject can be reduced and therefore the side-effects associated with statin administration can be reduced or abrogated entirely.

In addition, the compositions, including dual-domain peptides, disclosed herein can be used in combination with other peptides. Examples of other peptides that can be used in combination with the current compositions include, but are not limted to the peptides described in U.S. Patent Nos. 6,664,230; 6,933,279; 7,144,862; 7,166,578; 7,199,102; and 7,148,197. Other peptides that can be used in combination with the current compositions include, but are not limted to the peptides described in U.S. Patent.Application Nos. 60/494,449; 11/407,390; and 10/913,880. The compositions of the invention can be combined with any of these drugs. The combination of the peptides of the invention can generate an additive or a synergistic effect with current treatments. As such, the compositions, including dual-domain peptides, disclosed herein can be used in combination with other peptides in any of the methods disclosed herein.

Furthermore, the disclosed compostions can be administered in conjunction with a drug selected from the group consisting of CETP inhibitors, FTY720, Certican, DPP4 inhibitors, Calcium channel blockers, ApoA1 derivative or mimetic or agonist, PPAR agonists, Steroids, Gleevec, Cholesterol Absorption blockers (Zetia), Vytorin, Any Renin Angiotensin pathway blockers, Angiotensin II receptor antagonist (Diovan, *etc.*), ACE inhibitors, Renin inhibitors, MR antagonist and Aldosterone synthase inhibitor, Beta-blockers, Alpha-adrenergic antagonists, LXR agonist, FXR agonist, Scavenger Receptor B1 agonist, ABCA1 agonist, Adiponectic receptor agonist or adiponectin inducers, Stearoyl-CoA Desaturase I (SCD1) inhibitor, Cholesterol synthesis inhibitors (non-statins), Diacylglycerol Acyltransferase I (DGAT1) inhibitor, Acetyl CoA Carboxylase 2 inhibitor, PAI-1 inhibitor, LP-PLA2 inhibitor, GLP-1, Glucokinase activator, CB-1 agonist, AGE inhibitor/breaker, PKC inhibitors, Anti-thrombotic/coagulants:, Aspirin, ADP receptor blockers *e.g.*, Clopidigrel, Factor Xa inhibitor, GPIIb/IIIa inhibitor, Factor VIIa inhibitor, Warfarin, Low molecular weight heparin, Tissue factor inhibitor, Anti-inflammatory drugs:, Probucol and derivative, *e.g.*, AGI-1067 etc, CCR2 antagonist, CX3CR1 antagonist, IL-1 antagonist, Nitrates and NO donors, and Phosphodiesterase inhibitors.

For example, disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide and a statin to the subject, whereby the concentration of plasma glucose in the subject decreases, thereby treating diabetes in the subject.

Also disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide and a statin to the subject, whereby the concentration of plasma glucose in the subject decreases, thereby treating diabetic complications in the subject.

Compositions

As described above, apolipoprotein E-mimicking peptides can be used in a variety of methods. Human apolipoprotein E (apo E) consists of two distinct domains, the lipid-associating domain (residues 192-299) and the globular domain (1-191) which contains the LDL receptor binding site (residues 129-169). To test the hypothesis that a minimal arginine-rich apoE receptor binding domain (141-150) was sufficient to enhance low density lipoprotein (LDL) and very low density lipoprotein (VLDL) uptake and clearance when covalently linked to a class A amphipathic helix, Anantharamaiah et al. synthesized a

peptide in which the receptor binding domain of human apo E, LRKLRKRLLR (hApo E[141-150] also referred to as "hE", SEQ ID NO: 1), was linked to 18A, a well characterized high affinity lipid-associating peptide (DWLKAFYDKVAEKLKEAF, also referred to as "18A", SEQ ID NO: 4) to produce a peptide denoted as hApoE[141-150]-18A (also referred to as "hE-18A", SEQ ID NO: 11) (see U.S. Patent No. 6,506,880).

Also synthesiszed was an end protected analog of hE-18A, denoted Ac-hE18A-NH_2 (SEQ ID NO: 12). The importance of the lysine residues and the role of the hydrophobic residues in the receptor binding domain were also studied using two analogs, LRRLRRRLLR-18A (also referred to as "hE(R)-18A", SEQ ID NO: 13) and LRKMRKRLMR-18A (also referred to as "mE18A", SEQ ID NO: 14), whereby the receptor binding domain of human apo E was modified to substitute arginine (R) residues for lysine (K) residues at positions 143 and 146 (SEQ ID NO: 3) and whereby the receptor binding domain of mouse apo E (SEQ ID NO: 2), were linked to 18A, respectively. The effect of the dual character peptides was then determined.

Non-limiting Examples of Polypeptides and Peptides of the Invention

The present invention is directed to methods of using synthetic apolipoprotein-E mimicking peptides or polypeptides. Non-limiting examples of the synthetic apolipoprotein-E mimicking peptides or polypeptides that can be used in the disclosed methods are provided below.

Disclosed herein are synthetic apolipoprotein E-mimicking peptides, consisting of: a receptor binding domain of apolipoprotein E comprising the amino acid sequence of SEQ ID NO: 15; and a lipid-associating peptide, wherein said receptor binding domain is covalently linked to said lipid-associating peptide. As such, the receptor binding domain replaced the two leucine (L) residues at positions 148 and 149 of LRKLRKRLLR (hApo E[141-150], SEQ ID NO: 1) with two phenylalanine (F) residues. The lipid associating peptide for these synthetic apolipoprotein E-mimicking peptides can be the model class A amphipathic helical peptide 18A. For example the lipid-associating peptide can comprise the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 17.

Also disclosed herein are synthetic apolipoprotein E-mimicking peptides, comprising: a lipid binding domain of apolipoprotein E comprising the amino acid sequence of SEQ ID NO: 17; and a receptor binding domain peptide, wherein said lipid binding

domain is covalently linked to said receptor binding domain peptide. As such, the lipid binding domain replaced the two leucine (L) residues of DWLKAFYDKVAEKLKEAF (18A, SEQ ID NO: 16) with two phenylalanine (F) residues resulting in the sequence DWFKAFYDKVAEKFKEAF (SEQ ID NO: 17, also referred to as modified 18A or m18A). The receptor binding domain peptide for the synthetic apolipoprotein E-mimicking peptides can be a human receptor binding domain peptide of ApoE. For example, receptor binding domain peptide of the disclosed synthetic apolipoprotein E-mimicking peptides can comprise the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 15. The receptor binding domain peptide of such synthetic apolipoprotein E-mimicking peptides can also be from a species selected from the group consisting of mouse, rabbit, monkey, rat, bovine, pig, and dog.

The receptor binding domain peptide for the synthetic apolipoprotein E-mimicking peptides can also be the LDL receptor (LDLR) binding domain of apolipoprotein B (ApoB). The LDL receptor (LDLR) binding domain of ApoB can have the sequence RLTRKRGLK (SEQ ID NO. 104). ApoB-100 is a 550,000 Da glycoprotein with nine amino acids (3359–3367) serving as the binding domain for the LDL receptor (Segrest et al., J. Lipid. Res. 42, pp. 1346–1367 (2001)). Upon binding to LDLR in clathrin coated pits, LDL is internalized via endocytosis and moves into the endosome where a drop in pH causes the receptor to dissociate from the LDL. The receptor is recycled back to the surface of the cell while the LDL is moved into the lysosome where the particle is degraded (Goldstein *et al.*, Ann. Rev. Cell Biol. 1, pp. 1–39 (1985)). The LDL receptor (LDLR) binding domain of ApoB when used with the disclosed peptides can also be altered and/or modified as described throughout this application for ApoE. For example, LDL receptor (LDLR) binding domain of ApoB can be used with the the disclosed lipid-associating peptides, wherein the LDL receptor (LDLR) binding domain of ApoB is covalently linked to said lipid-associating peptide. In addition, the LDL receptor (LDLR) binding domain of ApoB can be scrambled, reverse-oriented, can be part of a domain switched peptide as described below.

As such, also disclosed are methods of methods of decreasing plasma glucose and plasma cholesterol in a subject, comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose and plasma cholesterol are decreased.

Also disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose and plasma cholesterol in the subject decreases, thereby treating diabetes in the subject.

Also disclosed are methods of reducing diabetic complications in a subject comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose and plasma cholesterol in the subject decreases, thereby reducing diabetic complications in the subject.

Examples of receptor binding domain peptides that can be used in the disclosed synthetic apolipoprotein E-mimicking peptides to be used in the disclosed methods are provided in Table 1.

Table 1 - Disclosed synthetic apolipoprotein E-mimicking peptides to be used in the disclosed methods

Species	Starting Residue NO:	Sequence	SEQ ID NO:
Human	141	**LRKLRKRLLR**	SEQ ID NO: 1
Rabbit	134	**LRKLRKRLLR**	SEQ ID NO: 5
Monkey	141	**LRKLRKRLLR**	SEQ ID NO: 6
Mouse	133	**LRK*M*RKRL*M*R**	SEQ ID NO: 2
Rat	133	**LRK*M*RKRL*M*R**	SEQ ID NO: 7
Bovine	140	**LRKL*P*KRLLR**	SEQ ID NO: 8
Pig	140	**LR*N*VRKRL*V*R**	SEQ ID NO: 9
Dog	133	***M***RKLRKR***V***LR	SEQ ID NO: 10
R Modified	141	**LR*R*LR*R*RLLR**	SEQ ID NO: 3
F Modified	141	**LRKLRKR*FF*R**	SEQ ID NO: 15
ApoB		***RLTRKRGLK***	SEQ ID NO: 104

The *italicized* residues in Table 1 indicate changes from the human sequence; however, the property of the amino acid is conserved. The ***bold-italicized*** residues in Table 1 indicate the difference from the human sequence at that position.

Also disclosed are synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods, consisting of a combination of the disclosed receptor binding domains of apolipoprotein E and the disclosed lipid-associating peptides, wherein said receptor binding domain is covalently linked to said lipid-associating peptide. Additional lipid-associating peptides that can be used in the disclosed compositions are described in U.S. Patent Application No. 11/407,390 (Fogelman et al.).

For example, the lipid-

associating peptides of Tables 2 - 6 of U.S. Patent Application No. 11/407,390 can be used in the disclosed compositions.

Also disclosed are synthetic apolipoprotein E-mimicking peptides, consisting of a combination of the disclosed receptor binding domains of apolipoprotein B and the disclosed lipid-associating peptides, wherein said receptor binding domain is covalently linked to said lipid-associating peptide. Non-limiting examples of the disclosed synthetic apolipoprotein E-mimicking peptides are provided in Table 2.

Also disclosed are synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods, consisting of a combination of the disclosed receptor binding domains of apolipoprotein E and the disclosed lipid-associating peptides, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation. Also disclosed are synthetic apolipoprotein E-mimicking peptides, consisting of a combination of the disclosed receptor binding domains of apolipoprotein B and the disclosed lipid-associating peptides, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation. These peptides can be referred to as "domain switched" "switched domain", or "switched" peptides. For example, disclosed are synthetic apolipoprotein E-mimicking peptides, consisting of a combination of the disclosed receptor binding domains of apolipoprotein E and the disclosed lipid-associating peptides, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation to those described above and in Table 2. Specifically, the lipid-associating peptide is covalently linked to the receptor binding domain of apolipoprotein E such that the lipid-associating peptide is at the N-terminus of the synthetic apolipoprotein E-mimicking peptide. Additional non-limiting examples of the disclosed synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods are provided in Table 3.

Table 2 - Non-limiting examples of the disclosed synthetic apolipoprotein E-mimicking peptides

Receptor Binding Domains of ApoE	**Lipid-Associating Peptides**	**SEQ ID NO:**
LRKLRKRLLR	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 18
LRKLRKRLLR	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 19
LRKLRKRLLR	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 20

LRK*M*RKRL*M*R	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 21
LRK*M*RKRL*M*R	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 22
LRKL*P*KRLLR	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 23
LR*N*VRKRL*V*R	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 24
*M*RKLRKR*V*LR	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 25
LR*R*LR*R*RLLR	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 26
LRKLRKR*FF*R	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 27
LRKLRKRLLR	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 28
LRKLRKRLLR	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 29
LRKLRKRLLR	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 30
LRK*M*RKRL*M*R	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 31
LRK*M*RKRL*M*R	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 32
LRKL*P*KRLLR	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 33
LR*N*VRKRL*V*R	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 34
*M*RKLRKR*V*LR	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 35
LR*R*LR*R*RLLR	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 36
LRKLRKR*FF*R	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 37

The disclosed synthetic apolipoprotein E-mimicking peptides can also be N-terminally protected using acetyl and amino groups.

Also disclosed are synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods, consisting of a combination of the disclosed receptor binding domains of apolipoprotein E and the disclosed lipid-associating peptides, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation. Also disclosed are synthetic apolipoprotein E-mimicking peptides, consisting of a combination of the disclosed receptor binding domains of apolipoprotein B and the disclosed lipid-associating peptides, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation. These peptides can be referred to as "domain switched" "switched domain", or "switched" peptides. For example, disclosed are synthetic apolipoprotein E-mimicking peptides, consisting of a combination of the disclosed receptor binding domains of apolipoprotein E and the disclosed lipid-associating peptides, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation to those described above and in Table 2. Specifically, the lipid-associating peptide is covalently linked to the receptor binding domain of apolipoprotein E such that the lipid-associating peptide is at the N-terminus of the synthetic apolipoprotein E-mimicking peptide.

Additional non-limiting examples of the disclosed synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods are provided in Table 3.

Table 3 - Additional non-limiting examples of the disclosed synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods

Lipid-associating peptides	Receptor Binding Domains of ApoE	SEQ ID NO:
DWLKAFYDKVAEKLKEAF	**LRKLRKRLLR**	SEQ ID NO: 38
DWLKAFYDKVAEKLKEAF	**LRKLRKRLLR**	SEQ ID NO: 39
DWLKAFYDKVAEKLKEAF	**LRKLRKRLLR**	SEQ ID NO: 40
DWLKAFYDKVAEKLKEAF	**LRK*M*RKRL*M*R**	SEQ ID NO: 41
DWLKAFYDKVAEKLKEAF	**LRK*M*RKRL*M*R**	SEQ ID NO: 42
DWLKAFYDKVAEKLKEAF	**LRKL*P*KRLLR**	SEQ ID NO: 43
DWLKAFYDKVAEKLKEAF	**LR*N*VRKRL*V*R**	SEQ ID NO: 44
DWLKAFYDKVAEKLKEAF	*M*RKLRKR*V*LR	SEQ ID NO: 45
DWLKAFYDKVAEKLKEAF	**LR*R*LR*R*RLLR**	SEQ ID NO: 46
DWLKAFYDKVAEKLKEAF	**LRKLRKR*FF*R**	SEQ ID NO: 47
DWFKAFYDKVAEKFKEAF	**LRKLRKRLLR**	SEQ ID NO: 48
DWFKAFYDKVAEKFKEAF	**LRKLRKRLLR**	SEQ ID NO: 49
DWFKAFYDKVAEKFKEAF	**LRKLRKRLLR**	SEQ ID NO: 50
DWFKAFYDKVAEKFKEAF	**LRK*M*RKRL*M*R**	SEQ ID NO: 51
DWFKAFYDKVAEKFKEAF	**LRK*M*RKRL*M*R**	SEQ ID NO: 52
DWFKAFYDKVAEKFKEAF	**LRKL*P*KRLLR**	SEQ ID NO: 53
DWFKAFYDKVAEKFKEAF	**LR*N*VRKRL*V*R**	SEQ ID NO: 54
DWFKAFYDKVAEKFKEAF	*M*RKLRKR*V*LR	SEQ ID NO: 55
DWFKAFYDKVAEKFKEAF	**LR*R*LR*R*RLLR**	SEQ ID NO: 56
DWFKAFYDKVAEKFKEAF	**LRKLRKR*FF*R**	SEQ ID NO: 57

The disclosed domain switched synthetic apolipoprotein E-mimicking peptides can also be N-terminally protected using acetyl and amino groups.

Also disclosed are synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods, consisting of a combination of the disclosed receptor binding domains of apolipoprotein E and the disclosed lipid-associating peptides, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a reversed orientation. For example, disclosed are synthetic apolipoprotein E-mimicking peptides, consisting of a combination of the disclosed receptor binding domains of apolipoprotein E and the disclosed lipid-associating peptides, wherein either the sequence of the receptor binding domain or the sequence of the lipid-associating peptide or both sequences are in the reversed oritentation . Also disclosed are synthetic apolipoprotein E-mimicking peptides,

consisting of a combination of the disclosed receptor binding domains of apolipoprotein B and the disclosed lipid-associating peptides, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a reversed orientation. Additional non-limiting examples of the disclosed synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods are provided in Table 4.

Table 4 - Additional non-limiting examples of the disclosed synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods

Receptor Binding Domains of ApoE	Lipid-Associating Peptides	SEQ ID NO:
RLLRKRLKRL	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 64
RLLRKRLKRL	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 65
RLLRKRLKRL	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 66
RMLRKRMKRL	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 67
RMLRKRMKRL	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 68
RLLRKPLKRL	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 69
RVLRKRVNRL	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 70
RLVRKRLKRM	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 71
RLLRRRLRRL	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 72
RFFRKRLKRL	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 73
RLLRKRLKRL	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 74
RLLRKRLKRL	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 75
RLLRKRLKRL	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 76
RMLRKRMKRL	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 77
RMLRKRMKRL	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 78
RLLRKPLKRL	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 79
RVLRKRVNRL	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 80
RLVRKRLKRM	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 81
RLLRRRLRRL	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 82
RFFRKRLKRL	DWFKAFYDKVAEKFKEAF	SEQ ID NO: 83
LRKLRKRLLR	FAEKLKEAVKDYFAKLWD	SEQ ID NO: 84
LRKLRKRLLR	FAEKLKEAVKDYFAKLWD	SEQ ID NO: 85
LRKLRKRLLR	FAEKLKEAVKDYFAKLWD	SEQ ID NO: 86
LRK*M*RKRL*M*R	FAEKLKEAVKDYFAKLWD	SEQ ID NO: 87
LRK*M*RKRL*M*R	FAEKLKEAVKDYFAKLWD	SEQ ID NO: 88
LRKL*P*KRLLR	FAEKLKEAVKDYFAKLWD	SEQ ID NO: 89
LR*N*VRKRL*V*R	FAEKLKEAVKDYFAKLWD	SEQ ID NO: 90
*M*RKLRKR*V*LR	FAEKLKEAVKDYFAKLWD	SEQ ID NO: 91
LR*R*LR*R*RLLR	FAEKLKEAVKDYFAKLWD	SEQ ID NO: 92
LRKLRKR*FF*R	FAEKLKEAVKDYFAKLWD	SEQ ID NO: 93
LRKLRKRLLR	FAEKFKEAVKDYFAKFWD	SEQ ID NO: 94
LRKLRKRLLR	FAEKFKEAVKDYFAKFWD	SEQ ID NO: 95
LRKLRKRLLR	FAEKFKEAVKDYFAKFWD	SEQ ID NO: 96

LRK*M*RKRL*M*R	FAEKFKEAVKDYFAKFWD	SEQ ID NO: 97
LRK*M*RKRL*M*R	FAEKFKEAVKDYFAKFWD	SEQ ID NO: 98
LRKL*P*KRLLR	FAEKFKEAVKDYFAKFWD	SEQ ID NO: 99
LR*N*VRKRL*V*R	FAEKFKEAVKDYFAKFWD	SEQ ID NO: 100
*M*RKLRKR*V*LR	FAEKFKEAVKDYFAKFWD	SEQ ID NO: 101
LR*R*LR*R*RLLR	FAEKFKEAVKDYFAKFWD	SEQ ID NO: 102
LRKLRKR*FF*R	FAEKFKEAVKDYFAKFWD	SEQ ID NO: 103

The disclosed reverse-oriented synthetic apolipoprotein E-mimicking peptides can also be N-terminally and C-terminally protected using acetyl and amide groups.

Also disclosed are synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods, consisting of: a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, wherein the receptor binding domain of apolipoprotein E is scrambled. For example, disclosed is a synthetic apolipoprotein E-mimicking peptide, consisting of: a receptor binding domain of apolipoprotein E comprising the amino acid sequence of SEQ ID NO: 58; and a lipid-associating peptide, wherein said receptor binding domain is covalently linked to said lipid-associating peptide. Also disclosed are synthetic apolipoprotein E-mimicking peptides, consisting of: a receptor binding domain of apolipoprotein B and a lipid-associating peptide, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, wherein the receptor binding domain of apolipoprotein B is scrambled.

Also disclosed are synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods, consisting of: a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, wherein the lipid-associating peptide is scrambled. For example, disclosed herein is a synthetic apolipoprotein E-mimicking peptides, comprising: a lipid binding domain of apolipoprotein E comprising the amino acid sequence of SEQ ID NO: 59 and a receptor binding domain peptide, wherein said lipid binding domain is covalently linked to said receptor binding domain peptide.

Also disclosed are synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods, consisting of: a receptor binding domain of apolipoprotein E and a lipid-associating peptide of apolipoprotein E, wherein receptor binding domain is covalently linked to said lipid-associating peptide, wherein both the receptor binding domain and the

lipid-associating peptide are scrambled. Additional non-limiting examples of the disclosed scrambled synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods are provided in Table 5.

Table 5 - Additional non-limiting examples of the disclosed scrambled synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods

Name	Receptor Binding Domains of ApoE	Lipid-Associating Peptides	SEQ ID NO:
hE-Sc18A (hE with Sc18A also referred to as Sc2F)	LRKLRKRLLR	KAFEEVLAKKFYDKALWD	SEQ ID NO: 60
SchE-18A	LRLLRKLKRR	DWLKAFYDKVAEKLKEAF	SEQ ID NO: 61

The disclosed scrambled synthetic apolipoprotein E-mimicking peptides can also be N-terminally and C-terminally protected using acetyl and amide groups. The disclosed scrambled synthetic apolipoprotein E-mimicking peptides can also be reverse-oriented as described above.

Also disclosed are single-domain synthetic apolipoprotein E-mimicking peptides that can be used in the disclosed methods. The single-domain synthetic apolipoprotein E-mimicking peptides can consist of a receptor binding domain of apolipoprotein E or a lipid-associating peptide. The receptor binding domain or the lipid-associating peptide can be modified or altered as described above. For example, the receptor binding domain or the lipid-associating peptide can be mutated, scrambeled, and/or reverse-oriented. Any other modifications or alterations disclosed herein for the dual-domain polypeptides can also be used for the single-domain peptides.

Numerous other variants or derivatives of the peptides disclosed herein that can be used in the disclosed methods are also contemplated. For example, scrambled peptides can also be reverse-oriented, or can be in a switched orientation. Additionally, reverse-oriented peptides can be in a switched orientation. All other combinations of the disclosed peptides are also contemplated. Non-limiting examples of the peptides have been described herein (see Tables 1 - 5, for example). As used herein, the term "analog" is used interchangeably with "variant" and "derivative." Variants and derivatives are well understood to those of skill in the art and can involve amino acid sequence modifications. Such, amino acid sequence modifications typically fall into one or more of three classes: substantial;

insertional; or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily are smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. These variants ordinarily are prepared by site-specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final derivative or analog. Substutitional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with Tables 6 and 7 and are referred to as conservative substitutions.

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 6, *i.e.*, selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties are those in which: (a) the hydrophilic residue, *e.g.*, seryl or threonyl, is substituted for (or by) a hydrophobic residue, *e.g.*, leucyl, isoleucyl, phenylalanyl, valyl or alanyl; Tryptophan, Tyrosinyl (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, *e.g.*, lysyl, arginyl, or hystidyl, is substituted for (or by) an electronegative residue, *e.g.*, glutamyl or aspartyl; or (d) a residue having a bulky side chain, *e.g.*, phenylalanine, is substituted for (or by) one not having a side chain, *e.g.*, glycine, in this case, or (e) by increasing the number of sites for sulfation and/or glycosylation.

It is understood that one way to define the variants and derivatives of the disclosed proteins herein is to define them in terms of homology/identity to specific known sequences. Specifically disclosed are variants of synthetic apolipoprotein E-mimicking peptides and other proteins or peptides herein disclosed which have at least, 70% or at least

75% or at least 80% or at least 85% or at least 90% or at least 95% homology to the synthetic apolipoprotein E-mimicking peptides specifically recited herein. Those of skill in the art readily understand how to determine the homology of two proteins.

Table 6 – Amino Acid Substitutions

Original Residue	Non-Limiting Exemplary Conservative Substitutions
Ala	Ser
Arg	Gly; Gln; Lys
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn; Lys
Glu	Asp
Gly	Ala
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

As this specification discusses various polypeptides and polypeptide sequences it is understood that the nucleic acids that can encode those polypeptide sequences are also disclosed. This would include all degenerate sequences related to a specific polypeptide sequence, i.e. all nucleic acids having a sequence that encodes one particular polypeptide sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every

sequence is in fact disclosed and described herein through the disclosed polypeptide sequences.

TABLE 7: Amino Acid Abbreviations	
Amino Acid	**Abbreviations**
Alanine	Ala (A)
Allosoleucine	Alle
Arginine	Arg (R)
Asparagines	Asn (N)
Aspartic Acid	Asp (D)
Cysteine	Cys (C)
Glutamic Acid	Glu (E)
Glutamine	Gln (Q)
Glycine	Gly (G)
Histidine	His (H)
Isolelucine	Ile (I)
Leucine	Leu (L)
Lysine	Lys (K)
Phenylalanine	Phe (F)
Praline	Pro (P)
Pyroglutamic Acid	PGlu (U)
Serine	Ser (S)
Threonine	Thr (T)
Tyrosine	Tyr (Y)
Tryptophan	Trp (W)
Valine	Val (V)

Blocking/Protecting Groups and D Residues

While the various compositions described herein may 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-

LRKLRKRLLRDWLKAFYDKVAEKLKEAF-NH_2 (SEQ ID NO:12 with blocking groups), either or both of these protecting groups can be eliminated and/or substituted with another protecting group as described herein.

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 can improve oral delivery and can also increase serum half-life.

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. For example, the protecting groups can include, but are not limited to alkyl chains as in fatty acids, propeonyl, formyl, and others. Carboxyl protecting groups include amides, esters, and ether-forming protecting groups can also be used. For example, an acetyl group can be used to protect the amino terminus and an amide group can be used to protect the carboxyl terminus. These blocking groups enhance the helix-forming tendencies of the peptides. Additioanl blocking groups include alkyl groups of various lengths, *e.g.*, groups having the formula: $CH_3(CH_2)_nCO$ 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.

Additionally, the protecting groups include, but are not limited to alkyl chains as in fatty acids, propeonyl, formyl, and others. For example, carboxyl protecting groups can include amides, esters, and ether-forming protecting groups. These blocking groups can enhance the helix-forming tendencies of the peptides. Blocking groups can include alkyl groups of various lengths, e.g. groups having the formula: $CH_3(CH_2)_nCO$ 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.

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).

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.). For example, acetylation can be 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.

The compositions disclosed herein can also comprise one or more D-form (dextro rather than levo) amino acids as described herein. For example, at least two enantiomeric amino acids, at least 4 enantiomeric amino acids or at least 8 or 10 enantiomeric amino acids can be in the "D" form amino acids. Additionally, every other, or even every amino acid (*e.g.*, every enantiomeric amino acid) of the peptides described herein is a D-form amino acid. Aditionally, at least 50% of the enantiomeric amino acids can be "D" form, at least 80% of the enantiomeric amino acids are "D" form, at least 90%, or even all of the enantiomeric amino acids can be in the "D" form amino acids.

Polypeptide Production

Polypeptides that can be used in the disclosed methods can be produced by any method known in the art. One method of producing the disclosed polypeptides is to link two or more amino acid residues, peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides are chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (tert -butyloxycarbonoyl) chemistry (Applied Biosystems, Inc., Foster City, CA). A peptide or polypeptide can be synthesized and not cleaved from its synthesis resin, whereas the other fragment of a peptide or protein can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group, which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, (Grant GA (1992) *Synthetic Peptides: A User Guide.* W.H. Freeman and Co., N.Y. (1992); Bodansky M and

Trost B., Ed. (1993) *Principles of Peptide Synthesis*. Springer-Verlag Inc., NY). Alternatively, the peptide or polypeptide is independently synthesized *in vivo*. Once isolated, these independent peptides or polypeptides may be linked to form a peptide or fragment thereof via similar peptide condensation reactions.

For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (Abrahmsen L et al., *Biochemistry*, 30:4151 (1991)). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide fragments. This method consists of a two-step chemical reaction (Dawson et al. *Science*, 266:776-779 (1994)). The first step is the chemoselective reaction of an unprotected synthetic peptide-thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester- linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site (Baggiolim M et al. (1992) *FEBS Lett.* 307:97-101; Clark-Lewis I *et al.*, *J.Biol.Chem.*, 269:16075 (1994); Clark-Lewis I et al., *Biochem.*, 30:3128 (1991); Rajarathnam K et al., *Biochem.* 33:6623-30 (1994)).

Alternatively, unprotected peptide segments are chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (non-peptide) bond (Schnolzer, M et al. *Science*, 256:221 (1992)). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton RC et al., *Techniques in Protein Chemistry IV*. Academic Press, New York, pp. 257-267 (1992)).

Also disclosed are the components to be used to prepare the disclosed APoE mimicking peptides that can be used in the disclosed methods as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular polynucleotide is disclosed and discussed and a number of modifications that can be made to a number of molecules including the polynucleotide are discussed, specifically

contemplated is each and every combination and permutation of polynucleotide and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

It is understood that one way to define any known variants and derivatives or those that might arise, of the disclosed genes and proteins herein is through defining the variants and derivatives in terms of homology to specific known sequences. Specifically disclosed are variants of the genes and proteins herein disclosed which have at least, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins or nucleic acids, such as genes. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), 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 inspection.

The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. Science 244:48-52, 1989, Jaeger *et al.* Proc. Natl. Acad.

Sci. USA 86:7706-7710, 1989, Jaeger *et al.* Methods Enzymol. 183:281-306, 1989.

For example, as used herein, a sequence recited as having a particular percent homology to another sequence refers to sequences that have the recited homology as calculated by any one or more of the calculation methods described above. For example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using the Zuker calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by any of the other calculation methods. As another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using both the Zuker calculation method and the Pearson and Lipman calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by the Smith and Waterman calculation method, the Needleman and Wunsch calculation method, the Jaeger calculation methods, or any of the other calculation methods. As yet another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using each of calculation methods (although, in practice, the different calculation methods will often result in different calculated homology percentages).

Delivery of Compositions

In the methods described herein, delivery of the compositions (for example, ApoE mimicking polypeptides) to cells can be via a variety of mechanisms. As defined above, disclosed herein are compositions comprising any one or more of the polypeptides, nucleic acids, vectors and/or antibodies described herein can be used to produce a composition of the invention which may also include a carrier such as a pharmaceutically acceptable carrier. For example, disclosed are pharmaceutical compositions, comprising the synthetic apolipoprotein E-mimicking peptides disclosed herein, and a pharmaceutically acceptable carrier

The polypeptide can be in solution or in suspension (for example, incorporated into microparticles, liposomes, or cells). These compositions can be targeted to a particular cell type via antibodies, receptors, or receptor ligands. One of skill in the art knows how to make and use such targeting agents with the compositions of the invention. A targeting

agent can be a vehicle such as an antibody conjugated liposomes; receptor mediated targeting of DNA through cell specific ligands, and highly specific retroviral targeting of cells *in vivo*. Any such vehicles can be part of the composition of the invention. In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clatrhin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, ligand valency, and ligand concentration.

For example, the compositions described herein can comprise s pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material or carrier that would be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art. Examples of carriers include dimyristoylphosphatidyl (DMPC), phosphate buffered saline or a multivesicular liposome. For example, PG:PC:Cholesterol:peptide or PC:peptide can be used as carriers in this invention. Other suitable pharmaceutically acceptable carriers and their formulations are described in Remington: The Science and Practice of Pharmacy (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an appropriate amount of pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Other examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution can be from about 5 to about 8, or from about 7 to about 7.5. Further carriers include sustained release preparations such as semi-permeable matrices of solid hydrophobic polymers containing the composition, which matrices are in the form of shaped articles, *e.g.*, films, stents (which are implanted in vessels during an angioplasty procedure), liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of composition being administered. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH.

Pharmaceutical compositions may also include carriers, thickeners, diluents, buffers, preservatives and the like, as long as the intended activity of the polypeptide, peptide, nucleic acid, vector of the invention is not compromised. Pharmaceutical compositions may also include one or more active ingredients (in addition to the composition of the invention) such as antimicrobial agents, anti-inflammatory agents, anesthetics, and the like. The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated.

Preparations of parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium choloride solution, Ringer's dextrose, dextrose and sodium choloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Formulations for optical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids, or binders may be desirable.

Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mon-, di-, trialkyl and aryl amines and substituted ethanolamines.

Methods for Making the Compositions of the Invention

The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted. For example, there are a variety of methods that can be used for making these compositions, such as synthetic chemical methods and standard molecular biology methods.

The peptide or polypeptides disclosed herein can be used to make certain other aspects of the invention. For example, the peptides and polypeptides of the invention can be used to produce the antibodies of the invention. Nucleic acids and vectors of the invention can be used to produce the peptides and polypeptides and other recombinant proteins of the invention. Host cells of the invention can be used to make nucleic acids, proteins, peptides, antibodies, and transgenic animals of the invention. These synthetic methods are described above.

As described above, the polypeptides or peptides of the invention may also be used to generate antibodies, which bind specifically to the polypeptides or fragments of the polypeptides. The resulting antibodies may be used in immunoaffinity chromatography procedures to isolate or purify the polypeptide or to determine whether the polypeptide is present in a biological sample. In such procedures, a protein preparation, such as an extract, or a biological sample is contacted with an antibody capable of specifically binding to one of the polypeptides of the invention, sequences substantially identical thereto, or fragments of the foregoing sequences.

In immunoaffinity procedures, the antibody is attached to a solid support, such as a bead or column matrix. The protein preparation is placed in contact with the antibody under conditions under which the antibody specifically binds to one of the polypeptides of the invention. After a wash to remove non-specifically bound proteins, the specifically bound polypeptides are eluted.

The ability of proteins in a biological sample to bind to the antibody may be determined using any of a variety of procedures familiar to those skilled in the art. For example, binding may be determined by labeling the antibody with a detectable label such as a fluorescent agent, an enzymatic label, or a radioisotope. Alternatively, binding of the antibody to the sample may be detected using a secondary antibody having such a

detectable label thereon. Particular assays include ELISA assays, sandwich assays, radioimmunoassays, and Western Blots.

The antibodies of the invention can be attached to solid supports and used to immobilize apolipoprotein E or polypeptides of the present invention. Polyclonal antibodies generated against the polypeptides of the invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal. The antibody so obtained will then bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies which may bind to the whole native polypeptide. Such antibodies can then be used to isolate the polypeptide from cells expressing that polypeptide.

The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

3. METHODS

DM is characterized by low HDL-C, high TG and high sdLDL. Moreover individuals with low HDL may also have hyperinsulinemia and insulin resistance and are at increased risk for developing DM. Clinical studies with drugs and lifestyle modification have demonstrated that increased HDL levels is associated with decrease in the diabetic or cardiovascular disease risk. DM-2 is not only associated with quantitative reduction in HDL but also qualitative changes (www.niddk.nih.gov; Knowler WC et al N Engl J Med. (2002) 346(6):393-403; Shaten BJ et al Diabetes Care. (1993) 16:1331-9; Betteridge DJ et al Diabetes Research and Clinical Practice (2005) 68S2:S15-2; and www.framinghamheartstudy.org). Compositional analyses of HDL isolated from DM-2 shows TG enrichment, depletion of cholesterol and enhanced oxidative crosslinking of apolipoprotein (apo) A-I (Betteridge DJ et al Diabetes Research and Clinical Practice (2005) 68S2:S15-22, Nicholls SJ et al J Am Coll Cardiol. (2006) 47(5):992-7). These changes are associated with attenuation of the anti-inflammatory, anti-oxidant and anti-atheroslerotic properties of HDL and its protein constituent apo A-I. There is increasing

evidence for an important role of inflammation in the onset and progression of DM-2. This is supported by the fact that various acute phase reactants such as CRP, IL-1, IL-6, TNF-α and serum amyloid A are elevated in DM-2. Nf-κB may be one of the central mediators of the inflammatory cascade resulting in DM-2. Reactive oxygen species (ROS) also have a causal role in multiple forms of insulin resistance. Houstis *et al.* demonstrated that increase in ROS precedes the onset of detectable insulin resistance. Further decrease in ROS is associated with improved insulin sensitivity and glucose homeostasis. HDL and its associated proteins such as apo A1 and Paraoxanase (PON) are potent anti-oxidants and may therefore also improved insulin sensitivity or prevent the onset or progression of glucose intolerance in similar fashion. However, DM-2 is associated with decreased levels of HDL and its more potent subspecies HDL-2. Further the HDL present in diabetics is not as potent for reverse cholesterol transport as that obtained from non-diabetics. Furthermore, HDL present in diabetics may not be as effective in preventing LDL oxidation as that from non diabetics. This indicates that DM-2 is characterized not only by significant pro-oxidant and pro-inflammatory state but also the normal homeostatic mechanisms to counter such mechanisms are dysfunctional at the very least. The oxidant, inflammatory and dyslipidemic effects also result in pancreatic beta cell apoptosis. Loss of beta cells results in decreased insulin secretion and progression of DM-2.

A potential therapeutic target in DM-2 can be lipoproteins, specifically HDL that can also alter the inflammatory milieu. An emerging area in the field of HDL therapy is the development of apo mimetic peptides (Linsel-Nitschke P et al Nat Rev Drug Discov. (2005) 4(3):193-205). In its dextro form, 4F is an orally active (due to synthesis with D-amino acids) apo A-I mimetic peptide that represents a modified form of the high affinity lipid-associating peptide 18A (DWLKAFYDKVAEKLKEAF) (Linsel-Nitschke P *et al* Nat Rev Drug Discov. (2005) 4(3):193-205; Otvos JD et al Circulation. (2006); 113(12):1556-63; Brown BG et al N Engl J Med. (2001) 345(22):1583-92; Nissen SE et al JAMA. (2007); 297(12):1362-73). This class A amphipathic helical peptide forms small HDL-like particles or pre-β HDL (Linsel-Nitschke P et al Nat Rev Drug Discov. (2005) 4(3):193-205). D-4F stimulates an increase in plasma HDL concentration and/or paraoxonase-1 (PON-1), an antioxidant enzyme that hydrolyzes oxidized phospholipids (Linsel-Nitschke P et al Nat Rev Drug Discov. (2005) 4(3):193-205). Incubation of human endothelial cells with an apo A-I mimetic peptide mimics the ability of native HDL to inhibit LDL oxidation (Brown BG

et al N Engl J Med. (2001) 345(22):1583-92; FIELD investigators Lancet (2005) 366: 1849-1861; Nissen SE et at al N Engl J Med. (2007); 356(13):1304-16). Apo A-I mimetic peptides also reduce LDL-induced monocyte chemotactic activity and macrophage infiltration into the aortic arch of hypercholesterolemic mice (Linsel-Nitschke P et al Nat Rev Drug Discov. (2005) 4(3):193-205). Other studies show that an apo A-I mimetic exerts anti-inflammatory effects by inhibiting interleukin-6 expression (Navab M et al Nat Clin Pract Endocrinol Metab. (2006) 2(9):504-11). As disclosed elsewhere herein, there is another class of peptides termed dual domain peptides. These peptides also inhibit superoixde production and improve endothelial function. In contrast to apo A-I mimetics like 4F, these peptides also clear the atherogenic lipoproteins from the plasma similar to apolipoprotein E. Therefore these peptides possess lipid lowering and also anti-oxidant and anti-inflammatory properties. For example, as described below, the peptide L-4F improves glucose homeostasis in ZDF rats, a well validated model of DM-2. Described below are methods that employ the use of the described dual domain peptides.

Disclosed herein are methods of decreasing the concentration of plasma glucose in a subject. For example, disclosed are methods of of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases.

Also disclosed are methods of of decreasing the concentration of plasma glucose in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, and wherein the synthetic apolipoprotein E-mimicking peptide is administered in a composition comprising a pharmaceutically acceptable carrier.

Also disclosed are methods for decreasing the concentration of plasma glucose in a subject. For example, disclosed are methods for decreasing the concentration of plasma glucose in a subject comprising: administering a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject, whereby the concentration of plasma glucose in the subject decreases.

Also disclosed are methods of treating a subject with diabetes. For example, disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases.

Further disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject, whereby the concentration of plasma glucose in the subject decreases.

The peptides can also be effective in treating a subject with diabetes and/or reducing diabetic complications in a subject, without an effect on the concentration of plasma glucose in the subject. For example, disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject, wherein the concentration of plasma glucose in the subject is unaltered.

Also disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject, wherein the concentration of plasma glucose in the subject is unaltered.

Also disclosed are methods of reducing diabetic complications in a subject comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject, wherein the concentration of plasma glucose in the subject is unaltered.

Also disclosed are methods of treating a subject with diabetes comprising: (a) selecting a subject with diabetes; and (b) administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject; thereby treating diabetes in the subject. Subjects can be selected using any of the known methods of identifying patients with diabetes. For example, subjects can be selected based on high HgbA1c levels, abnormal plasma glucose levels (for example, via random plasma glucose or fasting plasma glucose tests), the inability to metabolize glucose (for example via a glucose tolerance test), the inability of exogenous insulin to reduce plasma glucose levels (for example via an insulin tolerance test). Subjects can also be selected based on the presence of inflammatory markers such as CRP and SAA, or based on the subject's family history. For example, a subject with a random blood glucose concentration 11.1 mmol/L (200 mg/dL) or a fasting plasma glucose 7.0 mmol/L (126 mg/dL) or a two-hour plasma glucose 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test can be indicative of a subject with diabetes. A subject with Type 2 DM can be characterized or identified by three pathophysiologic

abnormalities: impaired insulin secretion, peripheral insulin resistance, and/or excessive hepatic glucose production.

Also disclosed are methods of treating a subject with diabetes comprising: (a) selecting a subject with diabetes; and (b) administering an effective amount of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject; thereby treating diabetes in the subject.

Also disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the concentration of plasma glucose in the subject decreases, thereby treating diabetes in the subject.

Also disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject, whereby the concentration of plasma glucose in the subject decreases, thereby treating diabetes in the subject.

Also disclosed are methods for of treating a subject with diabetes comprising: selecting a subject with diabetes; administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject; thereby treating diabetes in the subject.

Also disclosed are methods for of treating a subject with diabetes comprising: selecting a subject with diabetes; and administering an effective amount of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject; thereby treating diabetes in the subject.

Diabetic Complications

Diabetic complications affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease. Chronic complications can be divided into vascular and nonvascular complications. The vascular complications are further subdivided into microvascular (retinopathy, neuropathy, nephropathy) and macrovascular complications (coronary artery disease, peripheral arterial disease, cerebrovascular disease). Nonvascular complications include problems such as gastroparesis, infections, and skin changes. The risk of chronic complications increases as a function of the duration of hyperglycemia; they usually become apparent in the second

decade of hyperglycemia. Since type 2 DM often has a long asymptomatic period of hyperglycemia, many individuals with type 2 DM have complications at the time of diagnosis.

Diabetic complications include, but are not limited to, nephropathy, often necessitating dialysis or renal transplant; peripheral neuropathy; retinopathy leading to blindness; ulceration of the legs and feet, leading to amputation; fatty liver disease, sometimes progressing to cirrhosis; and vulnerability to coronary artery disease and myocardial infarction, gastroparesis, diseases associate with the autonomic nervous system, nerve condition abnormalities, i.v. contrast induced nephropathy, small vessel diseases (both within the brain and outside the brain), hypogonadism and heart failure.

As such, disclosed are methods of reducing or treating diabetic complications in a subject comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, wherein the diabetic complications in the subject are reduced. Also disclosed are methods as described elsewhere herein, wherein the synthetic apolipoprotein E-mimicking peptide can be used in combination with other with other well-known therapies and prophylactic vaccines already in use and/or in combination with drugs used to treat diabetic patients/treat low insulin levels/increase insulin levels or in combination with drugs used to treat diabetic patients/treat low insulin levels/increase insulin levels.

The synthetic apolipoprotein E-mimicking peptide to be used in the methods described herein can be one or more of any of the apolipoprotein E-mimicking peptides described above. For example, the synthetic apolipoprotein E-mimicking peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 11-14, 18-57, 60, 61, and 62-103. The synthetic apolipoprotein E-mimicking peptide can comprise a receptor binding domain peptide and a lipid-associating peptide, wherein said lipid binding domain peptide is covalently linked to said receptor binding domain peptide.

B-Cell Apoptosis

DM is classified on the basis of the pathogenic process that leads to hyperglycemia, as opposed to earlier criteria such as age of onset or type of therapy. As described above, the two broad categories of DM are designated type 1 and type 2. Type 1A DM results from autoimmune beta cell destruction, which leads to insulin deficiency. Individuals with type 1B DM lack immunologic markers indicative of an autoimmune destructive process of

the beta cells. However, they develop insulin deficiency by unknown mechanisms and are ketosis prone.

The disclosed peptides can also be used to inhibit β-cell apoptosis. By inhibiting β-cell apoptosis, β-cell populations can be maintained, thereby retaining insulin levels. By retaining insulin levels, oxidative stress that is often associated with increased plasma glucose levels can be reduced. In other words, by salvaging insulin levels, there is an antioxidant effect.

As such, disclosed are methods of reducing β-cell apoptosis in a subject. For example, disclosed are methods of reducing β-cell apoptosis in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby β-cell apoptosis in the subject is reduced. Also disclosed are methods of reducing β-cell apoptosis in a subject, comprising: administering a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject, whereby β-cell apoptosis in the subject is reduced. In addition, disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject, whereby β-cell apoptosis in the subject is reduced. Also disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject, whereby β-cell apoptosis in the subject is reduced. The subject can be a subject with diabetes or a subject with diabetic complications.

Also disclosed herein are of reducing oxidative stress in a subject. For example, disclosed are methods of reducing oxidative stress in a subject, comprising: administering a synthetic apolipoprotein E-mimicking peptide to the subject, whereby oxidative stress in the subject is reduced. Also disclosed are methods of reducing oxidative stress in a subject, comprising: administering a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject, whereby oxidative stress in the subject is reduced. In addition, disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject, whereby oxidative stress in the subject is reduced. Also disclosed are methods of treating a subject with diabetes comprising administering an effective amount of a pharmaceutical composition comprising

a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier to the subject, whereby oxidative stress in the subject is reduced. The subject can be a subject with diabetes or a subject with diabetic complications.

All the methods above can be carried out as described for the other methods described herein. In addition, the methods above can also be used to both reduce plasma glucose levels as well as to increase insulin levels. For example, plasma glucose levels can be reduced and insulin levels increased in a subject by reducing β-cell apoptosis and/or reducing the oxidative stress of the subject by administering one or more of the disclosed synthetic apolipoprotein E-mimicking peptides alone or in combination with another drug used to treat diabetic patients/treat low insulin levels/increase insulin levels as described above.

Transplantation

Chronic rejection in transplanted hearts or cardiac allograft vasculopathy (CAV) is the leading cause of late death among heart transplant recipients. Strategies to control CAV traditionally have focused on lymphocyte functions. Hsieh et al. have shown that D-4F, a single domain apoA-I mimetic peptide with potent anti-inflammatory/antioxidant properties, can attenuate CAV. (Transplantation (2007) 84(2):238-243). Hsieh *et al.* used a previously characterized murine model of CAV. B6.C-H2 hearts were heterotopically transplanted into C57BL/6 mice. Recipient mice were treated with either 20 mg of D-4F or carrier daily. Donor hearts were harvested on day 24 after transplantation. Treatment of recipients with D-4F reduced the severity of intimal lesions (62.5 +/- 3.4% vs. 31.1 +/- 8.7%, $p < 0.009$). Treatment also resulted in a decrease in the number of graft-infiltrating CD4 and CD8 lymphocytes and CXCR3+ T-lymphocyte subsets. Heme oxygenase-1 (HO-1) gene transcript in the donor hearts was up-regulated with D-4F treatment, and HO-1 blockade partially reversed the beneficial effects of D-4F. *In vitro* studies showed that D-4F reduced allogeneic T-lymphocyte proliferation and effector cytokine production. These processes were HO-1 independent. This study suggests that D-4F, a prototypical apoA-I mimetic peptide, is effective in controlling CAV via induction of HO-1 in the graft and a direct effect on T-lymphocyte function. This class of peptides with anti-inflammatory/antioxidant properties provides a novel strategy in the treatment of CAV. As such, the disclosed synthetic apolipoprotein E-mimicking peptides can also be used to treat CAV in a subject. For example, disclosed are methods of treating a subject with CAV comprising

administering an effective amount of a synthetic apolipoprotein E-mimicking peptide to the subject, whereby the number of graft-infiltrating CD4 and CD8 lymphocytes and CXCR3+ T-lymphocyte subsets is reduced, Heme oxygenase-1 (HO-1) gene transcript is increased, HO-1 blockade is reversed, and/or allogeneic T-lymphocyte proliferation and effector cytokine production are reduced.

The disclosed synthetic apolipoprotein E-mimicking peptides can also be used in pancreatic transplantation. As described above, the disclosed synthetic apolipoprotein E-mimicking peptides can be used to reduce β-cell apoptosis which has a value in β-cell transplantation. By allowing reducing β-cell apoptosis in a subject receiving a pancreas transplant, the subject's β-cells can remain functional and therefore insulin levels can be maintained. As such, oxidative strees can also be reduced in a subject receiving a pancreatic transplant.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific embodiments described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

EXAMPLES

Example 1

Previuous studies have been conducted with the apo mimetic peptides (4F, Ac-hE18A-NH_2) that have demonstrated their anti-oxidant and anti-inflammatory properties. The effects of Ac-hE18A-NH_2 in improving endothelial function in WHHL rabbits have also been demonstrated previously (Gupta H *et al.*, Circulation. (2005): 111(23):3112-8). These rabbits have defective LDL receptors and therefore have increased atherogenic lipoproteins (mainly LDL). It was found that a single administration of Ac-hE18A-NH_2 peptide resulted in dramatic decrease in total and LDL cholesterol. This was associated with improved aortic endothelial function. This improvement in endothelial function was mediated in part by increase in PON activity with associated decrease in plasma lipid hydroperoxide (Figure 1). Figure 1 also shows WHHL rabbits have defective LDL receptor and are therefore prone to atherosclerosis due to dyslipidemia. PON is an anti-oxidant

enzyme associated with HDL and is responsible for scavenging LOOH in plasma. The lipid lowering effects of Ac-hE18A-NH_2 in 1% cholesterol fed NZW-rabbits have also been shown. These animals have elevated cholesterol that are rich in VLDL type of particles.

Ac-hE18A-NH_2 was administered intravenously two times as shown in the figure (n = 4). At the end of 14 days (21 days after the initiation of atherogenic diet), while plasma cholesterol levels in the control rabbits were in the range of 2000 mg/dl (n = 4), the peptide administered rabbits showed cholesterol values in the range of 1000 mg/dl. Only 2 administrations of the peptide were effective in significantly reducing total cholesterol (Figure 2).

In another set of experiments it was noted that Ac-hE18A-NH_2 clears the plasma turbidity in 1% cholesterol fed NZW-rabbits. 3 mg/kg of peptide was administered intravenously/ week. The rabbits were sacrificed after 51 days from the start of diet. Aortas were harvested and en face analysis was done on Oil Red O stained tissue samples. The results showed that Ac-hE18A- NH_2 inhibits atherosclerois in 1% Cholesterol fed NZW rabbits. This was associated with decrease in atherogenic lipoproteins and inhibition of atherosclerosis at day 51.

The anti-inflammatory effects of 4F in preventing LPS induced VCAM-1 expression and also sepsis pathways in a rodent model has also been previously reported, as wells as a number of anti-inflammatory and anti-oxidant properties of 4F. One of the important mechanisms of action of 4F can be related to the formation of pre-β HDL as depicted in Figure 3. It was determined that both D-4F and scrambled D-4F are highly water-soluble. Two milligrams of D-4F or scrambled D-4F (Sc D-4F) was weighed and dissolved in 500 µL of apoE-null mouse plasma and diluted with additional plasma to a final concentration of 500 µg/mL and incubated for 20 minutes at 37 °C with gentle mixing. Plasma was fractionated by agarose electrophoresis in first dimension, and native PAGE in second dimension, and subjected to Western analysis with anti-mouse ApoA-I. Figure 3 shows *in vitro*, in apoE-null mouse plasma, D-4F causes a major redistribution of apoA-I from α-migrating to pre-ß migrating particles.

This example outlies studies carried out for the evaluation of the effects of these peptides in the prevention of onset and progression of DM-2. Figure 4 shows (A) 5-6 week old male ZDF(fa/fa) with defective leptin receptor were administered peptides (5 mg/kg i.v.) that mimic the properties of HDL (Ac-hE18A- NH_2 and L-4F respectively) or vehicle

(control) alone (n = 7-8/group). Baseline fasting plasma was collected prior to peptide administration. Biweekly injections (6 for Ac-hE18A-NH_2 group and 5 for L-4F group were administered before day 18). From day 18 - day 33, no additional peptide/ vehicle injection were performed. Control animals demonstrated increasing fasting plasma glucose levels. In comparison, peptide-treated animals demonstrated only mild increase in plasma glucose at day 18 and day 33. (B) Corresponding insulin levels are depicted in the control and Ac-hE18A-NH_2 group only. Control animals become relatively insulin resistant at day 18 as depicted by hyperinsulinemia and hyperglycemia. By day 33, the control animals demonstrate decrease in plasma insulin despite even higher plasma glucose and indicate a loss of beta cell function. In contrast, the Ac-hE18A-NH_2-treated animals demonstrate much less insulin resistance at day 18 as depicted by lower plasma insulin levels and normal plasma glucose levels. Despite no additional administration of peptides to these animals, they continue to demonstrate relatively preserved beta cell function with increase in plasma insulin and milder increase in plasma glucose. Data are expressed as Mean ± SEM; * $p < 0.05$.

The results below show that apo-mimetic peptides are extremely potent in preventing the onset and progression of DM-2 (Figure 4). Relatively infrequent injections (biweekly) of the peptides as compared to vehicle were able to improve glucose homeostasis in the ZDF rats. Form the studies it was determined that adiponectin levels in the peptide treated animals were much higher than in controls at day 33 (5.7 ± 1 vs. 3 ± 0.2 μl/ml, $p < 0.05$). Adiponectin levels have previously been shown to correlate with insulin sensitivity. Adiponectin also prevents the production and action of pro-inflammatory TNF-α and IL-6 and induces anti-inflammatory cytokine IL-10 and IL-1 receptor antagonist. A summary of the potential effects of the peptides on liver, pancreas, peripheral tissue, blood and blood vessel are depicted in Figure 5.

It is known that elevated plasma glucose results in secretion of insulin by the pancreatic β- cells which is the result of influx of Ca ions into the cell (Figure 6). Increased cholesterol in the β-cells can result in inhibition of insulin secretion. Further, inflammatory insults (cytokines, free fatty acids (FFA) and glucose) can inhibit reverse cholesterol transport. The same factors can also promote apoptosis of β-cells and insulin resistance in other tissues. Apolipoproteins and apo-mimetics can inhibit the action of inflammatory insults (cytokines), FFA and glucose by promoting reverse cholesterol transport by

stimulating ABCA-1 and formation of pre-β HDL particles. Similarly these mechanisms elsewhere (in blood vessel, peripheral tissue and blood) can cause anti-inflammatory and anti-oxidant effects with increased reverse cholesterol transport, scavenging of lipid hydroperoxides and upregulation of anti-oxidant enzymes such as PON. Some of these peptides also mobilize the atherogenic particles for clearance via liver.

Example 2

Whether apo A-I, HDL lipoproteins and apo-mimetic peptides (4F, Ac-hE18A-NH_2) that modulate HDL function can inhibit the onset and progression of DM-2 in rodent models can also be determined. DM-2, as previously described, is characterized by low HDL-C levels with poor HDL quality. This is reflected by impaired anti-inflammatory and anti-oxidant effects. These changes are seen early in the disease process where insulin resistance without elevation in the plasma glucose is noted. Inflammation and oxidant stress are important mediators of insulin resistance. These mechanisms eventually lead to decrease in pancreatic β-cell mass in later stages of DM-2. There are many rodent models of DM-2. ZDF rats with defective leptin receptor are commonly used models of insulin resistance and DM-2. These animals are hyperleptinemic but show impaired leptin actions. Homozygous ZDF (fa/fa) male rats develop insulin resistance early on and when fed a standard diet these animals, demonstrate hyperglycemia by 7 weeks of age. The rats are hyperinsulinemic between 7-10 weeks of age and subsequently the insulin levels drop. By 12 weeks of age these animals demonstrate hypoinsulinemia and hyperglycemia. There is loss of Glut-2 transporters in the pancreatic β-cells and Glut-4 transporters in skeletal muscle of these animals that results in impaired insulin secretion and impaired peripheral glucose uptake. Overall these rodents also demonstrate loss of pancreatic β-cell mass due to apoptosis, as well as other manifestations of DM-2 including hyperlipidemia and multi-organ involvement due to DM-2. Heterozygote ZDF male rats do not demonstrate a diabetic phenotype on standard diet and therefore serve as a good control.

Whether apo A-I and HDL prevent the onset and progression of DM-2 in ZDF (fa/fa) male rats and whether apo- mimetic synthetic peptides (4F, Ac-hE18A-NH_2) prevent the onset and progression of DM-2 in ZDF (fa/fa) male rats can also be determined. For such studies, Apo A-I can be isolated from rodents and human plasma using HPLC. HDL can be isolated by centrifugation. Test peptides can be synthesized and scrambled peptide and vehicle will serve as the control for such experiments.

Example 3

As previously described, preliminary observations support the anti-diabetic effects of the apo-mimetic peptides. These effects of the peptides are likely due to three major mechanisms: (i) improved insulin secretion; (ii) decrease in pancreatic β-cell apoptosis or cell death; and/or (iii) improved insulin sensitivity of peripheral tissues. These effects of the peptides are mediated by their anti-inflammatory, anti-oxidant and reverse cholesterol promoting mechanisms and are summarized in Figures 5 and 6. As such, whether apo A-I, HDL and apo-mimetic peptides (4F and Ac-hE18A-NH_2) prevent apoptosis in pancreatic β-cells, whether apo A-I, HDL and apo-mimetic peptides (4F and Ac-hE18A-NH_2) improve peripheral insulin sensitivity, and whether apo A-I, HDL and apo-mimetic peptides (4F and Ac-hE18A-NH_2) promote reverse cholesterol transport can also be studied.

WHAT IS CLAIMED IS:

1. Use of a synthetic apolipoprotein E-mimicking peptide for decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

2. Use of a synthetic apolipoprotein E-mimicking peptide for the preparation of a medicament for decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

3. The use of claim 1 or 2, wherein the synthetic apolipoprotein E-mimicking peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 11-14, 18-57, 60, 61, and 62-103.

4. The use of claim 1 or 2, wherein the receptor binding domain is from a species selected from the group consisting of human, mouse, rabbit, monkey, rat, bovine, pig and dog.

5. The use of claim 1 or 2, wherein the receptor binding domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1-2, 3, 5-10, 15, and 58.

6. The use of claim 1 or 2, wherein the receptor binding domain is in a reversed orientation.

7. The use of any one of claims 4-6, wherein the lipid-associating peptide is model class A amphipathic helical peptide 18A.

8. The use of any one of claims 4-6, wherein said lipid-associating peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 4, 16, 17, and 59.

9. The use of any one of claims 4-6, wherein the lipid-associating peptide is in a reversed orientation.

10. The use of any one of claims 4-9, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation.

11. The use of any one of claims 1-10, wherein the synthetic apolipoprotein E-mimicking peptide is for administration in a composition comprising a pharmaceutically acceptable carrier.

12. Use of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier for decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

13. Use of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier for the preparation of a medicament for decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

14. The use of claim 12 or 13, wherein the synthetic apolipoprotein E-mimicking peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 11-14, 18-57, 60, 61, and 62-103.

15. The use of claim 12 or 13, wherein the receptor binding domain is from a species selected from the group consisting of human, mouse, rabbit, monkey, rat, bovine, pig and dog.

16. The use of claim 12 or 13, wherein the receptor binding domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1-2, 3, 5-10, 15, and 58.

17. The use of claim 12 or 13, wherein the receptor binding domain is in a reversed orientation.

18. The use of any one of claims 15-17, wherein the lipid-associating peptide is model class A amphipathic helical peptide 18A.

19. The use of any one of claims 15-17, wherein said lipid-associating peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 4, 16, 17, and 59.

20. The use of any one of claims 15-17, wherein the lipid-associating peptide is in a reversed orientation.

21. The use of any one of claims 15-20, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation.

22. A synthetic apolipoprotein E-mimicking peptide for use in decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

23. The peptide of claim 22, wherein the synthetic apolipoprotein E-mimicking peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 11-14, 18-57, 60, 61, and 62-103.

24. The peptide of claim 22, wherein the receptor binding domain is from a species selected from the group consisting of human, mouse, rabbit, monkey, rat, bovine, pig and dog.

25. The peptide of claim 22, wherein the receptor binding domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1-2, 3, 5-10, 15, and 58.

26. The peptide of claim 22, wherein the receptor binding domain is in a reversed orientation.

27. The peptide of any one of claims 24-26, wherein the lipid-associating peptide is model class A amphipathic helical peptide 18A.

28. The peptide of any one of claims 24-26, wherein said lipid-associating peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 4, 16, 17, and 59.

29. The peptide of any one of claims 24-26, wherein the lipid-associating peptide is in a reversed orientation.

30. The peptide of any one of claims 24-29, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation.

31. The peptide of any one of claims 22-30, wherein the synthetic apolipoprotein E-mimicking peptide is for administration in a composition comprising a pharmaceutically acceptable carrier.

32. A pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier for decreasing the concentration of plasma glucose in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an

acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

33. The composition of claim 32, wherein the synthetic apolipoprotein E-mimicking peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 11-14, 18-57, 60, 61, and 62-103.

34. The composition of claim 32, wherein the receptor binding domain is from a species selected from the group consisting of human, mouse, rabbit, monkey, rat, bovine, pig and dog.

35. The composition of claim 32, wherein the receptor binding domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1-2, 3, 5-10, 15, and 58.

36. The composition of claim 32, wherein the receptor binding domain is in a reversed orientation.

37. The composition of any one of claims 32-36, wherein the lipid-associating peptide is model class A amphipathic helical peptide 18A.

38. The composition of any one of claims 32-36, wherein said lipid-associating peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 4, 16, 17, and 59.

39. The composition of any one of claims 32-36, wherein the lipid-associating peptide is in a reversed orientation.

40. The composition of any one of claims 32-39, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation.

41. Use of a synthetic apolipoprotein E-mimicking peptide for reducing β-cell apoptosis in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said

lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

42. Use of a synthetic apolipoprotein E-mimicking peptide for the preparation of a medicament for reducing β-cell apoptosis in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

43. The use of claim 41 or 42, wherein the synthetic apolipoprotein E-mimicking peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 11-14, 18-57, 60, 61, and 62-103.

44. The use of claim 41 or 42, wherein the receptor binding domain is from a species selected from the group consisting of human, mouse, rabbit, monkey, rat, bovine, pig and dog.

45. The use of claim 41 or 42, wherein the receptor binding domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1-2, 3, 5-10, 15, and 58.

46. The use of claim 41 or 42, wherein the receptor binding domain is in a reversed orientation.

47. The use of any one of claims 43-46, wherein the lipid-associating peptide is model class A amphipathic helical peptide 18A.

48. The use of any one of claims 43-46, wherein said lipid-associating peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 4, 16, 17, and 59.

49. The use of any one of claims 43-46, wherein the lipid-associating peptide is in a reversed orientation.

50. The use of any one of claims 43-49, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation.

51. The use of any one of claims 41-50, wherein the synthetic apolipoprotein E-mimicking peptide is for administration in a composition comprising a pharmaceutically acceptable carrier.

52. Use of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier for reducing β-cell apoptosis in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

53. Use of a pharmaceutical composition comprising a synthetic apolipoprotein E-mimicking peptide and a pharmaceutically acceptable carrier for the preparation of a medicament for reducing β-cell apoptosis in a subject, wherein the synthetic apolipoprotein E-mimicking peptide consists of a receptor binding domain of apolipoprotein E and a lipid-associating peptide, wherein the lipid-associating peptide is a model class A amphipathic helical peptide or derivative thereof, wherein said receptor binding domain is covalently linked to said lipid-associating peptide, and wherein the receptor binding domain contains an acetyl group on the N-terminus and the lipid-associating peptide contains an amide group on the C-terminus.

54. The use of claim 52 or 53, wherein the synthetic apolipoprotein E-mimicking peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 11-14, 18-57, 60, 61, and 62-103.

55. The use of claim 52 or 53, wherein the receptor binding domain is from a species selected from the group consisting of human, mouse, rabbit, monkey, rat, bovine, pig and dog.

56. The use of claim 52 or 53, wherein the receptor binding domain comprises a sequence selected from the group consisting of SEQ ID NOs: 1-2, 3, 5-10, 15, and 58.

57. The use of claim 52 or 53, wherein the receptor binding domain is in a reversed orientation.

58. The use of any one of claims 54-57, wherein the lipid-associating peptide is model class A amphipathic helical peptide 18A.

59. The use of any one of claims 54-57, wherein said lipid-associating peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 4, 16, 17, and 59.

60. The use of any one of claims 54-57, wherein the lipid-associating peptide is in a reversed orientation.

61. The use of any one of claims 52-60, wherein said receptor binding domain is covalently linked to said lipid-associating peptide in a domain switched orientation.

62. The use of any one of claims 41, 42, 52 and 53, wherein the receptor binding domain peptide comprises the sequence of SEQ ID NO:1 and the lipid-associating peptide comprises the sequence of SEQ ID NO:4.

63. The use of any one of claims 41, 42, 52 and 53, wherein the receptor binding domain peptide comprises the sequence of SEQ ID NO:3 and the lipid-associating peptide comprises the sequence of SEQ ID NO:4.

64. The use of any one of claims 41, 42, 52 and 53, wherein the receptor binding domain peptide comprises the sequence of SEQ ID NO:15 and the lipid-associating peptide comprises the sequence of SEQ ID NO:4.

65. The use of any one of claims 41, 42, 52 and 53, wherein the receptor binding domain peptide comprises the sequence of SEQ ID NO:1 and the lipid-associating peptide comprises the sequence of SEQ ID NO:17.

66. The use of any one of claims 41, 42, 52 and 53, wherein the receptor binding domain peptide comprises the sequence of SEQ ID NO:3 and the lipid-associating peptide comprises the sequence of SEQ ID NO:17.

67. The use of any one of claims 41, 42, 52 and 53, wherein the receptor binding domain peptide comprises the sequence of SEQ ID NO:15 and the lipid-associating peptide comprises the sequence of SEQ ID NO:17.

68. The use of any one of claims 41, 42, 52 and 53, wherein the subject has diabetes.

69. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 1 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

70. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

71. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

72. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

73. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

74. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

75. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

76. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

77. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

78. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

79. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

80. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

81. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

82. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

83. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

84. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

85. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

86. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

87. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

88. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

89. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

90. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

91. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

92. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

93. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

94. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

95. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

96. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

97. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

98. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

99. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

100. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

101. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

102. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

103. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

104. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

105. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

106. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

107. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

108. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

109. The use of any one of claims 1, 2, 12 and 13, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

110. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 1 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

111. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

112. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

113. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

114. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

115. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

116. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

117. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

118. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

119. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

120. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

121. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

122. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

123. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

124. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

125. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

126. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

127. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

128. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

129. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

130. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

131. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

132. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

133. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

134. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

135. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

136. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

137. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

138. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

139. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

140. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

141. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

142. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

143. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

144. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

145. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

146. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

147. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

148. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

149. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

150. The peptide of claim 22, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

151. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 1 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

152. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

153. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

154. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

155. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

156. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

157. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

158. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

159. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

160. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

161. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 4.

162. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

163. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

164. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

165. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

166. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

167. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

168. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

169. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

170. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

171. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 16.

172. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

173. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

174. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

175. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

176. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

177. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

178. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

179. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

180. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

181. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 17.

182. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 2 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

183. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 3 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

184. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 5 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

185. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 6 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

186. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 7 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

187. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 8 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

188. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 9 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

189. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 10 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

190. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 15 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

191. The composition of claim 32, wherein the receptor binding domain comprises the sequence of SEQ ID NO: 58 and the lipid-associating peptide comprises the sequence of SEQ ID NO: 59.

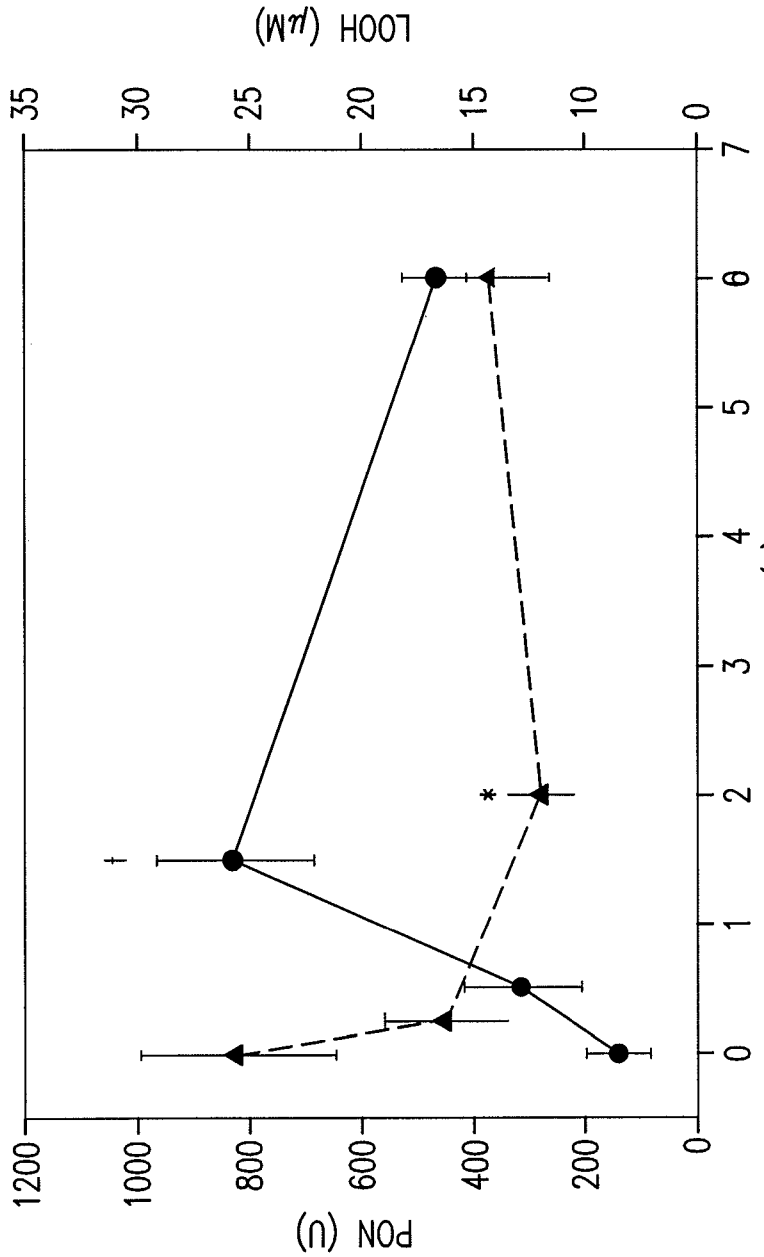
LOOH (μM)
35
30
25
20
15
10
0
7
6
5
4
3
2
1
0
Time (h)
*
†
1200
1000
800
600
400
200
0
PON (U)

FIG.1

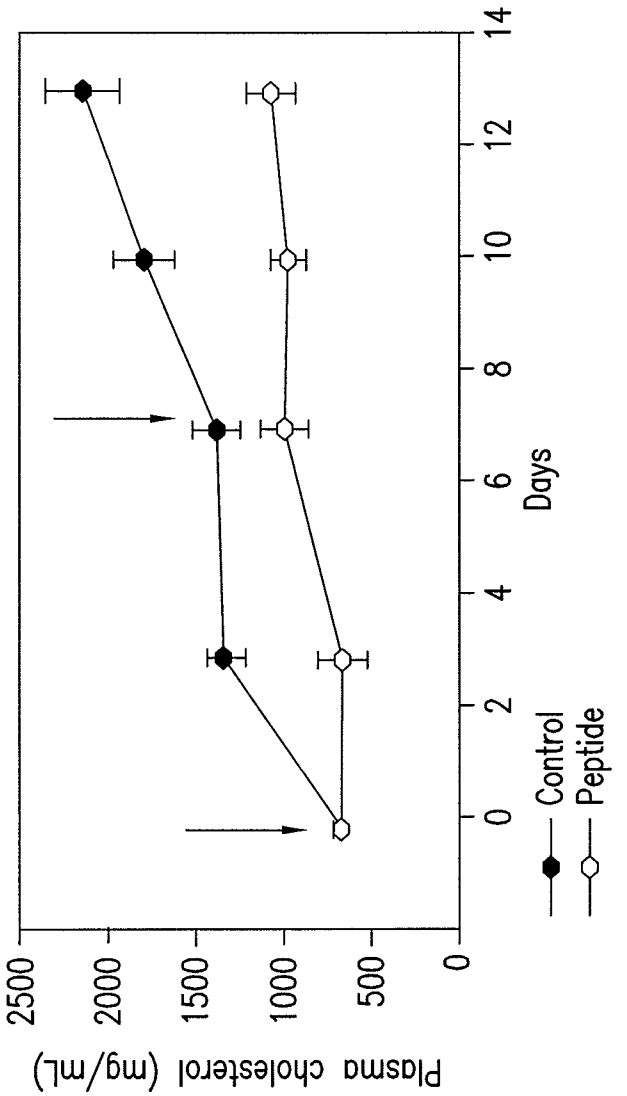
Plasma cholesterol (mg/mL)
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
Days
Control
Peptide

FIG.2

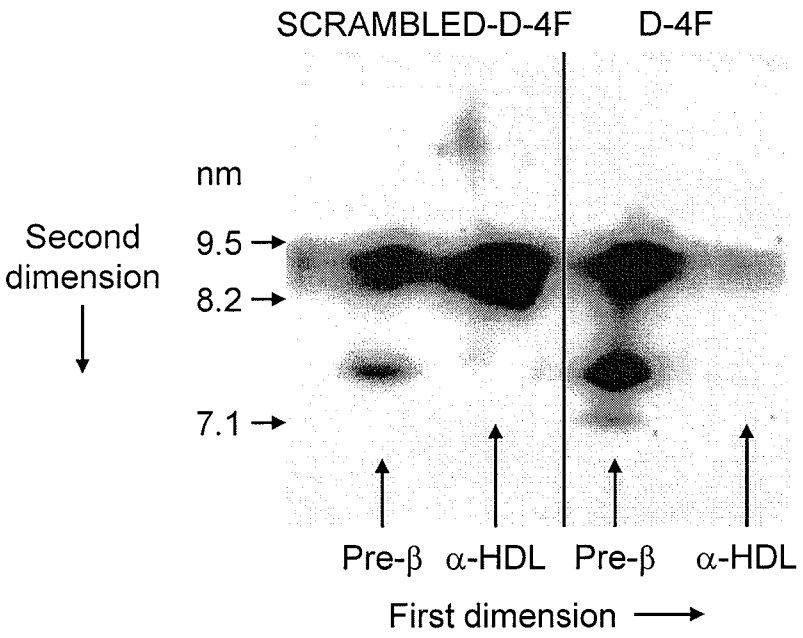
SCRAMBLED-D-4F
D-4F
nm
Second dimension
9.5
8.2
7.1
Pre-β
α-HDL
Pre-β
α-HDL
First dimension

FIG.3

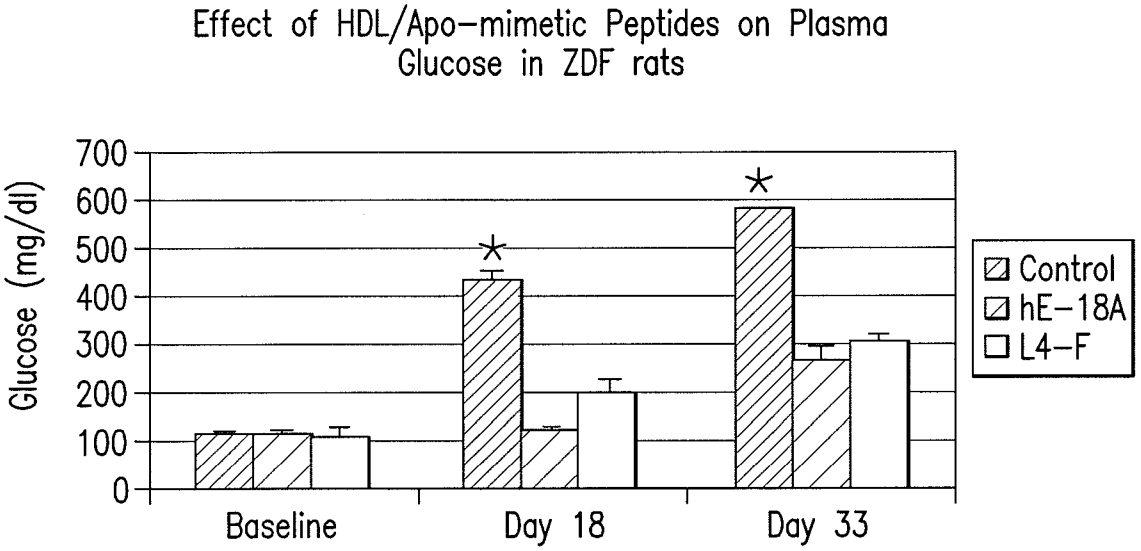
Effect of HDL/Apo-mimetic Peptides on Plasma Glucose in ZDF rats
Glucose (mg/dl)
700
600
500
400
300
200
100
0
Baseline
Day 18
Day 33
Control
hE-18A
L4-F

FIG.4A

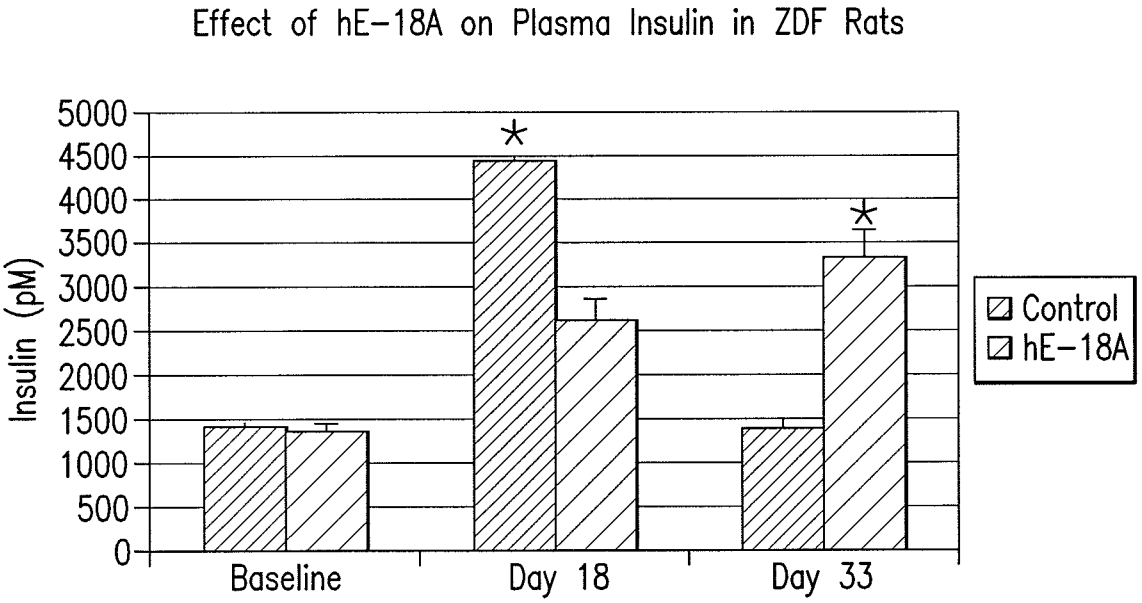
Effect of hE-18A on Plasma Insulin in ZDF Rats
Insulin (pM)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Baseline
Day 18
Day 33
Control
hE-18A

FIG.4B

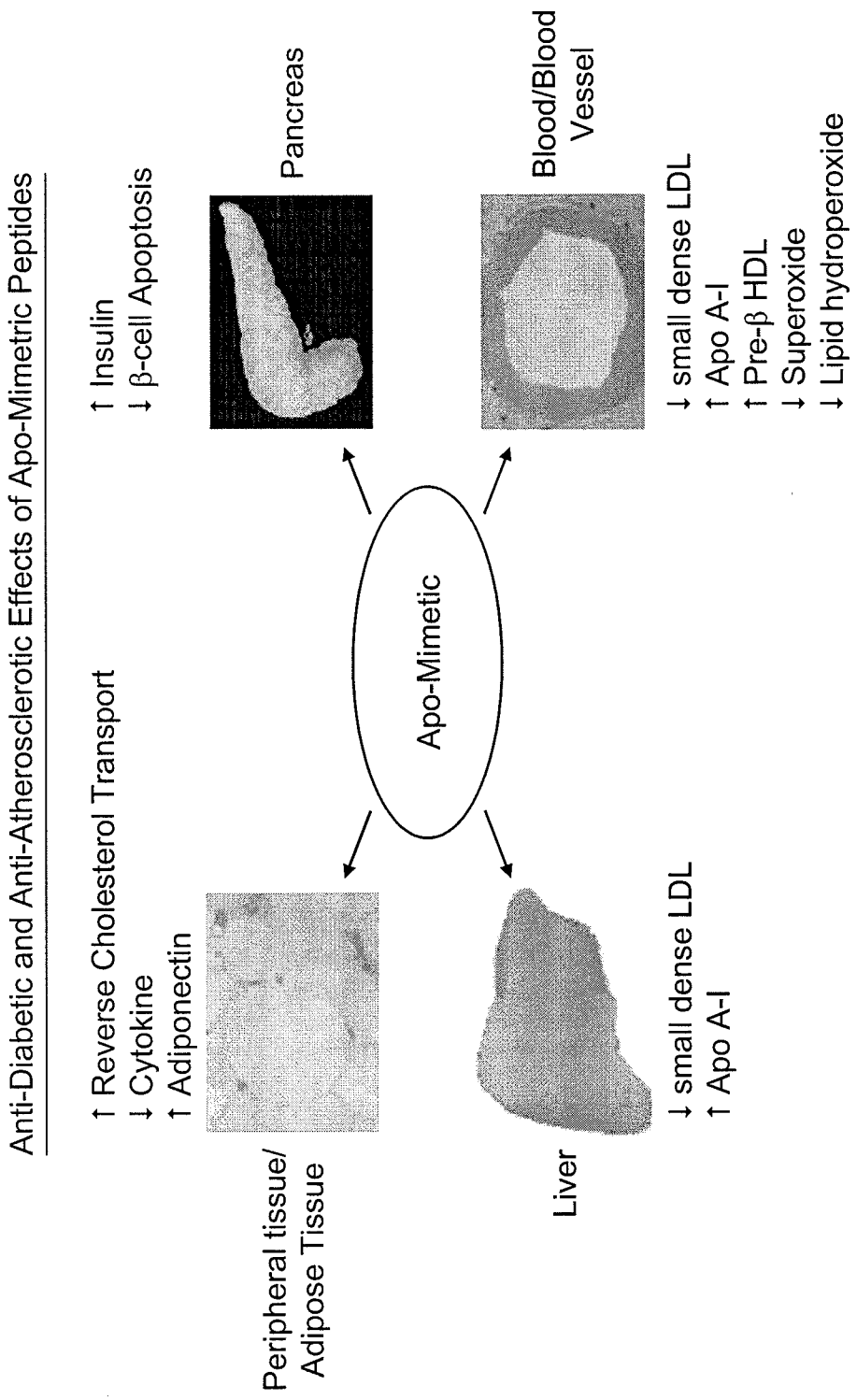
Anti-Diabetic and Anti-Atherosclerotic Effects of Apo-Mimetic Peptides
↑ Reverse Cholesterol Transport
↓ Cytokine
↑ Adiponectin
Peripheral tissue/
Adipose Tissue
↑ Insulin
↓ β-cell Apoptosis
Pancreas
Apo-Mimetic
↓ small dense LDL
↑ Apo A-I
Liver
Blood/Blood
Vessel
↓ small dense LDL
↑ Apo A-I
↑ Pre-β HDL
↓ Superoxide
↓ Lipid hydroperoxide

FIG.5

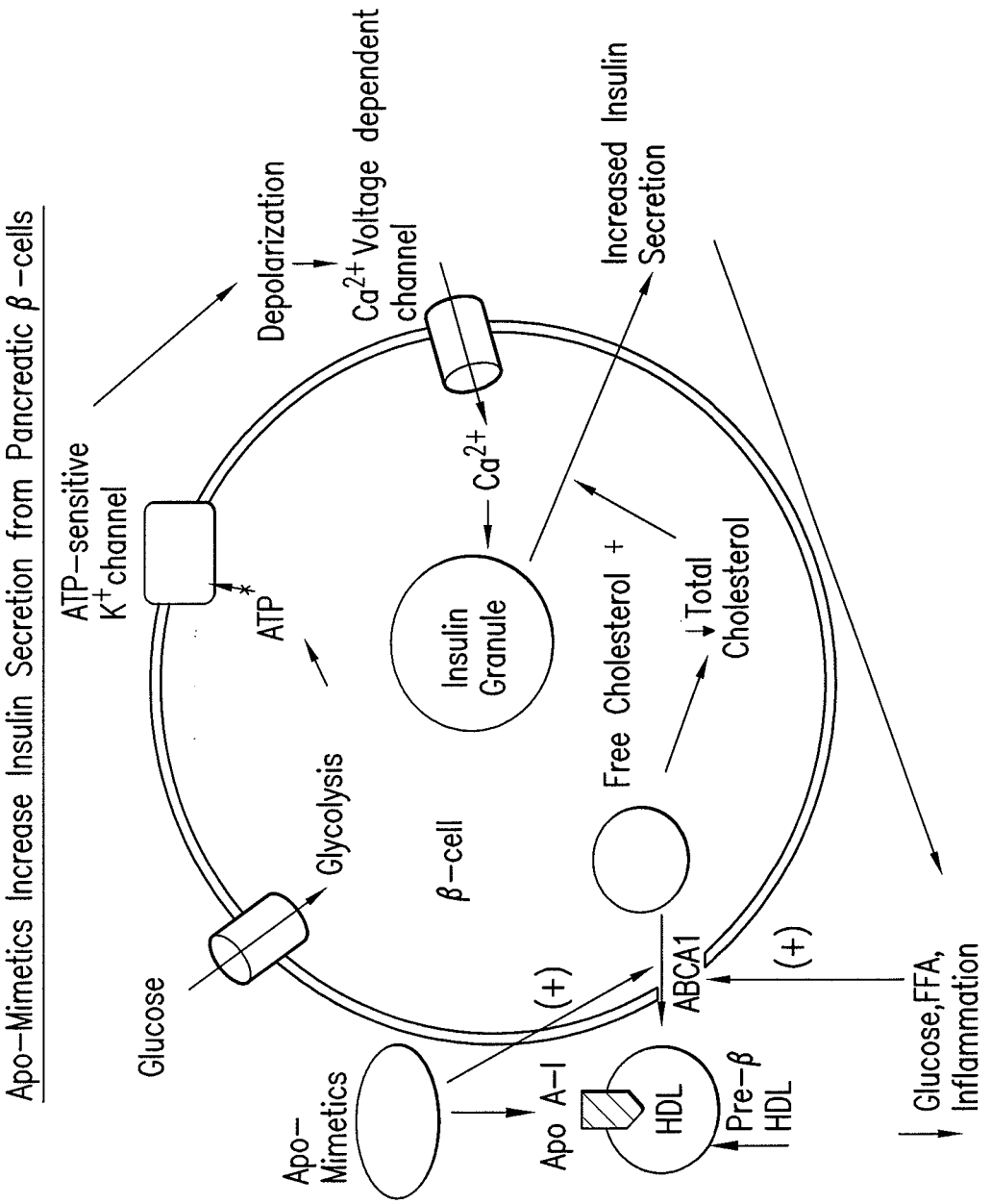
Apo–Mimetics Increase Insulin Secretion from Pancreatic β–cells
ATP–sensitive K+ channel
Depolarization
Ca2+ Voltage dependent channel
Increased Insulin Secretion
ATP
Ca2+
Insulin Granule
Glycolysis
β–cell
Free Cholesterol +
Total Cholesterol
Glucose
(+)
ABCA1
(+)
Apo–Mimetics
Apo A–I
HDL
Pre–β HDL
Glucose,FFA, Inflammation

FIG.6