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(54) **Title**: METHOD OF TREATING HYPERGLYCEMIC DISORDERS USING APOLIPROTEIN AIV

(57) **Abstract**: Methods for treating hyperglycemia disorders in a subject in need thereof and pharmaceutical compositions for the treatment of hyperglycemia disorders are disclosed. The methods include administering an effective amount of apolipoprotein A-IV to the subject. Also disclosed are methods for substantially restoring glucose tolerance in a subject in need thereof to a normal level and methods for lowering blood glucose levels in a subject having hyperglycemic disorders, including insulin resistant disorders, such as prediabetes, metabolic syndrome, polycystic ovary disease, type A syndrome, gestational diabetes, and endocrine conditions associated with hyperglycemia, including Cushing's Disease, glucagon excess (glucagon secreting tumors) and acromegaly.

# METHOD OF TREATING HYPERGLYCEMIC DISORDERS USING APOLIPOPROTEIN AIV

## TECHNICAL FIELD

**[0001]** The present disclosure relates to a method of treating conditions related hyperglycemia. More particularly, the present disclosure relates to a method of treating hyperglycemic disorders, including insulin resistant and endocrine disorders, by administering an effective amount of apolipoprotein A-IV.

## RELATED APPLICATIONS

**[0002]** This application claims priority to U.S. Provisional Patent Appln. No. 61/675,549, filed on July 25, 2012. The contents of the priority application is hereby incorporated by reference in its entirely.

## BACKGROUND

**[0003]** The occurrence of diabetes is widespread, with approximately 8% of the population in the United States suffering from diabetes. Diabetes is a chronic disease characterized by high blood sugar due to the body's inability to effectively produce and/or use insulin. Diabetes can lead to a variety of physical complications, including but not limited to renal failure, blindness, nerve damage, heart disease, sleep apnea, and celiac disease. For example, in the United States, diabetes is the leading cause of renal failure, blindness, amputation, stroke, and heart attack. Also in the United States, diabetes is the sixth leading cause of death and has been shown to reduce the life expectancy of middle-aged adults by about five to ten years.

**[0004]** The most common form of diabetes is type two diabetes mellitus which is associated with hyperglycemia, insulin resistance, β-cell dysfunction, and dysregulated hepatic gluconeogenesis. Persons suffering from type two diabetes experience a loss of glucose-stimulated insulin secretion related to the impaired release of stored insulin granules from β-cells in the first phase of insulin secretion. In the second phase of insulin secretion, persons suffering from type two diabetes experience a gradual loss of the ability to actively synthesize insulin in response to glucose stimuli.

**[0005]** In addition to type II diabetes, there are a number of related conditions defined by hyperglycemia that are also increasing in the general population. For

example, from 2005 to 2008, 35 percent of U.S. adults ages 20 years or older had prediabetes (defined by a fasting glucose or A1C levels; see CDC). As such, new therapies for effectively treating conditions related to hyperglycemia are needed.

## SUMMARY OF INVENTION

**[0006]** The present disclosure is based on the surprising discovery that apolipoprotein A-IV is effective for treating disorders characterized by hyperglycemia (or hyperglycemia disorders).

**[0007]** Apolipoprotein A-IV is a key gut hormone which contributes to post-prandial glucose tolerance and acts as a previously unappreciated mediator in the improvement of glucose tolerance. Accordingly, in one embodiment, methods of treating a hyperglycemia disorder in a subject in need thereof are disclosed. In one embodiment, methods of treating hyperglycemia disorders including insulin resistant disorders or endocrine disorders associated with hyperglycemia in a subject in need thereof are disclosed. In one embodiment, methods of treating endocrine disorders associated with hyperglycemia including Cushing's disease, glucagon secreting tumors, glucagon excess, and acromegaly in a subject in need thereof are disclosed. In another embodiment, methods of treating hyperglycemia in a subject having polycystic ovary disease are disclosed. In another embodiment, methods of treating hyperglycemia in a subject having an insulin resistant disorder selected from the group consisting of prediabetes, metabolic syndrome, polycystic ovary disease, type A syndrome, and gestational diabetes are disclosed. The method comprises administering to the subject an effective amount of an apolipoprotein A-IV or a biologically active analogue thereof having at least 90, 95, 96, 97, 98 or 99% identity to the apolipoprotein A-IV.

**[0008]** In another embodiment, a pharmaceutical composition comprising apolipoprotein A-IV is disclosed. The pharmaceutical composition comprises an apolipoprotein A-IV or a biologically active analogue thereof having at least 90, 95, 96, 97, 98 or 99% identity to the apolipoprotein A-IV formulated for administration to a subject for the treatment of hyperglycemia disorders.

**[0009]** In yet another embodiment, a method for substantially restoring glucose tolerance in a subject in need thereof to a normal level is disclosed. The method comprises administering to the subject an effective amount of an apolipoprotein A-IV

or a biologically active analogue thereof having at least 90, 95, 96, 97, 98 or 99% identity to an apolipoprotein A-IV , for example, by systemic administration of the apolipoprotein A-IV or the biologically active analogue thereof.

**[0010]** In yet still another embodiment, a method for lowering blood glucose level in a subject in need thereof is disclosed. The method comprises administering to the subject an effective amount of apolipoprotein A-IV or a biologically active analogue thereof having at least 90, 95, 96, 97, 98 or 99% identity to the apolipoprotein A-IV to the subject in need, for example, by systemic administration. An "effective amount" is as described below and includes, for example, about 0.25 to 2 μg/g of the apoA-IV or the biologically active analogue thereof. In one embodiment the effective amount is about 0.1 mg/kg to 25 mg/kg. In another embodiment, the effective amount is a fixed dose of about 1 to 1000 mg. In a further embodiment, the effective amount is a fixed dose of about 1 to 10 mg.

**[0011]** In a further embodiment, the apolipoprotein A-IV, or biologically active analogue thereof, is non-glycosylated.

**[0012]** In one embodiment, the methods and compositions disclosed herein include an apoA-IV protein comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 1, 3, 4 or 6-51, or biologically active fragments thereof. In one embodiment, the methods and compositions disclosed herein include an apoA-IV protein comprising an amino acid sequence which is 95%, 96%, 97%, 98%, or 99% identical to a sequence as set forth in SEQ ID NOs: 1, 3, 4 or 6-51, or a biologically active fragment thereof.

**[0013]** These and other features and advantages of these and other various embodiments according to the present disclosure will become more apparent in view of the drawings, detailed description, and claims provided herein.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0014]** The following detailed description of the embodiments of the present disclosure can be better understood when read in conjunction with the following drawings, where like structure is indicated with like reference numerals, and in which:

**[0015]** FIG. 1 is a side perspective view of a device having a reservoir of a pharmaceutical composition and a syringe according to an embodiment of the present disclosure.

**[0016]** FIG. 2 is a graph of plasma glucose (mg/dL) in male apolipoprotein A-IV knockout and wild-type mice with respect to time (min) for an intraperitoneal glucose tolerance test.

**[0017]** FIG. 3 is a graph of plasma glucose (mg/dL) with respect to time (min) for an intraperitoneal glucose tolerance test in apolipoprotein A-IV wild-type and knockout animals at 16 months of age.

**[0018]** FIG. 4 is a graph of plasma glucose (mg/dL) with respect to time (min) in male apolipoprotein A-IV knockout mice following the intraperitoneal administration of recombinant apolipoprotein A-IV (μg/g) or saline solution for an intraperitoneal glucose tolerance test.

**[0019]** FIG. 5 is a graph of plasma glucose (mg/dL) with respect to time (min) in apolipoprotein A-IV knockout mice following the intraperitoneal administration of recombinant apolipoprotein A-I or saline solution for an intraperitoneal glucose tolerance test.

**[0020]** FIG. 6 is a graph of insulin secretion (ng/mL) with respect to time (min) in apolipoprotein A-IV knockout mice following the intraperitoneal administration of recombinant apolipoprotein A-I or saline solution.

**[0021]** FIG. 7 is graph of plasma glucose (mg/mL) with respect to time (min) in apolipoprotein A-IV knockout and wild-type mice on a chronically high-fat diet for an intraperitoneal glucose tolerance test.

**[0022]** FIG. 8 is a graph of plasma glucose (mg/mL) with respect to time (min) in apolipoprotein A-IV knockout mice on a chronically high-fat diet following the intraperitoneal administration of recombinant mouse apolipoprotein A-IV (1 μg/g) or saline solution for an intraperitoneal glucose tolerance test.

**[0023]** FIG. 9 is a graph of plasma glucose (mg/dL) with respect to time (h) in diabetic mice following the intraperitoneal administration of recombinant mouse apolipoprotein A-IV ( 1 μg/g) or saline solution for an intraperitoneal glucose tolerance test.

**[0024]** FIG. 10 depicts the results of a Western blot analysis of the level of serum amyloid A protein component in apolipoprotein A-IV knockout mice, wild-type mice, and apolipoprotein A-I knockout mice.

**[0025]** FIG. 11 is a graph of plasma glucose (mg/dL) in female apolipoprotein A-IV knockout and wild-type mice with respect to time (min) during an intraperitoneal glucose tolerance test (IPGTT).

**[0026]** FIG 12. is a graph of plasma glucose (mg/dL) with respect to time (min) in wild type mice following the intraperitoneal administration of 1.0 μg/g human apolipoprotein A-IV or saline solution during an intraperitoneal glucose tolerance test.

**[0027]** FIG. 13 is a graph of plasma glucose (mg/dL) with respect to time (min) in female wild type mice following the intraperitoneal administration of 1.0 μg/g recombinant mouse apolipoprotein A-IV or saline solution during an intraperitoneal glucose tolerance test.

**[0028]** FIG. 14 is a bar graph showing the effect of 10 μg/g human apoA-IV on human islets depolarized by 30mM KCl and 250μM diazoxide in the presence of 3mM or 20mM glucose.

**[0029]** FIG. 15 is a protein with the amino acid sequence of full length wild type human apolipoprotein A-IV (SEQ ID NO. 1).

**[0030]** FIG. 16 is a protein with the amino acid sequence of full length wild type mouse apolipoprotein A-IV (SEQ ID NO. 2).

**[0031]** FIG. 17 is a protein with the amino acid sequence of full length wild type human apolipoprotein A-IV wit the addition of glycine at the *N*-terminus (SEQ ID NO. 3).

**[0032]** FIG. 18 is a protein with the amino acid sequence of human apolipoprotein A-IV showing polymorphic substitutions T347S, Q360H, and/or E165K and the optional addition of glycine, alanine or valine to the *N*-terminus (SEQ ID NO. 4).

**[0033]** FIG.19 is a polynucleotide (SEQ ID NO. 5) encoding full length wild type human apolipoproteom A-IV.

**[0034]** Skilled artisans appreciate that elements in the figures are illustrated for simplicity and clarity and are not necessarily drawn to scale. For example, the

dimensions of some of the elements in the figures may be exaggerated relative to other elements, as well as conventional parts removed, to help to improve understanding of the various embodiments of the present disclosure.

## DETAILED DESCRIPTION

**[0035]** The following terms are used in the present application:

**[0036]** As used herein, the term "hyperglycemic disorders" or "disorder associated with hyperglycemia" refers to a disorder in which a subject's blood sugar is elevated. The resulting increase in blood glucose may raise levels outside the normal range and cause adverse health effects for the subject. Examples of hyperglycemic disorders include, but are not limited to, diseases associated with insulin resistance (including, but not limited to, prediabetes, metabolic syndrome, Type A Insulin Resistance Syndrome, hyperglycemia or insulin resistance associated with polycystic ovarian disease, gestational diabetes), and hyperglycemia associated with endocrine abnormalities (including but not limited to Cushing's Disease, glucagon secreting tumors, and Acromegaly). In one embodiment, a hyperglycemic disorder does not include type II diabetes.

**[0037]** As used herein, the term "disorder associated with insulin resistance" refers to a condition where insulin becomes less effective at lowering blood sugar. Examples include, but are not limited to, prediabetes, metabolic syndrome, Type A Insulin Resistance Syndrome, hyperglycemia or insulin resistance associated with polycystic ovarian disease, gestational diabetes.

**[0038]** As used herein, the term "endocrine disorder associated with hyperglycemia" refers to disorders of the endocrine system characterized by high blood sugar. Examples of endocrine disorders associated with hyperglycemia include, but are not limited to Cushing's Disease, glucagon secreting tumors, and Acromegaly.

**[0039]** As used herein, the term "prediabetes" means a condition characterized by one or more of the following factors: the presence of anti-islets of Langerhans cells immunological markers, an impairment in the number of islets of Langerhans cells, suppression of the early peak of insulin secretion, glucose intolerance, an impairment tin fasting glycaemia and/or any diabetic risk factor. Prediabetes is characterized

either by impaired glucose tolerance or impaired fasting glucose and often described as the “gray area” between the normal level and diabetic levels of blood sugar. Prediabetes often precedes type two diabetes.

**[0040]** As used herein, the term "effective amount" describes the amount necessary or sufficient to realize a desired biologic effect. The effective amount for any particular application may vary depending on a variety of factors, including but not limited to the particular composition being administered, the size of the subject, and/or the severity of the disease and/or condition being treated. In one embodiment, an “effective amount” is a dose of about 0.25 to 10 μg/g of an apolipoprotein A-IV or biologically active analogue thereof. Alternatively, an “effective amount of an apoA-IV or a biologically active analogue thereof is about 1 to 10 μg/g, about 0.25 to 2 μg/g, about 1 μg/g, or 0.1 mg/kg to 25 mg/kg. In another embodiment, the effective amount is a fixed dose of about 1 to 1000 mg. In a further embodiment, the effective amount is a fixed dose of about 1 to 10 mg. An apoA-IV or a biologically active analogue is administered one time daily. Alternatively, an apoA-IV or a biologically active analogue thereof is administered about 2 times per day. In yet another alternative, an apoA-IV or a biologically active analogue thereof is administered more than twice a day, for example, three times per day. In yet another alternative, apoA-IV is administered once every second, third, fourth, fifth or sixth day, or once weekly.

**[0041]** As used herein, the term "desired biologic effect" describes reducing the effects of, counteracting, and/or eliminating a disease or condition. For example, in the context of hyperglycemic disorders, desired biologic effects include, but are not limited to lowering blood glucose, improving glucose tolerance, substantially restoring glucose tolerance to a normal level, improving insulin secretion, and/or substantially restoring insulin secretion to a normal level.

**[0042]** As used herein, the term "normal level" describes a level that is substantially the same as the level in a subject who is not in need of treatment. For example, in the context of treating a hyperglycemic disorder, a normal level of blood glucose is from about 70 mg/dL to about 130 mg/dL before meals and less than about 180 mg/dL about one to two hours after meals, or from about 70 mg/dL to about 100 mg/dL before meals and less than about 140 mg/dL about one to two hours after meals. In another example in the context of treating a hyperglycemic disorder, a normal level of

glucose tolerance describes the ability of the subject to metabolize carbohydrates such that the level of blood glucose is from about 70 mg/dL to about 130 mg/dL before meals and less than about 180 mg/dL about one to two hours after meals, or from about 70 mg/dL to about 100 mg/dL before meals and less than about 140 mg/dL about one to two hours after meals. In still another example in the context of treating a hyperglycemic disorder, the normal level of insulin secretion is the amount required to maintain a normal level of glucose tolerance, wherein the level of insulin secretion is greater than about 1 ng/mL about fifteen hours after meals. In a further embodiment, a normal level of blood glucose is from about 70 mg/dl to 100 mg/dl for a morning fasting blood sugar test.

**[0043]** In the context of blood glucose level, the term "restore" describes changing the blood glucose level of a subject to a normal level. Similarly, in the context of glucose tolerance, the term "restore" describes changing the glucose tolerance of a subject to a normal level. Also, in the context of insulin secretion, "restore" describes changing the insulin secretion of a subject to a normal level or to one sufficient to lower blood glucose.

**[0044]** In the context of apolipoprotein A-IV, the term "biologically active fragment" describes a fragment of apolipoprotein A-IV which is capable of realizing a desired biologic effect in a subject with a hyperglycemic disorder, *e.g.,* restore glucose tolerance. The term "biologically active analogue " describes an analogue of an apolipoprotein A-IV which is capable of realizing a desired biologic effect in a subject with a hyperglycemic disorder, *e.g.,* restore glucose tolerance. In one example, a desired biological effect is to restore glucose tolerance in apoA-IV knockout mice as described in Example 2. Another example of a desired biological effect is to cause a statistically significant lowering of abnormal glucose levels in an animal model of type 2 diabetes (T2DM), such as the mouse model described in Example 7.

**[0045]** The phrase "percent identical" or "percent identity" refers to the similarity (e.g., 95%, 96%, 97%, 98%, or 99%) between at least two different sequences. This percent identity can be determined by standard alignment algorithms, for example, the Basic Local Alignment Search Tool (BLAST) described by Altshul et al. ((1990) J. Mol. Biol. 215:403-10); the algorithm of Needleman et al. ((1970) J. Mol. Biol. 48:444-53); or the algorithm of Meyers et al. ((1988) Comput. Appl. Biosci. 4:11-17).

A set of parameters may be, for example, the Blosum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The percent identity between two amino acid or nucleotide sequences can also be determined using the algorithm of Meyers and Miller ((1989) CABIOS 4:11-17), which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4.

**[0046]** The term "recombinant protein" refers to a protein molecule that is expressed from recombinant DNA. For example, a recombinant ApoA-IV protein is one that is expressed in a recombinant host cell.

**[0047]** As used herein, the term "obese" describes a condition in which a subject is well above a normal weight. In one specific example, the term obese describes a condition in which a subject is more than about 20% over their ideal weight and/or has a body mass index of about thirty or greater than about thirty. In one embodiment, the subject being treated is obese; in another embodiment, the subject being treated is not obese; and in yet another embodiment, the subject being treated has a normal body weight.

**[0048]** Embodiments of the present disclosure relate to methods for treating a hyperglycemic disorder in a subject in need thereof and pharmaceutical compositions for the treatment of hyperglycemic disorder. In one embodiment, a method of treating diabetes is disclosed. In one particular embodiment, a method of treating a hyperglycemic disorder in a subject in need thereof is disclosed, wherein the method comprises administering an effective amount of an apoA-IV or a biologically active analogue thereof to the subject.

**[0049]** In one embodiment, the method of treating a hyperglycemic disorder results in a lower blood glucose level of a subject. In one particular embodiment, the method is effective to lower blood glucose level of a subject by about 20 to 60%. In a further embodiment, the method is effective to lower the blood glucose level of a subject by about 70 %. In a further embodiment, the method is effective to lower the blood glucose level of a subject by about 40%. In still a further embodiment, the method is effective to substantially restore blood glucose level to a lower than previous levels or to normal level.

**[0050]** In one embodiment, the method of treating a hyperglycemic disorder results in a lower blood glucose level of a subject. In one particular embodiment, the method is effective to lower blood glucose level of a subject by about 1 mg/dl, 2 mg/dl, 3 mg/dl, 4 mg/dl, 5 mg/dl, 6 mg/dl, 7 mg/dl, 8 mg/dl, 9 mg/dl, 10 mg/dl, 11 mg/dl, 12 mg/dl, 13 mg/dl, 14 mg/dl, 15 mg/dl, 16 mg/dl, 17 mg/dl, 18 mg/dl, 19 mg/dl, 20 mg/dl, 40 mg/dl, 60 mg/dl, 80 mg/dl, 100 mg/dl, 120 mg/dl, 140 mg/dl, 160 mg/dl, 180 mg/dl, 200 mg/dl, 220 mg/dl, or 240 mg/dl, from a baseline level over the course of the dosing interval.

**[0051]** In another embodiment, the method of treating a hyperglycemia disorder is effective to substantially restore glucose tolerance of a subject to a normal level. In one particular embodiment, the method is effective to substantially restore glucose tolerance of a subject to a normal level within about two hours after administration of a dose of an apoA-IV or a biologically active analogue thereof . In another embodiment, the method is effective to substantially restore glucose tolerance of a subject to a normal level within about three hours or within about four hours after administration of a dose of an apoA-IV or a biologically active analogue thereof . In another embodiment, the glucose tolerance of a subject is substantially restored to a normal level for about eight to twelve hours.

**[0052]** In yet another embodiment, the treatment is effective to substantially restore insulin secretion to a normal level. In one particular embodiment, the treatment is effective to substantially restore insulin secretion to a normal level within about two hours after the administration of a dose of an apoA-IV or a biologically active analogue thereof . In another embodiment, insulin secretion is substantially restored to a normal level for about eight to twelve hours. In still another embodiment, the treatment is effective to lower the level of C-reactive protein.

**[0053]** As described above, examples of hyperglycemic disorders include, but are not limited to, disorders associated with insulin resistance. Examples of insulin resistant disorders include, but are not limited to, prediabetes, metabolic syndrome, polycystic ovary disease, type A syndrome and other insulin resistant genetic disorders, and gestational diabetes.

**[0054]** In one embodiment, the invention provides a method of treating prediabetes in a subject by administering apoA-IV. A person having prediabetes has blood sugar

levels that are higher than normal, but not yet high enough to be classified as type 2 diabetes. Prediabetes can be characterized through a number of tests. For example, the glycated hemoglobin (A1C) test may be used to diagnose prediabetes. The A1C blood test indicates the average blood sugar level for the past two to three months of the subject. An A1C level between 6 and 6.5 percent is considered prediabetic, whereas a level of 6.5 percent or higher on two separate tests indicates diabetes.

**[0055]** Other tests for prediabetes include the fasting blood sugar test and the glucose tolerance test. Impaired fasting glucose is determined by measuring glucose levels after a fast of at least eight hours or overnight. Impaired glucose fasting indicating prediabetes is defined as 100 to 125 mg/dL (5.6 to 6.9 mmol/L), whereas a blood sugar level lower than 100 milligrams per deciliter (mg/dL) — 5.6 millimoles per liter (mmol/L) — is normal. A blood sugar level of 126 mg/dL (7.0 mmol/L) or higher may indicate diabetes mellitus.

**[0056]** Alternatively, prediabetes may be characterized by the impaired glucose tolerance test where glucose levels are measured following the consumption of a sugary solution (generally two hours following ingestion). For the impaired glucose tolerance test, a blood sugar level less than 140 mg/dL (7.8 mmol/L) is normal, whereas a blood sugar level from 140 to 199 mg/dL (7.8 to 11.0 mmol/L) is considered prediabetic and often referred to as impaired glucose tolerance (IGT). A blood sugar level of 200 mg/dL (11.1 mmol/L) or higher may indicate diabetes mellitus. As such, the invention provides a means for treating prediabetes by administration of apoA-IV.

**[0057]** In one embodiment, the invention provides a method of treating metabolic syndrome in a subject by administering apoA-IV. Metabolic Syndrome (also referred to as Syndrome X or insulin resistance syndrome), is the name for a group of risk factors that occur together and increase a person's risk for coronary artery disease, stroke, and type 2 diabetes. While metabolic syndrome is becoming more and more common in the United States, it is unknown whether the syndrome is due to one single cause, but all of the risks for the syndrome are related to obesity. The two most important risk factors for metabolic syndrome are 1. extra weight around the middle and upper parts of the body (central obesity) of the body and 2. insulin resistance, in which the body cannot use insulin effectively. According to the American Heart

Association and the National Heart, Lung, and Blood Institute, metabolic syndrome is present if you have three or more of the following signs: 1. blood pressure equal to or higher than 130/85 mmH; 2. a fasting blood sugar (glucose) equal to or higher than 100 mg/d; 3. a large waist circumference (length around the waist), defined as 40 inches or more for men and 35 inches or more for women; 4. low HDL cholesterol levels, defined as under 40 mg/dl for men and under 50 mg/dl for women; and 5. triglycerides equal to or higher than 150 mg/dL. As such, the invention provides a means for treating metabolic syndrome by administration of apoA-IV.

**[0058]** In one embodiment, the invention provides a method of treating insulin resistance and hyperglycemia in a subject having polycystic ovary disease by administering apoA-IV. Polycystic ovary disease (also referred to as Stein-Leventhal syndrome or polyfollicular ovarian disease) is a condition in which a woman has an imbalance of a female sex hormones, and may lead to menstrual cycle changes, cysts in the ovaries, fertility issues, and other health changes. While the causes of polycystic ovary disease are not fully understood, one of the major biochemical features of polycystic ovary syndrome is insulin resistance accompanied by compensatory hyperinsulinemia (elevated fasting blood insulin levels). Indeed, type II diabetes is often a long-term complication associated with the disease. Thus, the invention provides a means for treating insulin resistance and hyperglycemia in a subject having polycystic ovary disease by administration of apoA-IV.

**[0059]** In a further embodiment, the methods and compositions of the invention may also be used to treat genetic disorders associated with extreme insulin resistance, including, but not limited to, Donohue syndrome, Rabson-Mendenhall syndrome, and type A insulin resistance. Donohue syndrome is characterized by intrauterine growth restriction, failure to thrive after birth, loss of glucose homeostasis and hyperinsulinemia, among other conditions. Patients diagnosed with Donohue syndrome have a shortened life expectancy, and generally do not live more than a year. Rabson-Mendenhall syndrome is characterized by growth retardation and hyperinsulinemia, among other symptoms. Type A insulin resistance may present with hirsutism, reduced subcutaneous fat, diabetes mellitus, acanthosis nigricans, and hyperinsulinemia, as well as amenorrhea and polycystic ovaries in females. Hyperinsulinemia, a biological marker for insulin resistance, is often associated with glucose tolerance defects over the course of the disease, and diabetes progressively

sets in. As such, apoA-IV may be used to treat patients having inherited diseases associated with extreme insulin resistance, such as type A insulin resistance syndrome.

**[0060]** In a further embodiment, the methods and compositions of the invention may also be used to treat gestational diabetes. Gestational diabetes is characterized as high blood sugar levels (see tests described above for prediabetes which can be used to determine gestational diabetes as well). While gestational diabetes generally disappears upon the birth of the child, sugar levels must be maintained during pregnancy for the health of the mother and baby. Moreover, gestational diabetes is often a precursor to prediabetes. Thus, apoA-IV may be used to lower sugar levels in subjects having gestational diabetes.

**[0061]** As described above, examples of hyperglycemic disorders include, but are not limited to, endocrine disorders associated hyperglycemia. Examples of endocrine disorders associated hyperglycemia include, but are not limited to, Cushing's Disease, increased glucagon secretion, and Acromegaly

**[0062]** In one embodiment, the methods and compositions of the invention may be used to treat Cushing's disease. Cushing's disease is characterized by an excess cortisol production by adrenal glands. Symptoms include high blood sugar levels along with high blood pressure, weariness, obesity in the upper part of body and slenderness in legs and arms.

**[0063]** In another embodiment, the methods and compositions of the invention may be used to treat glucagon secreting tumors. Aglucagon secreting tumor is also called a glucagonoma, and is a rare tumor of the alpha cells of the pancreas that results in up to a 1000-fold overproduction of the hormone glucagon. The primary physiological effect of glucagonoma is an overproduction of the peptide hormone glucagon, which enhances blood glucose levels through the activation of anabolic and catabolic processes including gluconeogenesis and lipolysis, respectively.

**[0064]** In yet another embodiment, the methods and compositions of the invention may be used to treat acromegaly. Acromegaly is a metabolic disorder caused by a pituitary gland tumor, which results in an overproduction of human growth hormone.

**[0065]** In one embodiment, the invention includes a method of first selecting a subject having one of the aforementioned disorders and administering ApoA-IV, or a biologically active fragment thereof, for the treatment of said disorder.

**[0066]** In one embodiment, an apoA-IV or a biologically active analogue thereof is administered systemically. Systemic administration of the apoA-IV or the analogue thereof is selected from the group consisting of oral, subcutaneous, intravenous, intramuscular, and intraperitoneal administration.

**[0067]** In another embodiment, a pharmaceutical composition is disclosed. In one particular embodiment, the pharmaceutical composition comprises an apoA-IV or a biologically active analogue thereof . In another embodiment, the apoA-IV or analogue thereof is formulated for administration to a subject for the treatment of hyperglycemic disorders. In this particular embodiment, a method for treating hyperglycemic disorders in a subject in need thereof is also provided, wherein the method comprises administering an effective amount of the pharmaceutical composition to the subject.

**[0068]** An "apolipoprotein A-IV" (also referred to herein as "apoA-IV") refers to mammalian apoA-IV and includes full-length apoA-IV and biologically active fragments of apoA-IV. The full-length human apoA-IV is a 376 amino acid protein (SEQ ID NO: 1), the amino acid sequence of which is shown in FIG. 15; the amino acid sequence of full length mouse apoA-IV (SEQ ID NO. 2) is shown in FIG. 16. Also encompassed by the term "apolipoprotein A-IV" is the known analogue in which a glycine is added to *N*-terminus of the apolipoprotein A-IV of the full length human sequence (SEQ ID NO. 3, as shown in FIG. 17), and analogues thereof having conservative substitutions for the *N*-terminal glycine (such as alanine and valine). An "apolipoprotein A-IV" also includes polymorphic forms thereof, including the T347S, Q360H, or E165K substitutions to the human sequence represented by SEQ ID NO. 1 or the corresponding positions of SEQ ID NO. 3. As such, "apolipoprotein A-IV" includes the protein of SEQ ID NO. 4, shown in FIG. 18. Variant versions of apoA-IV are also included in the methods and compositions of the invention, and include variants containing the following missense mutations: P393H, Q385K, Q381K, Q380H, Q377P, T367S, S353A, N352Y, V336M, D335H, G311R, V307L, R305C, R304Q, E291G, V274M, V274A, R264Q, A260T, E250K, N235S, Q231K, R220C, Q214H, E207K, T202M, R200C, D191N, D184N, P181L, A172T, R169W, A161S, R154W, T148M, S147N, A139E, N127K, S95L, R90C, T85A, Q77H, G74S, V13M, or V6M (see SEQ ID Nos: 6-50). SEQ ID NO: 51 represents the amino acid sequence of ApoA-IV having the signal sequence. ApoA-IV proteins having 95%, 96%, 97%,

98%, or 99% identity the amino acid sequences described in SEQ ID NOs: 1, 3, 4, and 6-51, or biologically active fragments thereof, are also contemplated in the methods of the invention.

**[0069]** A biologically active analogue of apolipoprotein A-IV has at least 90, 95, 96, 97, 98 or 99% identity to an apolipoprotein A-IV. As described in the previous paragraph, an apolipoprotein A-IV includes full length mammalian apolipoprotein A-IV (e.g., human or mammalian), polymorphic forms thereof, the protein of SEQ ID NOS. 3 and 4, and biologically active fragments of any of the foregoing. Amino acid variations in the biologically active analogues preferably have conservative substitutions relative to the wild type sequences. A "conservative substitution" is the replacement of an amino acid with another amino acid that has the same net electronic charge and approximately the same size and shape. Amino acid residues with aliphatic or substituted aliphatic amino acid side chains have approximately the same size when the total number of carbon and heteroatoms in their side chains differs by no more than about four. They have approximately the same shape when the number of branches in their side chains differs by no more than one. Amino acid residues with phenyl or substituted phenyl groups in their side chains are considered to have about the same size and shape. Listed below are five groups of amino acids. Replacing an amino acid residue with another amino acid residue from the same group results in a conservative substitution:

Group I: glycine, alanine, valine, leucine, isoleucine, serine, threonine, cysteine, and non-naturally occurring amino acids with C1-C4 aliphatic or C1-C4 hydroxyl substituted aliphatic side chains (straight chained or monobranched).

Group II: glutamic acid, aspartic acid and non-naturally occurring amino acids with carboxylic acid substituted C1-C4 aliphatic side chains (unbranched or one branch point).

Group III: lysine, ornithine, arginine and non-naturally occurring amino acids with amine or guanidino substituted C1-C4 aliphatic side chains (unbranched or one branch point).

Group IV: glutamine, asparagine and non-naturally occurring amino acids with amide substituted C1-C4 aliphatic side chains (unbranched or one branch point).

Group V: phenylalanine, phenylglycine, tyrosine and tryptophan.

**[0070]** Apolipoprotein A-IV or a biologically active analogue thereof can be glycosylated or unglycosylated. The preparation of recombinant unglycosylated human and mouse apolipoprotein A-IV is described in Example 11. The polynucleotide sequence of full length wild type human apolipoprotein (SEQ ID NO. 1) is shown as SEQ ID NO. 5 in Figure 19. Apolipoprotein A-IV used in examples 1-11 is unglycosylated. ApoA-IV may be prepared according to a method known in the molecular biology field. For example, apoA-IV may be prepared via traditional molecular cloning techniques.

**[0071]** In one embodiment, a bacterial host may be used to produce non-glycosylated apoA-IV. Examples of bacterial hosts include, but are not limited to, *E. coli* BL-21, BL-21 (DE3), BL21-AI™, BL21(DE3)pLysS, BL21(DE3)pLysE, BL21 Star™(DE3), and BL21 Star™ (DE3)pLysS, (Invitrogen). Corynebacterium may also be used as a host cell for expressing apoA-IV. Prior to transformation into the bacterial host, the DNA segment encoding ApoA-IV or its analogue may be incorporated in any of suitable expression vectors for transformation into the bacterial host. Suitable expression vectors include plasmid vectors, cosmid vectors, and phage vectors variously known to those of skill in the art, for example, as described in Sambrook, et al., Molecular Cloning Manual, 2d Edition, 1989. Examples of the expression vector include pET Vectors (Invitrogen), pDEST vectors (Invitrogen), pRSET vectors (Invitrogen), and pJexpress Vector (DNA2.0 Inc.). In one embodiment, *E. Coli* BL-21 (DE3) is transformed with pET30 expression vector which contains the gene encoding the ApoA-IV.

**[0072]** In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for apoA-IV-encoding vectors. Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as Schizosaccharomyces pombe; Kluyveromyces hosts such as, e.g., K. lactis, K fragilis

(ATCC 12,424), K. bulgaricus (ATCC 16,045), K. wickeramii (ATCC 24,178), K. waltii (ATCC 56,500), K. drosophilarum (ATCC 36,906), K. thermotolerans, and K. marxianus; yarrowia (EP 402,226); Pichia pastoris (EP 183,070); Candida; Trichoderma reesia (EP 244,234); Neurospora crassa; Schwanniomyces such as Schwanniomyces occidentalis; and filamentous fungi such as, e.g., Neurospora, Penicillium, Tolypocladium, and Aspergillus hosts such as A. nidulans and A. niger.

**[0073]** Suitable host cells for the expression of apoA-IV are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as Spodoptera frugiperda (caterpillar), Aedes aegypti (mosquito), Aedes albopictus (mosquito), Drosophila melanogaster (fruitfly), and Bombyx mori have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of Autographa californica NPV and the Bm-5 strain of Bombyx mori NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of Spodoptera frugiperda cells.

**[0074]** Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

**[0075]** Another suitable host cell for production of apoA-IV protein is a vertebrate cell. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (e.g., 293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, e.g., ATCC CCL 10); Chinese hamster ovary cells/-DHFR(CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)), including, but not limited to CHO K1, CHO pro3-, CHO DG44, CHO DUXB11, Lec13, B-Ly1, and CHO DP12 cells, preferably a CHO DUX (DHFR-) or subclone thereof (herein called "CHO DUX"); C127 cells, mouse L cells; Ltk.sup.- cells; mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HeLa, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse myeloma cells; NS0; hybridoma cells such as mouse hybridoma cells;

COS cells; mouse mammary tumor (MMT 060562, ATCC CCL51); TR1 cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

**[0076]** Host cells are transformed with expression or cloning vectors for production of the apoA-IV protein, and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

**[0077]** Apolipoprotein A-IV knockout mice used in the examples were generated according to procedures disclosed in J Lipid Res. 1997 Sep;38(9):1782-94 , the entire teachings of which are incorporated herein by reference.

**[0078]** In one particular embodiment, the pharmaceutical composition may further comprise a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers include a wide range of known diluents (i.e., solvents), fillers, extending agents, binders, suspending agents, disintegrates, surfactants, lubricants, excipients, wetting agents and the like commonly used in this field. The pharmaceutical composition is preferably aqueous, i.e., is a liquid formulation, and preferably comprises pyrogen free water.These carriers may be used singly or in combination according to the form of the pharmaceutical preparation. The resulting preparation may incorporate, if necessary, one or more solubilizing agent, buffers, preservatives, colorants, perfumes, flavorings and the like that are widely used in the field of pharmaceutical preparation.

**[0079]** The apolipoprotein A-IV or biologically active analogue thereof may be formulated into a dosage form selected from the group consisting of tablets, capsules, granules, pills, injections, solutions, emulsions, suspensions, and syrups. The form and administration route for the pharmaceutical composition are not limited and can be suitably selected. For example, tablets, capsules, granules, pills, syrups, solutions, emulsions, and suspensions may be administered orally. Additionally, injections (e.g. subcutaneous, intravenous, intramuscular, and intraperitoneal) may be administered intravenously either singly or in combination with a conventional replenisher containing glucose, amino acid and/or the like, or may be singly administered intramuscularly, intracutaneously, subcutaneously and/or intraperitoneally.

**[0080]** The pharmaceutical composition of the invention for treating hyperglycemia may be prepared according to a method known in the pharmaceutical field of this kind

using a pharmaceutically acceptable carrier. For example, oral forms such as tablets, capsules, granules, pills and the like are prepared according to known methods using excipients such as saccharose, lactose, glucose, starch, mannitol and the like; binders such as syrup, gum arabic, sorbitol, tragacanth, methylcellulose, polyvinylpyrrolidone and the like; disintegrates such as starch, carboxymethylcellulose or the calcium salt thereof, microcrystalline cellulose, polyethylene glycol and the like; lubricants such as talc, magnesium stearate, calcium stearate, silica and the like; and wetting agents such as sodium laurate, glycerol and the like.

**[0081]** Injections, solutions, emulsions, suspensions, syrups and the like may be prepared according to a known method suitably using solvents for dissolving the active ingredient, such as ethyl alcohol, isopropyl alcohol, propylene glycol, 1,3-butylene glycol, polyethylene glycol, sesame oil and the like; surfactants such as sorbitan fatty acid ester, polyoxyethylenesorbitan fatty acid ester, polyoxyethylene fatty acid ester, polyoxyethylene of hydrogenated castor oil, lecithin and the like; suspending agents such as cellulose derivatives including carboxymethylcellulose sodium, methylcellulose and the like, natural gums including tragacanth, gum arabic and the like; and preservatives such as parahydroxybenzoic acid esters, benzalkonium chloride, sorbic acid salts and the like.

**[0082]** The proportion of the active ingredient to be contained in the pharmaceutical composition of the invention for treating a hyperglycemic disorder can be suitably selected from a wide range.

**[0083]** Also included in the methods of the invention are combination therapies for treating hyperglycemic disorders. Examples of additional therapeutic agents that may be used in combination with apolipoprotein A-IV include, but are not limited to, sulfonylureas, meglitinides, biguanides, thiazolidinediones, alpha-glucosidase inhibitors, DPP-4 inhibitors, incretin mimetics, and insulin. An additional therapeutic agent may be administered prior to, concurrently with, or subsequent to administration of apoA-IV to the subject in need thereof.

**[0084]** The effective amount or apoA-IV administered to a subject for the treatment of a disorder associated with hyperglycemia may, for example, be a weight-based dose (e.g., mg/kg) or, in another example, be a fixed dose (non-weight dependent). In one embodiment, about 1 to 10 mg/kg, about 0.25 to 2 mg/kg, about 1 mg/kg, or 0.1 mg/kg

to 25 mg/kg of apoA-IV is administered to a subject in need thereof. In another embodiment, the effective amount of apoA-IV administered to a subject in need thereof is a fixed dose of about 1 to 1000 mg. In a further embodiment, the effective amount is a fixed dose of apoA-IV administered to a subject in need thereof, is about 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11, mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 120 mg, 140 mg, 160 mg, 180 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, or 1000 mg.

**[0085]** In one particular embodiment, the subject in need of treatment of a hyperglycemic disorder is a mammal. The mammal may be selected from the group consisting of humans, non-human primates, canines, felines, murines, bovines, equines, porcines, and lagomorphs. In one specific embodiment, the mammal is human. In another embodiment, apoA-IV or a biologically active analogue thereof may be administered to a subject for the treatment of a hyperglycemic disorder wherein the subject is obese. Alternatively, apoA-IV may be administered to a subject for the treatment of a hyperglycemic disorder wherein the subject is not obese.

**[0086]** Referring to FIG. 1, in yet another embodiment, a device 1 is disclosed. In one embodiment, the device 1 comprises a reservoir 10 of the pharmaceutical composition previously discussed above. In a further embodiment, the reservoir 10 comprises a vial 12. The vial 12 may be formed of any material that does not inhibit the function of the pharmaceutical composition. For example, the vial 12 may comprise glass and/or plastic. Additionally, the vial 12 may comprise a pierceable septum 14 through which the pharmaceutical composition may be removed. In use, the septum 14 of the vial is pierced by the needle 22 of a syringe 20, the pharmaceutical composition is removed by the syringe 20 from the vial 12, and the pharmaceutical composition is administered via injection to a subject in need.

## EXAMPLES

**[0087]** The following non-limiting examples illustrate the methods of the present disclosure.

**Example 1: Glucose Intolerance of ApoA-IV Knockout Mice**

**[0088]** *Experimental Protocol.* Male apoA-IV knockout ("hereinafter "KO") mice were obtained. Wild-type (hereinafter "WT") mice served as controls. ApoA-IV KO and WT mice were obtained from a colony kept at the University of Cincinnati (Cincinnati, OH). ApoA-IV KO and WT mice were fed a chow diet. Prior to performing the glucose tolerance tests, ApoA-IV KO mice and WT mice were fasted for five hours. In the glucose tolerance tests, the apoA-IV KO mice and WT mice were injected intraperitoneally with a dose of about 2 mg/g body weight of glucose and plasma glucose was measured at about 0, 15, 30, 60, and 120 minutes following the injection of glucose. The glucose tolerance tests were performed twice, once at three months of age and again at sixteen months of age.

**[0089]** *Experimental Results.* As shown in FIG. 2, apoA-IV KO mice were glucose intolerant relative to the WT mice. Specifically, FIG. 2 shows that plasma glucose levels in WT mice were lower than plasma glucose levels in apoA-IV KO mice for two hours following intraperitoneal injection with glucose. While not being bound by the theory, the implication of these studies was that apoA-IV is necessary for normal glucose homeostasis (at least in males). Moreover, as shown in FIG. 3, apoA-IV KO mice demonstrated an increased glucose intolerance when tested at sixteen months of age. Specifically, FIG. 3 shows that plasma glucose levels in apoA-IV KO mice tested at sixteen months of age were higher than the plasma glucose levels in apoA-IV KO tested at three months of age. While not being bound by the theory, the implication of these studies was that glucose tolerance of apoA-IV KO mice worsens with age.

**[0090]** *Experiment with Female Wild Type and ApoA-IV Knockout Mice*

. Female ApoA-IV wildtype and knockout mice were subjected to the same inraperitoneal glucose itolerance test as was used for the male apoA-IV KO and WT mice, as described earlier in this Example 1. The results are shown in Figure 11.

Female apoA-IV $^{-/-}$mice, when challenged intraperitoneally with glucose, have increased plasma glucose levels compared with female WT animals, but there is no statistical significant difference. On the other hand, the males have a significant difference between WT and KO animals.

**Example 2: Restoration of Glucose Tolerance in ApoA-IV Knockout Mice**

**[0091]** *Experimental Protocol.* Upon demonstrating that apoA-IV KO mice are glucose intolerant, a series of extensive studies were performed to determine whether administration of apoA-IV to apoA-IV KO mice would restore glucose tolerance to a normal level. Specifically, a series of studies were performed to determine not only the amount of apoA-IV to be administered but also the optimal time in which to administer apoA-IV prior to conducting glucose tolerance tests.

**[0092]** ApoA-IV male KO mice were injected intraperitoneally with doses of about 0.25, 0.5, 1, and 2 μg/g by weight of apoA-IV. ApoA-IV KO mice were also injected intraperitoneally with saline solution to serve as a control. Following injection with mouse apoA-IV or saline solution, glucose tolerance tests were conducted at three months of age as previously discussed. Specifically, glucose tolerance tests were conducted about two hours following injection with apoA-IV or saline solution. Experimental results indicated that the optimal time to restore glucose tolerance in apoA-IV KO mice was to administer apoA-IV about two hours prior to conducting glucose tolerance tests.

**[0093]** *Experimental Results.* As shown in FIG. 4, the administration of apoA-IV to apoA-IV KO mice restored glucose tolerance to a normal level. Specifically, FIG. 4 shows that plasma glucose levels in apoA-IV KO mice injected with apoA-IV were lower than plasma glucose levels in apoA-IV KO mice injected with saline solution. Moreover, as shown in FIG. 4, plasma glucose levels in apoA-IV KO mice injected with apoA-IV were the lowest in the apoA-IV KO mice injected with the highest dosage of apoA-IV; similarly, plasma glucose levels in apoA-IV KO mice injected with apoA-IV were the highest in the apoA-IV KO mice injected with the lowest dosage of apoA-IV. Accordingly, it was discovered that the degree of improvement of glucose tolerance was dependent on the dose of apoA-IV administered, with higher doses resulting in improved glucose tolerance.

**Example 3: Specificity of ApoA-IV in Restoring Glucose Tolerance in ApoA-IV Knockout Mice**

**[0094]** *Experimental Protocol.* In order to assess the specificity of apoA-IV, we administered apolipoprotein AI (hereinafter "apoA-I") to apoA-IV KO mice. ApoA-I is a protein made by the small intestinal epithelial cells which also produce apoA-IV.

ApoA-I shares many of the functions of apoA-IV. ApoA-IV KO mice were injected intraperitoneally with a dose of 1 μg/g by weight of apoA-I. ApoA-IV KO mice were also injected intraperitoneally with saline solution to serve as a control. Following injection with apoA-I or saline solution, glucose tolerance tests were conducted at three months of age as previously discussed. Specifically, glucose tolerance tests were conducted about two hours following injection with apoA-I or saline solution.

**[0095]** *Experimental Results.* As shown in FIG. 5, the administration of apoA-I to apoA-IV KO mice failed to restore or improve glucose tolerance.

**Example 4: Mechanism of Restoration of Glucose Tolerance in ApoA-IV Knockout Mice**

**[0096]** *Experimental Protocol.* In order to assess the mechanism by which ApoA-IV improves glucose tolerance in apoA-IV KO mice, we measured glucose-induced insulin secretion in apoA-IV KO mice. More specifically, we measured glucose-induced insulin secretion during glucose tolerance tests at three months of age as previously discussed. In this study, apoA-IV KO mice were injected intraperitoneally with a dose of about 1 μg/g by weight of mouse apoA-IV two hours prior to conducting the glucose tolerance tests. ApoA-IV KO mice were injected with saline solution about two hours prior to conducting glucose tolerance tests to serve as a control.

**[0097]** *Experimental Results.* As shown in FIG. 6, phase I insulin secretion was absent in apoA-IV KO mice injected with saline solution. However, as shown in FIG. 6, phase I insulin secretion was restored in apoA-IV KO mice when apoA-IV was injected intraperitoneally two hours prior to performing the glucose tolerance tests.

**Example 5: Efficacy of ApoA-IV in ApoA-IV Knockout and Wild-Type Mice on High Fat Diets**

**[0098]** *Experimental Protocol.* ApoA-IV KO and WT mice were chronically fed a high-fat semi-purified, nutritionally complete experimental diets (AIN-93M) purchased from Dyets (Bethlehem, PA) for 10 weeks. The high-fat diets contain about 20 g of fat (i.e. about 19 g of butter fat and 1 g of soybean oil to provide essential fatty acids) per 100 g of diet. The apoA-IV KO and WT mice were housed in individual tub cages with corncob bedding in a temperature- (about 22 ± 1° C) and light- (about 12 h light/12 dark) controlled vivarium. Glucose tolerance tests were performed at

three months of age as previously discussed. Prior to performing the glucose tolerance tests, apoA-IV KO mice and WT mice were fasted for five hours. In the glucose tolerance tests, the apoA-IV KO mice and WT mice were injected intraperitoneally with a dose of about 2 mg/g body weight of glucose.

**[0099]** *Experimental Results.* As shown in FIG. 7, apoA-IV KO mice displayed greater glucose intolerance relative to the WT mice. Specifically, FIG. 7 shows that plasma glucose levels in WT mice were lower than plasma glucose levels in apoA-IV KO mice for two hours following intraperitoneal injection with glucose.

**Example 6: Restoration of Glucose Tolerance in ApoA-IV Knockout and Wild-Type Mice on High Fat Diets**

**[00100]** *Experimental Protocol.* A series of studies were performed related to the administration of apoA-IV to apoA-IV KO and WT mice on high-fat diets for 14 weeks at three months of age (20% by weight of fat, 19% of butter fat and 1% of safflower oil). . Specifically, apoA-IV KO and WT mice were injected intraperitoneally with a dose of about 1 μg/g body weight of mouse apoA-IV. ApoA-IV KO and WT mice were also injected intraperitoneally with saline solution to serve as a control. Following injection with apoA-IV or saline solution, glucose tolerance tests were conducted. Specifically, glucose tolerance tests were conducted two hours following injection with apoA-IV or saline solution.

**[00101]** *Experimental Results.* As shown in FIG. 8, the administration of apoA-IV in apoA-IV KO mice significantly improved glucose tolerance. Specifically, FIG. 8 shows that plasma glucose levels in apoA-IV KO mice injected with apoA-IV were lower than plasma glucose levels in apoA-IV KO mice injected with saline solution. [the previous sentence is redundant since the next sentence describes the same thing. Although the data is not included herein, it was also discovered that the administration of apoA-IV in WT mice fed chronically a high fat diet also significantly improved glucose tolerance.

**Example 7: Restoration of Glucose Tolerance in Mice with Type 2 Diabetes**

**[00102]** *Experimental Protocol.* In order to confirm that apoA-IV is effective in promoting glucose tolerance in animals with type 2 diabetes, heterozygous KK Cg-A/J (hereinafter "Cg-A/J") mice were obtained from Jackson Laboratories (Bar Harbor, Maine). Cg-A/J mice develop hyperglycemia, hyperinsulinemia, obesity, and glucose

intolerance by eight weeks of age. The main cause of diabetes in these mice is insulin resistance produced by the polygenic interactions with the $A^y$ mutation, which encodes the agouti related protein and antagonist of the melanocortin-IV receptor. The Cg-A/J mice were fed chow diet. Additionally, there was a marked increase in blood glucose from ten to fourteen weeks of feeding the chow diet.

**[00103]** At fourteen weeks of age, the Cg-A/J mice were administered either mouse apoA-IV (about 1 μg/g body weight) or saline solution (to serve as a control) via intraperitoneal injection. Plasma glucose was then determined at about 0, 0.5, 1, 2, 3, 4, 5, 7, 11, and 24 hours.

**[00104]** *Experimental Results.* As shown in FIG. 9, apoA-IV has a marked effect in lowering the blood sugar level of the Cg-A/J mice relative to the saline control. While the Cg-A/J mice injected with saline solution maintained a steady plasma glucose level throughout the 24 hour period of study, the Cg-A/J mice injected with apoA-IV experienced a decrease in plasma glucose for over 10 hours, and, during most of this period, the plasma glucose level was comparable to the C57BL/6J animals we have been studying. From this study, we conclude that the administration of apoA-IV is effective in lowering the plasma glucose in Cg-A/J mice.

**Example 8: Level of Serum Amyloid P Component in ApoA-IV KO, ApoA-I KO, and WT Mice**

**[00105]** *Experimental Protocol.* A series of studies were performed in related to determining the level of serum amyloid A protein component (hereinafter "SAP") in apoA-IV KO, apoA-I KO, and WT mice on atherogenic diets. The apoA-IV KO, apoA-I KO, and WT mice were obtained from the University of Cincinnati. SAP is a serum form of amyloid P component (hereinafter "AP"), a 25 kDa pentameric protein first identified as the pentagonal constituent of *in vivo* pathological deposits called amyloid. SAP behaves like C-reactive protein in humans. Specifically, the level of plasma SAP in apoA-IV KO, apoA-I KO, and WT mice was determined in apoA-IV KO, apoA-I KO, and WT mice after 12 weeks on an atherogenic diet. The level of plasma SAP was determined via Western blot analysis.

**[00106]** *Experimental Results.* As shown in FIG. 10, the level of SAP in apoA-IV KO mice (corresponding to mouse numbers 1, 8, and 10) increased relative to the level

of SAP in apoA-I KO mice (corresponding to mouse numbers 28, 29, and 30) and WT mice (corresponding to mouse numbers 19, 20, and 25).

**[00107]** For the purposes of describing and defining the present disclosure it is noted that the terms "about" and "substantially" are utilized herein to represent the inherent degree of uncertainty that may be attributed to any quantitative comparison, value, measurement, or other representation. The terms "about" and "substantially" are also utilized herein to represent the degree by which a quantitative representation may vary from a stated reference without resulting in a change in the basic function of the subject matter at issue.

**[00108]** The above description and drawings are only to be considered illustrative of exemplary embodiments, which achieve the features and advantages of the present disclosure. Modification and substitutions the features and steps described can be made without departing from the intent and scope of the present disclosure. Accordingly, the disclosure is not to be considered as being limited by the foregoing description and drawings, but is only limited by the scope of the appended claims.

**Example 9: Human ApoA-IV Lowers Blood Glucose Levels in Wild-Type Mice Undergoing Intraperitoneal Glucose Tolerance Testing**

**[00109]** *Experimental Protocol.* Studies were performed to determine whether administration of human apoA-IV to wild type mice would affect blood glucose levels in mice undergoing glucose tolerance testing.

**[00110]** Three month old wild type mice were injected intraperitoneally with doses of about 1 μg/g by weight of human apoA-IV. As a control, another group of wild type mice was injected intraperitoneally with saline solution. Following injection with human apoA-IV or saline solution, glucose tolerance tests were conducted. Specifically, glucose tolerance tests were conducted about two hours following injection with apoA-IV or saline solution and after five hours of fasting. Tail blood was collected and measure by glucometer.

**[00111]** *Experimental Results.* As shown in Figure 12, human apoA-IV was effective in lowering blood glucose in wild type mice undergoing glucose tolerance testing.

**Example 10 Effect of Mouse ApoA-IV in Wild-Type Female Mice Undergoing Intraperitoneal Glucose Tolerance Testing**

**[00112]** *Experimental Protocol.* Studies were performed to determine whether administration of mouse apoA-IV to female wild type mice would affect blood glucose levels in mice undergoing glucose tolerance testing.

**[00113]** Three month old female wild type mice were injected intraperitoneally with doses of about 1 μg/g by weight of mouse apoA-IV. As a control, another group of female wild type mice were injected intraperitoneally with saline solution. Following injection with human apoA-IV or saline solution, glucose tolerance tests were conducted. Specifically, glucose tolerance tests were conducted about two hours following injection with apoA-IV or saline solution and after five hours of fasting. Tail blood was collected and measure by glucometer.

**[00114]** *Experimental Results.* As shown in Figure 13, mouse apoA-IV was effective in lowering blood glucose in wild type female mice undergoing glucose tolerance testing.

**Example 11 Human ApoA-IV Stimulates Insulin Release in Human Islets**

**[00115]** High purity human islets were provided by University of Virginia, Axon Cells. Islets were cultured in RPMI 1640, containing 10 % FBS and 11mM glucose at 37° C in a humidified atmosphere of 95 % air and 5% $CO_2$ for 48 hours. Four Groups of 50 IEQ islets were then pre-incubated at 37° C for 1 h in regular KRB (129mM NaCl, 4.8mM KCl, 2.5mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2mM $KH_2PO_4$, 5mM $NaHCO_3$, 10mM HEPES and 0.2% BSA)containing 3.0 mM glucose. Islets in the first two groups were then incubated in regular KRB containing 3.0mM glucose for an hour in the presence or absence of 10μg/ml human A-IV and were further incubated with 20 mM glucose for an additional hour in the presence or absence of 10 μg/ml human A-IV. Islets in the last two groups were incubated in 30mM KCl KRB (103.8 mM NaCl, 30mM KCl, 2.5mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2mM $KH_2PO_4$, 5mM $NaHCO_3$, 10mM HEPES and 0.2% BSA) plus 250μmol/l diazoxide containing 3.0mM glucose for an hour in the presence or absence of 10μg/ml human A-IV and were further incubated with 20mM glucose for an additional hour in the presence or

absence of 10μg/ml human A-IV. Media were collected at the end of each one-hour incubation. Insulin levels were measured by ELISA kit (Millipore).

**[00116]** As can be seen from FIG. 14, when the human islets were maximally depolarized by 30mM KCl plus 250μM diazoxide, 10 μg/ml hA-IV showed a significant stimulatory effect on insulin secretion.

**Example 12 Preparation of Unglycosylated ApoA-IV**

**[00117]** Human and mouse apoA-IV cDNA was contained in a pSP65 maintenance vector, and an *Afl* III restriction site was engineered immediately 5' of the coding sequence for the mature apoA-IV protein. The gene was excised from the maintenance vector and ligated into the pET30 expression vector. The construct was transfected into *E. Coli BL-21* (DE3) cells and grown in Luria- Bertani cultures supplemented with kanamycin (30 μg/ml) at 37℃. After induction of apoA-IV protein synthesis in the cells, the cells were harvested and sonicated. ApoA-IV protein from the lysate was purified by His-bind affinity column chromatography and dialysis. The resultant apoA-IV protein was diluted to a final concentration of 1.0 mg/ml in saline.

**Incorporation by Reference**

The contents of all references and patents cited herein are hereby incorporated by reference in their entirety.

**Equivalents**

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

What is claimed is:

1. A method for treating a hyperglycemic disorder in a subject in need thereof, the method comprising administering to the subject an effective amount of an apolipoprotein A-IV protein, or a biologically active analogue thereof having at least 90% identity to the apolipoprotein A-IV protein, such that the hyperglycemic disorder is treated.

2. The method of claim 1, wherein the hyperglycemic disorder is an insulin resistant disorder or an endocrine disorder associated with hyperglycemia.

3. The method of claim 2, wherein the insulin resistant disorder is selected from the group consisting of prediabetes, metabolic syndrome, polycystic ovary disease, type A syndrome, and gestational diabetes.

4. The method of claim 2, wherein the endocrine disorder associated with hyperglycemia is selected from the group consisting of Cushing's Disease, a glucagon secreting tumor, and acromegaly.

5. A method of lowering blood glucose level in a subject having a hyperglycemia disorder, the method comprising administering to the subject an effective amount of an apolipoprotein A-IV protein, or a biologically active analogue thereof having at least 90% identity to the apolipoprotein A-IV protein, such that the blood glucose level is lowered.

6. The method of claim 5, wherein the hyperglycemic disorder is an insulin resistant disorder or an endocrine disorder associated with hyperglycemia.

7. The method of claim 6, wherein the insulin resistant disorder is selected from the group consisting of prediabetes, metabolic syndrome, polycystic ovary disease, type A syndrome, and gestational diabetes.

8. The method of claim 6, wherein the endocrine disorder associated with hyperglycemia is selected from the group consisting of Cushing's Disease, a glucagon secreting tumor, and acromegaly.

9. The method of Claim 3 or 7, wherein the prediabetes is characterized either by impaired glucose tolerance or impaired fasting glucose.

10. A method of treating hyperglycemia or insulin resistance associated with polycystic ovary disease in a subject in need thereof, the method comprising administering to the subject an effective amount of an apolipoprotein A-IV protein, or a biologically active analogue thereof having at least 90% identity to the apolipoprotein A-IV protein, such that hyperglycemia or insulin resistance associated with polycystic ovary disease is treated.

11. The method of any one of Claims 1-10, wherein the biologically active analogue thereof has at least 95% identity to the apolipoprotein A-IV protein.

12. The method of any one of Claims 1-10, wherein the biologically active analogue thereof has at least 99% identity to the apolipoprotein A-IV protein.

13. The method of any one of Claims 1-12, wherein the subject is a human.

14. The method of any one of Claims 1-10, wherein the amino acid sequence of the apolipoprotein A-IV protein is

$X_1$EVSADQVATVMWDYFSQLSNNAKEAVEHLQKSELTQQLNALFQDK LGEVNTYAGDLQKKLVPFATELHERLAKDSEKLKEEIGKELEELRARLLPHAN EVSQKIGDNLRELQQRLEPYADQLRTQVNTQAEQLRRQLTPYAQRMERVLRE NADSLQASLRPHADX$_2$LKAKIDQNVEELKGRLTPYADEFKVKIDQTVEELRRS LAPYAQDTQEKLNHQLEGLTFQMKKNAEELKARISASAEELRQRLAPLAEDV RGNLRGNTEGLQKSLAELGGHLDQQVEEFRRRVEPYGENFNKALVQQMEQL RQKLGPHAGDVEGHLSFLEKDLRDKVNSFFSTFKEKESQDKX$_3$LSLPELEQQQ EQX$_4$QEQQQEQVQMLAPLES (SEQ ID NO. 4)

wherein, $X_1$ is G, A, V or absent;

$X_2$ is E or K;

$X_3$ is T or S; and

$X_4$ is Q or H,

Or a biologically active fragment thereof.

15. The method of any one of Claims 1-10, wherein the apolipoprotein A-IV protein is a full length human apolioprotein A-IV protein.

16. The method of Claim 15, wherein the amino acid of the apolipoprotein A-IV protein is

EVSADQVATVMWDYFSQLSNNAKEAVEHLQKSELTQQLNALFQDKLGEVNT
YAGDLQKKLVPFATELHERLAKDSEKLKEEIGKELEELRARLLPHANEVSQKI
GDNLRELQQRLEPYADQLRTQVNTQAEQLRRQLTPYAQRMERVLRENADSL
QASLRPHADELKAKIDQNVEELKGRLTPYADEFKVKIDQTVEELRRSLAPYAQ
DTQEKLNHQLEGLTFQMKKNAEELKARISASAEELRQRLAPLAEDVRGNLRG
NTEGLQKSLAELGGHLDQQVEEFRRRVEPYGENFNKALVQQMEQLRQKLGP
HAGDVEGHLSFLEKDLRDKVNSFFSTFKEKESQDKTLSLPELEQQQEQQQEQQ
QEQVQMLAPLES (SEQ ID NO. 1).

17. The method of any one of Claims 1-10, wherein the amino acid sequence of the apolipoprotein A-IV protein is

GEVSADQVATVMWDYFSQLSNNAKEAVEHLQKSELTQQLNALFQDK
LGEVNTYAGDLQKKLVPFATELHERLAKDSEKLKEEIGKELEELRARLLPHAN
EVSQKIGDNLRELQQRLEPYADQLRTQVNTQAEQLRRQLTPYAQRMERVLRE
NADSLQASLRPHADELKAKIDQNVEELKGRLTPYADEFKVKIDQTVEELRRSL
APYAQDTQEKLNHQLEGLTFQMKKNAEELKARISASAEELRQRLAPLAEDVR
GNLRGNTEGLQKSLAELGGHLDQQVEEFRRRVEPYGENFNKALVQQMEQLR
QKLGPHAGDVEGHLSFLEKDLRDKVNSFFSTFKEKESQDKTLSLPELEQQQEQ
QQEQQQEQVQMLAPLES (SEQ ID NO. 3).

18. The method of any one of Claims 1-10, wherein the apolipoprotein A-IV protein comprises an amino acid sequence as set forth in any one of SEQ ID NOs: 1, 3, 4, or 6-50, or a biologically active fragment thereof.

19. The method of any one of Claims 1-10, wherein the apolipoprotein A-IV protein comprises an amino acid sequence which is 95% identical to any one of the

sequence set forth in any one of SEQ ID NOs: 1, 3, 4, or 6-50, or a biologically active fragment thereof.

20. The method of any one of Claims 1-10, wherein the apolipoprotein A-IV protein comprises an amino acid sequence which is 96% identical to any one of the sequence set forth in any one of SEQ ID NOs: 1, 3, 4, or 6-50, or a biologically active fragment thereof.

21. The method of any one of Claims 1-10, wherein the apolipoprotein A-IV protein comprises an amino acid sequence which is 97% identical to any one of the sequence set forth in any one of SEQ ID NOs: 1, 3, 4, or 6-50, or a biologically active fragment thereof.

22. The method of any one of Claims 1-10, wherein the apolipoprotein A-IV protein comprises an amino acid sequence which is 98% identical to any one of the sequence set forth in any one of SEQ ID NOs: 1, 3, 4, or 6-50, or a biologically active fragment thereof.

23. The method of any one of Claims 1-10, wherein the apolipoprotein A-IV protein comprises an amino acid sequence which is 99% identical to any one of the sequence set forth in any one of SEQ ID NOs: 1, 3, 4, or 6-50, or a biologically active fragment thereof.

24. The method of any one of Claims 1-23, wherein the apolipoprotein A-IV, or biologically active analogue thereof, is non-glycosylated.

25. The method according to any one of Claims 1-24, wherein the apolipoprotein A-IV protein, or the biologically active analogue thereof, is administered systemically.

26. The method according to Claim 25, wherein the systemic administration of the apolipoprotein A-IV protein, or the biologically active analogue thereof, is selected

from the group consisting of oral, subcutaneous, intravenous, intramuscular, and intraperitoneal administration.

27. The method according to any one of Claims 1-26, wherein the apolipoprotein A-IV protein, or the biologically active analogue thereof, is administered in a dose of about 1 to about 10 μg/g.

28. The method according to any one of Claims 1-26, wherein the apolipoprotein A-IV protein, or the biologically active analogue thereof, is administered in a dose of about 0.25 to about 2 μg/g .

29. The method according to any one of Claims 1-26, wherein the apolipoprotein A-IV protein, or the biologically active analogue thereof, is administered in a dose of about 1 μg/g.

30. The method according to any one of Claims 1-26, wherein the apolipoprotein A-IV protein, or the biologically active analogue thereof, is administered as a fixed dose of about 1 to 1000 mg.

31. The method according to any one of Claims 1-30, wherein the apolipoprotein A-IV protein, or the biologically active analogue thereof, is administered once daily.

32. The method according to any one of Claim 1-30, wherein the apolipoprotein A-IV protein, or the biologically active analogue thereof, is administered about 2 times per day.

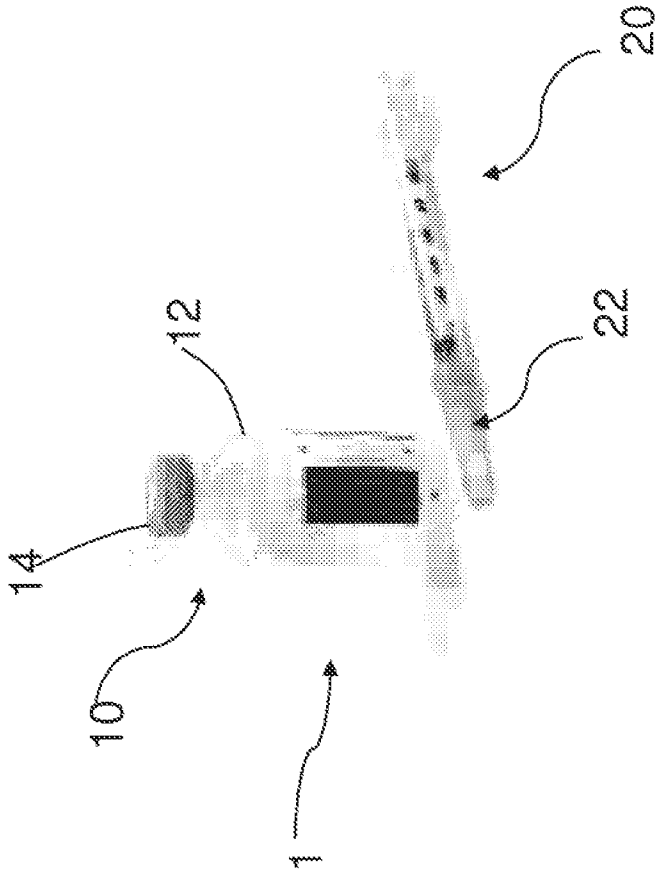



FIG. 1

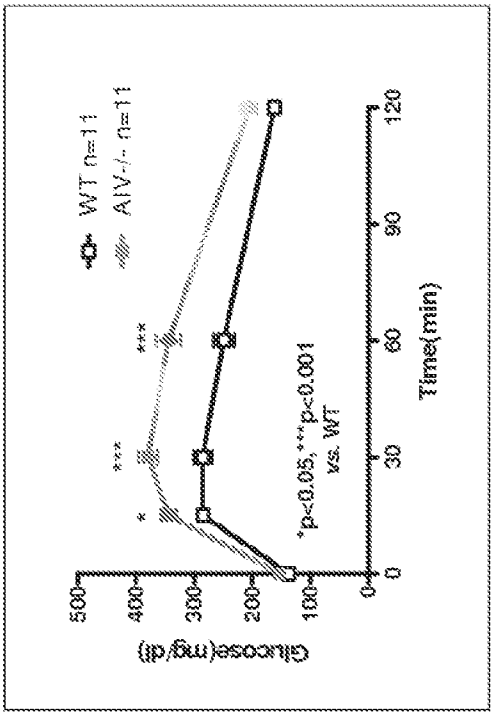

WT n=11
AIV-/- n=11
Glucose(mg/dl)
500
400
300
200
100
0
0
30
60
90
120
Time(min)
*p<0.05, ***p<0.001
vs. WT
*
***
***

FIG. 2

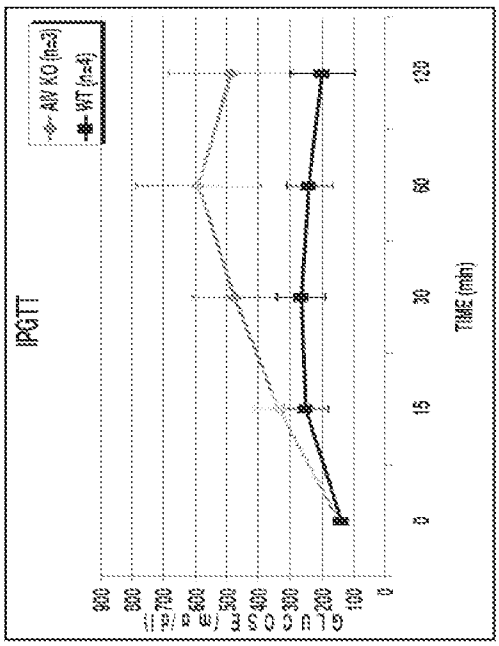



FIG. 3

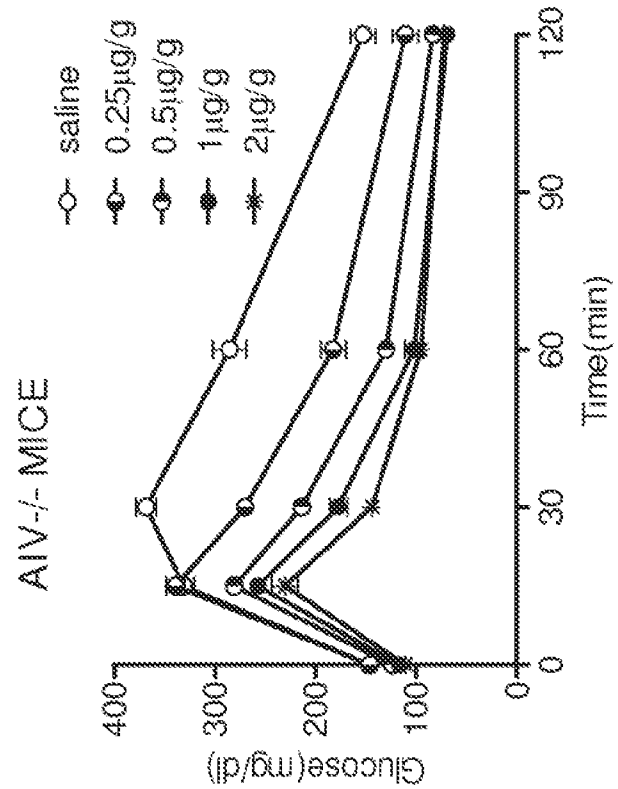

AIV-/- MICE
saline
0.25μg/g
0.5μg/g
1μg/g
2μg/g
Glucose(mg/dl)
400
300
200
100
0
0
30
60
90
120
Time(min)

FIG. 4

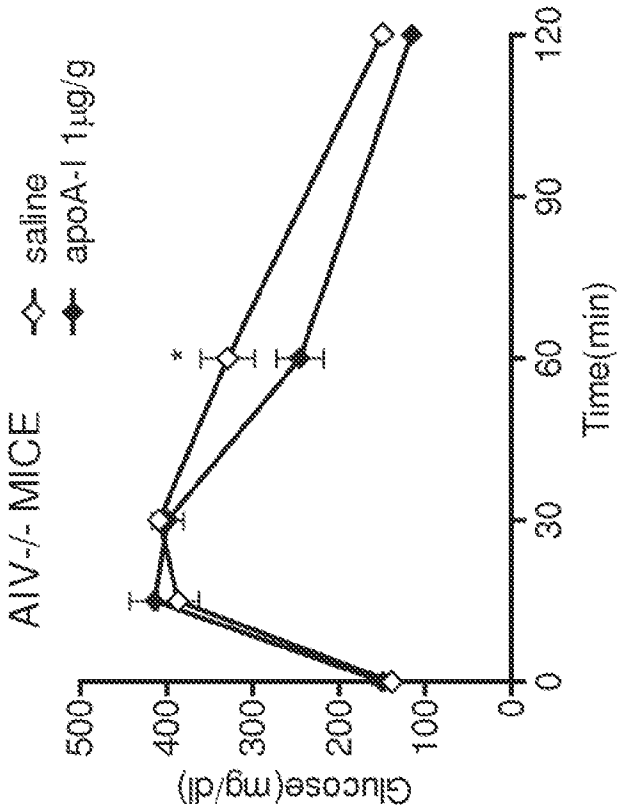

AIV-/- MICE
saline
apoA-I 1μg/g
Glucose(mg/dl)
500
400
300
200
100
0
0
30
60
90
120
Time(min)
*

FIG. 5

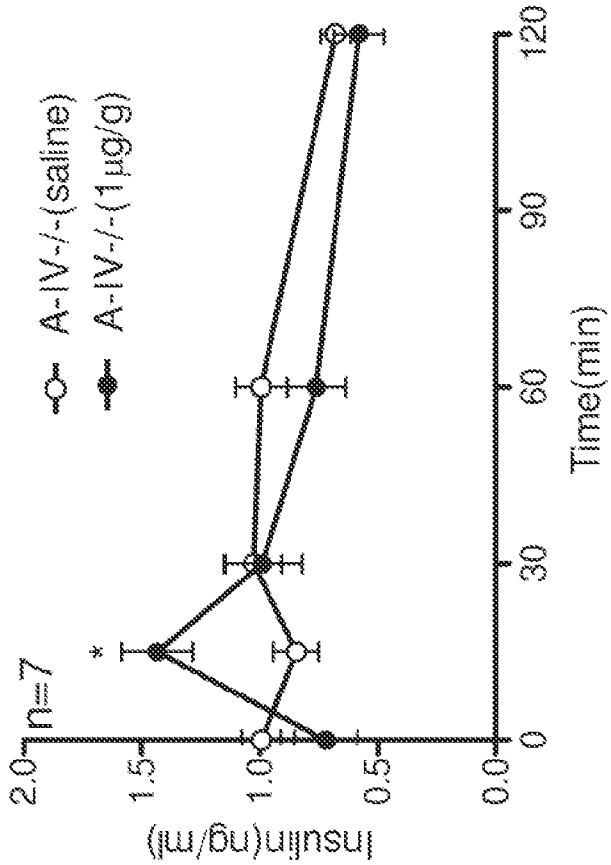

A-IV-/-(saline)
A-IV-/-(1 μg/g)
n=7
*
Insulin(ng/ml)
2.0
1.5
1.0
0.5
0.0
0
30
60
90
120
Time(min)

FIG. 6

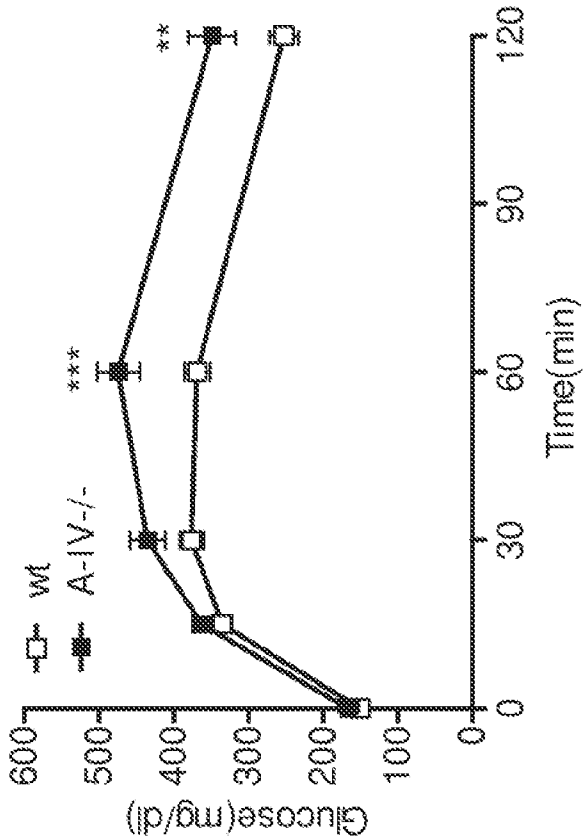

Glucose(mg/dl)
600
500
400
300
200
100
0
0
30
60
90
120
Time(min)
wt
A-1V-/-
***
**

FIG. 7

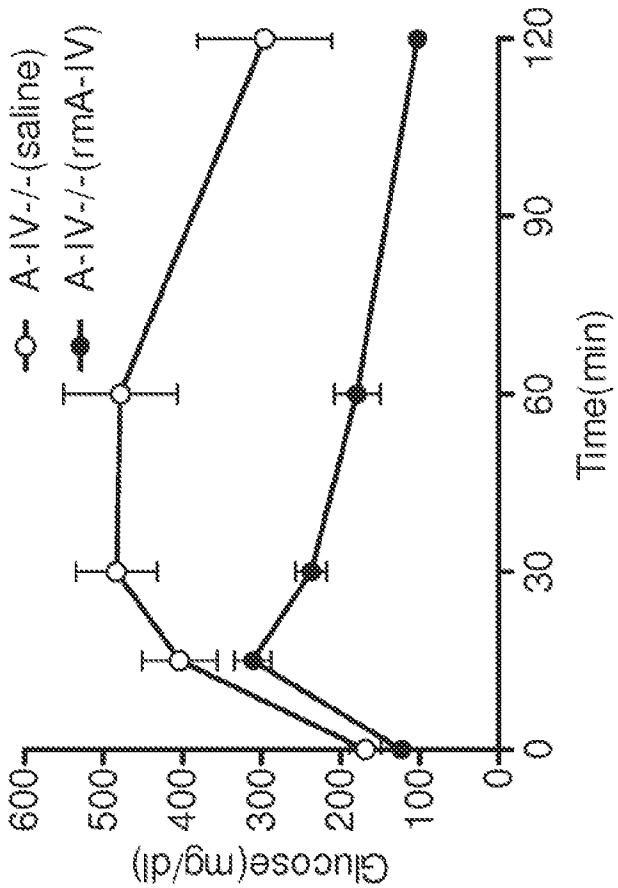

A-IV-/-(saline)
A-IV-/-(rmA-IV)
Glucose(mg/dl)
600
500
400
300
200
100
0
0
30
60
90
120
Time(min)

FIG. 8

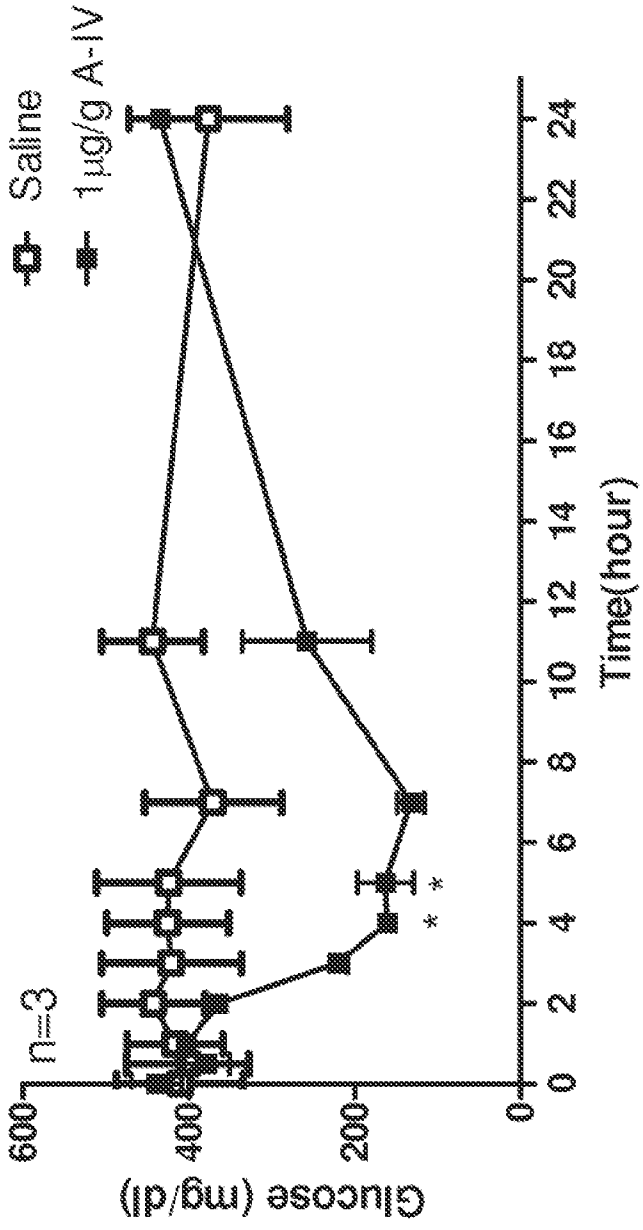

Saline
1μg/g A-IV
n=3
Glucose (mg/dl)
600
400
200
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Time(hour)
*
*

FIG. 9

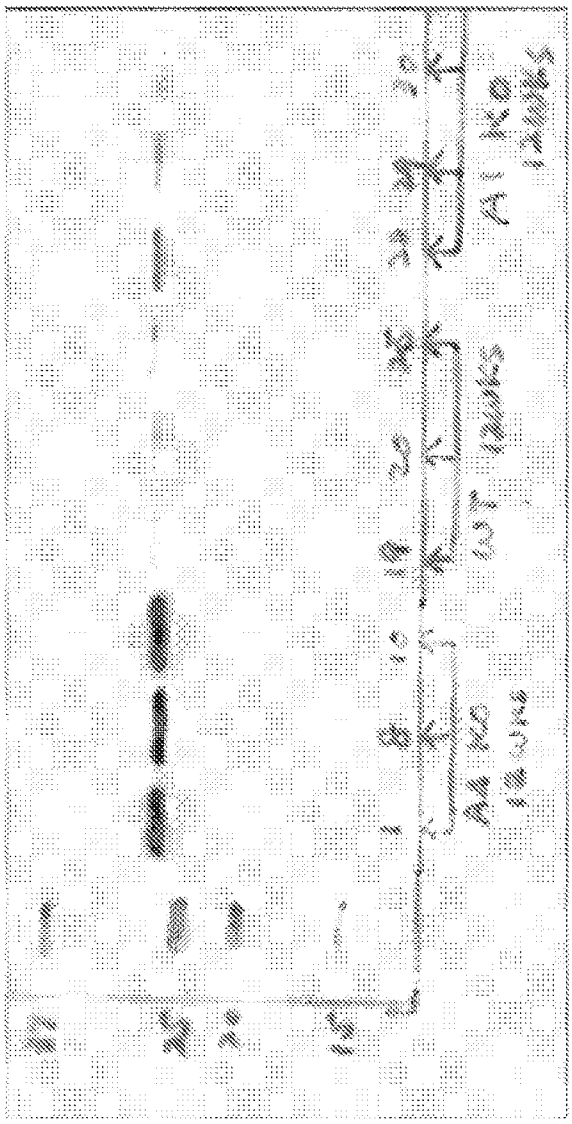


FIG. 10

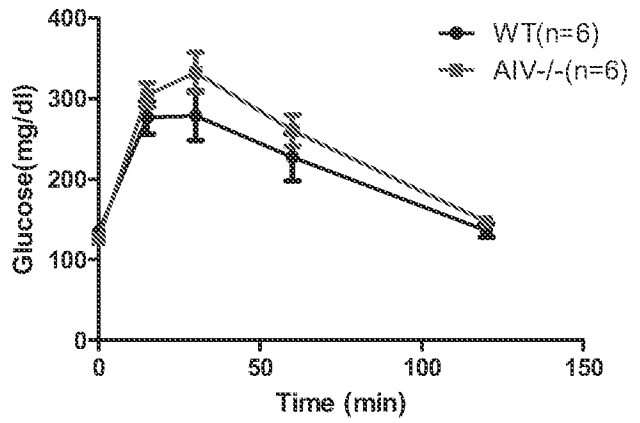

IPGTT in female WT and AIV-KO mice
WT(n=6)
AIV-/-(n=6)
Glucose(mg/dl)
400
300
200
100
0
0
50
100
150
Time (min)

FIG. 11

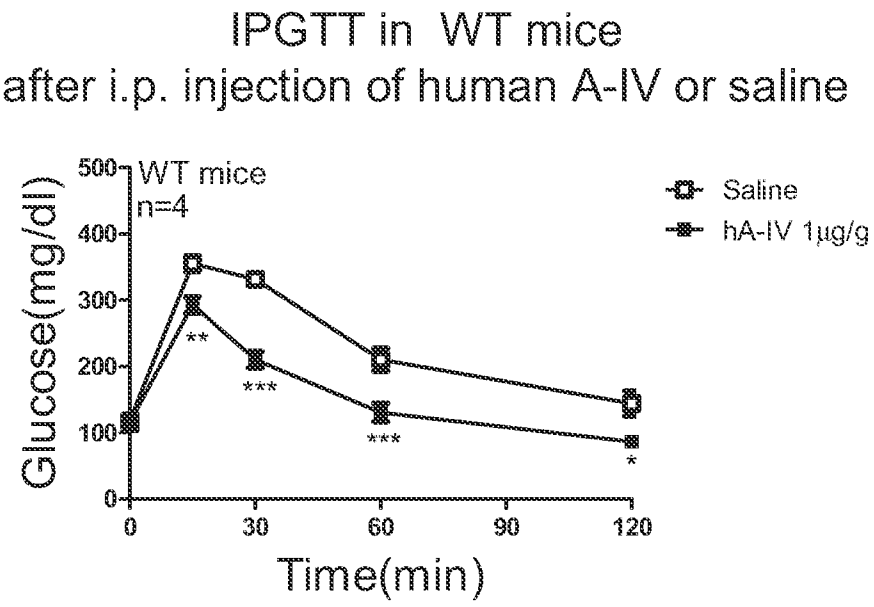

IPGTT in WT mice
after i.p. injection of human A-IV or saline
WT mice
n=4
Saline
hA-IV 1μg/g
Glucose(mg/dl)
500
400
300
200
100
0
**
***
***
*
0
30
60
90
120
Time(min)

FIG. 12

WT female mice

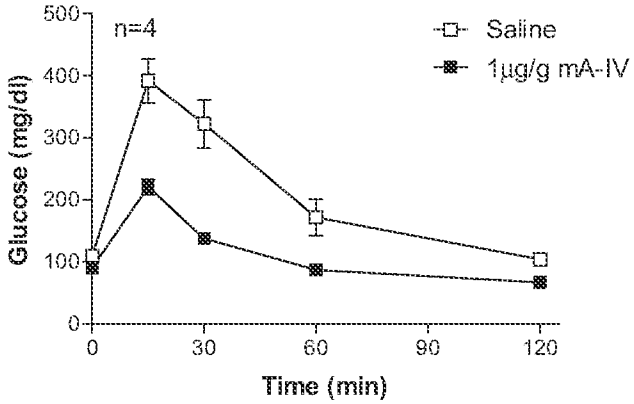
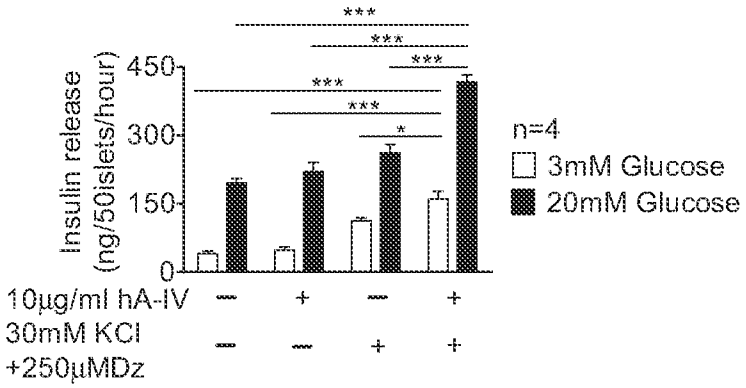



FIG. 13

# human islets treated with human A-IV




When the human islets were depolarized by 30mM KCl plus 250μM Dz, 10μg/ml hA-IV showed a significantly stimulatory effect on insulin secretion.

**FIG. 14**

EVSADQVATVMWDYFSQLSNNAKEAVEHLQKSELTQQLNALFQDKLGEVNTYAGDLQ
KKLVPFATELHERLAKDSEKLKEEIGKELEELRARLLPHANEVSQKIGDNLRELQQRLEP
YADQLRTQVNTQAEQLRRQLTPYAQRMERVLRENADSLQASLRPHADELKAKIDQNVE
ELKGRLTPYADEFKVKIDQTVEELRRSLAPYAQDTQEKLNHQLEGLTFQMKKNAEELK
ARISASAEELRQRLAPLAEDVRGNLRGNTEGLQKSLAELGGHLDQQVEEFRRRVEPYGE
NFNKALVQQMEQLRQKLGPHAGDVEGHLSFLEKDLRDKVNSFFSTFKEKESQDKTLSLP
ELEQQQEQQQEQQQEQVQMLAPLES

SEQ ID NO. 1

FIG. 15

EVTSDQVANVVWDYFTQLSNNAKEAVEQFQKTDVQQLSTLFASTYADGVHNKLVPFV
VQLSGHLAQETERVKEEIKKELEDLRDRKTQTFGENMQKLQEHLKPYAVDLQDQINTQ
TQEMKLQLTPYIQRMQTTIKENVDNLHTSMMPLATNLKDKFNRNMEELKGHLTPRANE
LKATIDQNLEDLRRSLAPLTVGVQEKLNHQMEGLAFQMKKNAEELQTKVSAKIDQLQK
NLAPLVEDVQSKVKGNTEGLQKSLEDLNRQLEQQVEEFRRTVEPMGEMFNKALVQQLE
QFRQQLGPNSGEVESHLSFLEKSLREKVNSFMSTLEKKGSPDQPQALPLPEQAQEQAQE
QAQEQVQPKPLES

SEQ ID NO. 2

FIG. 16

GEVSADQVATVMWDYFSQLSNNAKEAVEHLQKSELTQQLNALFQDKLGEVNTYAGDL
QKKLVPFATELHERLAKDSEKLKEEIGKELEELRARLLPHANEVSQKIGDNLRELQQRLE
PYADQLRTQVNTQAEQLRRQLTPYAQRMERVLRENADSLQASLRPHADELKAKIDQNV
EELKGRLTPYADEFKVKIDQTVEELRRSLAPYAQDTQEKLNHQLEGLTFQMKKNAEEL
KARISASAEELRQRLAPLAEDVRGNLRGNTEGLQKSLAELGGHLDQQVEEFRRRVEPYG
ENFNKALVQQMEQLRQKLGPHAGDVEGHLSFLEKDLRDKVNSFFSTFKEKESQDKTL*S*
LPELEQQQEQQ*Q*EQQQEQVQMLAPLES

SEQ ID NO. 3

FIG. 17

$X_1$EVSADQVATVMWDYFSQLSNNAKEAVEHLQKSELTQQLNALFQDKLGEVNTYAGD
LQKKLVPFATELHERLAKDSEKLKEEIGKELEELRARLLPHANEVSQKIGDNLRELQQRL
EPYADQLRTQVNTQAEQLRRQLTPYAQRMERVLRENADSLQASLRPHADX$_2$LKAKIDQ
NVEELKGRLTPYADEFKVKIDQTVEELRRSLAPYAQDTQEKLNHQLEGLTFQMKKNAE
ELKARISASAEELRQRLAPLAEDVRGNLRGNTEGLQKSLAELGGHLDQQVEEFRRRVEP
YGENFNKALVQQMEQLRQKLGPHAGDVEGHLSFLEKDLRDKVNSFFSTFKEKESQDKX
$_3$L*S*LPELEQQQEQX$_4$*Q*EQQQEQVQMLAPLES

$X_1$ is G, A, V or absent

$X_2$ is E or K

$X_3$ is T or S

$X_4$ is Q or H

SEQ ID NO. 4

FIG. 18

GTCAGTGCTGACCAGGTGGCCACAGTGATGTGGGACTACTTCAGCCAGCTGAGCAA
CAATGCCAAGGAGGCCGTGGAACATCTCCAGAAATCTGAACTCACCCAGCAACTCA
ATGCCCTCTTCCAGGACAAACTTGGAGAAGTGAACACTTACGCAGGTGACCTGCAG
AAGAAGCTGGTGCCCTTTGCCACCGAGCTGCATGAACGCCTGGCCAAGGACTCGGA
GAAACTGAAGGAGGAGATTGGGAAGGAGCTGGAGGAGCTGAGGGCCCGGCTGCTG
CCCCATGCCAATGAGGTGAGCCAGAAGATCGGGGACAACCTGCGAGAGCTTCAGCA
GCGCCTGGAGCCCTACGCGGACCAGCTGCGCACCCAGGTCAACACGCAGGCCGAGC
AGCTGCGGCGCCAGCTGACCCCCTACGCACAGCGCATGGAGAGAGTGCTGCGGGAG
AACGCCGACAGCCTGCAGGCCTCGCTGAGGCCCCACGCCGACGAGCTCAAGGCCAA
GATCGACCAGAACGTGGAGGAGCTCAAGGGACGCCTTACGCCCTACGCTGACGAAT
TCAAAGTCAAGATTGACCAGACCGTGGAGGAGCTGCGCCGCAGCCTGGCTCCCTAT
GCTCAGGACACGCAGGAGAAGCTCAACCACCAGCTTGAGGGCCTGACCTTCCAGAT
GAAGAAGAACGCCGAGGAGCTCAAGGCCAGGATCTCGGCCAGTGCCGAGGAGCTG
CGGCAGAGGCTGGCGCCCTTGGCCGAGGACGTGCGTGGCAACCTGAGGGGCAACAC
CGAGGGGCTGCAGAAGTCACTGGCAGAGCTGGGTGGGCACCTGGACCAGCAGGTGG
AGGAGTTCCGACGCCGGGTGGAGCCCTACGGGGAAAACTTCAACAAAGCCCTGGTG
CAGCAGATGGAACAGCTCAGGCAGAAACTGGGCCCCCATGCGGGGGACGTGGAAG
GCCACCTGAGCTTCCTGGAGAAGGACCTGAGGGACAAGGTCAACTCCTTCTTCAGC
ACCTTCAAGGAGAAAGAGAGCCAGGACAAGACTCTCTCCCTCCCTGAGCTCGAGCA
ACAGCAGGAACAGCAGCAGGAGCAGCAGCAGGAGCAGGTGCAGATGCTGGCCCCT
TTGGAGAGC

SEQ ID NO. 5

FIG. 19

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/066334

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   [ ] on paper

   [X] in electronic form

   b. (time)

   [X] in the international application as filed

   [ ] together with the international application in electronic form

   [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/066334

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/066334

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 C07K14/775
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WANG FEI ET AL: "Apolipoprotein A-IV improves glucose homeostasis by enhancing insulin secretion", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 109, no. 24, June 2012 (2012-06), pages 9641-9646, XP002696576, ISSN: 0027-8424 | 1-3,5-7, 10-13 |
| Y | the whole document | 4,8,9, 14-32 |
| | ----- | |
| X,P | WO 2012/100010 A1 (UNIV CINCINNATI [US]; TSO PATRICK [US]; DAVIDSON SEAN [US]; WOODS STEP) 26 July 2012 (2012-07-26) claims 1-24 | 1-32 |
| | ----- | |
| | -/-- | |

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 6 May 2013 | 24/05/2013 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Vollbach, Silke |

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Form PCT/ISA/210 (second sheet) (April 2005)

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/066334

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | DATABASE Biosis, [Online]<br><br>1 July 2002 (2002-07-01),<br>"Physiology of the small intestine in the glycemic control and the treatment of diabetes mellitus",<br>XP002676412,<br>retrieved from Biosis<br>Database accession no. PREV200200415101 | 1-3,5-7,<br>10-13 |
| Y | the whole document | 4,8,9,<br>14-32 |

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/066334

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 2012100010 A1 | 26-07-2012 | NONE | |

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/** 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-32

   Method of treating prediabetes

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2. claims: 1-32(partially)

   Method of treating metabolic syndrome

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3. claims: 1-32(partially)

   Method of treating polycysitc ovary disease

   ---

4. claims: 1-32(partially)

   Method of treating type A syndrome

   ---

5. claims: 1-32(partially)

   Method of treating gestational diabetes

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6. claims: 1-32(partially)

   Method of treating Cushing's Disease

   ---

7. claims: 1-32(partially)

   Method of treating glucagen secreting tumor

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8. claims: 1-32(partially)

   Method of treating acromegaly

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