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(54) Title: METHODS FOR TREATING METABOLIC DISORDERS AND OBESITY WITH GIP AND GLP-1 RECEPTOR-ACTIVE GLUCAGON-BASED PEPTIDES

(57) Abstract:

METHODS FOR TREATING METABOLIC DISORDERS AND OBESITY WITH GIP AND GLP-1 RECEPTOR-ACTIVE GLUCAGON-BASED PEPTIDES

PRIORITY CLAIM

[0001] This application claims priority to U.S. Provisional Application No. 61/500,229, filed June 23, 2011, and U.S. Provisional Application No. 61/426,338, filed December 22, 2010.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 285,688 byte ASCII (Text) file named "45708A_SeqListing.txt," created on December 22, 2011.

BACKGROUND OF THE INVENTION

Field of the Disclosure

[0003] This disclosure relates generally to methods of administering an extended half-life GLP-1/GIP coagonist peptide. More particularly, the disclosure relates to methods for reducing weight gain or inducing weight loss, and methods for treating hyperglycemia, reducing blood glucose levels, or normalizing blood glucose levels through the administration of an extended half-life GLP-1/GIP coagonist peptide.

Brief Description of Related Technology

[0004] Diabetes mellitus type II (i.e., type 2 diabetes) is a heterogeneous group of conditions that constitute approximately 90% of diabetes in the United States. Type 2 diabetes is caused by a combination of insulin resistance and diminished insulin secretion. Weight reduction in obese patients is associated with improvement of insulin resistance and amelioration of diabetes symptoms.

[0005] There are five widely recognized classes of oral anti-diabetic agents, sulfonylureas, biguanides, meglitinides, thiazolidinediones, and alpha-glucosidase inhibitors. Treatment of type 2 diabetes usually involves choosing one or more of these oral agents as initial therapy (see, e.g., Charpentier G. Diabetes Metab. Res. Rev. 18(Supp. 3):S70-S76 (2002)). Despite the use of multiple drugs, however, the all-over control of diabetes remains inadequate. Only 49.8% of persons with diabetes achieve the National Diabetes Association target HbA_{1c} of

less than 7%. Instead, 29.7% of persons with diabetes have an HbA_{1c} of greater than 8% (see, e.g., Resnick et al., Diabetes Care 29:531-537 (2006)).

[0006] The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic peptide (GIP), are naturally-occurring peptide hormones. Both GLP-1 and GIP stimulate insulin synthesis and secretion in a glucose-dependent manner and do not produce hypoglycemia (see, e.g., Nauck et al., J. Clin. Endocrinol. Metab. 76:912-917 (1993) and Irwin et al., Regul. Pept. 153:70-76 (2009)).

[0007] GLP-1 has been shown to be effective as adjunctive therapy for diabetes. Although GLP-1 therapy is associated with weight loss, it is also associated with nausea, which occurs in over 20% of patients that are treated with GLP-1 analogs.

SUMMARY

[0008] One aspect of the invention provides methods for reducing weight gain or inducing weight loss in an adult human in need thereof (e.g., the adult human has excess body weight, diabetes mellitus type II, insulin resistance, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), or metabolic syndrome) by administering to the adult human an extended half-life GLP-1/GIP coagonist peptide at a total weekly dosage of about 1 mg to about 40 mg, or about 4 mg to about 30 mg, or about 4 to about 20 mg, or about 10 to about 20 mg, or about 2 to about 10 mg.

[0009] Another aspect of the invention provides methods for treating hyperglycemia, reducing blood glucose levels, or normalizing blood glucose levels in an adult human in need thereof (e.g., the adult human has diabetes mellitus type I, diabetes mellitus type II, or gestational diabetes) by administering to the adult human an extended half-life GLP-1/GIP coagonist peptide at a total weekly dosage of about 1 mg to about 40 mg, or about 4 mg to about 30 mg, or about 4 to about 20 mg, or about 10 to about 20 mg, or about 2 to about 10 mg.

[0010] The extended half-life GLP-1/GIP coagonist peptide typically has a GIP percentage potency of at least 1%, or a GLP-1 percentage potency of at least 1%. In some embodiments, the extended half-life GLP-1/GIP coagonist peptide exhibits a GLP-1 percentage potency within about 10-fold of the GIP percentage potency. Alternatively, in some embodiments, the extended half-life GLP-1/GIP coagonist peptide exhibits an EC_{50} at the GLP-1 receptor within about 10-fold of the EC_{50} at the GIP receptor. In any of these embodiments, for example, the extended half-life GLP-1/GIP coagonist peptide exhibits a half-life of about 4 to

about 10 days, or about 4 to about 7 days. In any of these embodiments, the administration of the extended half-life GLP-1/GIP coagonist peptide can result in an increase in insulin level, a decrease in glucose level, an increase in C-peptide level, a decrease in HbA_{1c} level, or a decrease in fructosamine level, or any combination thereof.

[0011] The extended half-life GLP-1/GIP coagonist peptide can be administered alone or in combination with a second therapeutic agent (e.g., an anti-diabetic agent and/or an anti-obesity agent), and at any frequency that results in the recited total weekly dose (e.g., administered once per week, twice per week).

[0012] In some embodiments, the extended half-life GLP-1/GIP coagonist peptide includes one or more hydrophilic polymer moieties that have a collective molecular weight of about 30,000 Daltons to about 60,000 Daltons (e.g., about 40,000 Daltons). Nonlimiting examples of the extended half-life GLP-1/GIP coagonist peptide include any of SEQ ID NOs: 5-94, 99-169, 173-413, an amino acid sequence that has up to 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid modifications relative to SEQ ID NOs: 5-94, 99-169, 173-413 that retains, e.g., at least 10% of the GIP or GLP-1 activity, or a pegylated derivative of SEQ ID NOs: 5-94, 99-169, 173-413. For example, the extended half-life GLP-1/GIP coagonist peptide can include any of SEQ ID NOs: 75, 99-103, 140, 153, 166, and 261.

[0013] The extended half-life GLP-1/GIP coagonist peptide can be administered in a sterile pharmaceutical composition, such as through subcutaneous, intravenous or intramuscular injection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1 depicts the results of a Phase I, randomized, placebo-controlled, positive-controlled two-part study performed in healthy male and female subjects to evaluate the effect of a range of doses on beta cell response to a glucose load. Human subjects were administered placebo or 4 mg, 8 mg, or 16 mg of extended half-life GLP-1/GIP coagonist peptide and insulin secretion rates were measured. A dose dependent, dose proportional, increase in insulin secretion rate (ISR) was observed after treatment with the extended half-life GLP-1/GIP coagonist peptide in comparison to placebo.

[0015] Figure 2 depicts the results of a Phase I, randomized, placebo-controlled, positive-controlled two-part study performed in healthy male and female subjects to evaluate the effect of a range of doses on beta cell response to a glucose load. Human subjects were administered placebo or 4 mg (Figure 2A) or 8 mg (Figure 2B) of extended half-life GLP-

1/GIP coagonist peptide and a glucose infusion at a rate between 0 and 12 mg/kg/min and plasma glucose levels were measured.

[0016] Figure 3 illustrates the results of an experiment comparing C-peptide levels in subjects administered 4 mg, 8 mg, or 16 mg of extended half-life GLP-1/GIP coagonist peptide and a glucose infusion between 0 and 12 mg/kg/min.

[0017] Figure 4 compares the appearance of plasma acetaminophen over a 4 hour time period in subjects administered BYETTA, or 4 mg, 8 mg, or 16 mg of extended half-life GLP-1/GIP coagonist peptide.

DETAILED DESCRIPTION

[0018] Extended half-life GLP-1/GIP coagonist peptides, as described in PCT Patent Application Publication No. WO 2010/011439, incorporated herein by reference in its entirety, were found to be useful for treating hyperglycemia, including diabetes, as well as for reducing weight gain or inducing weight loss. These peptides provide greater weight reduction effects compared to GLP-1 alone. The extended half-life GLP-1/GIP coagonist peptides have a prolonged duration of action, e.g. due to a prolonged half-life in circulation (e.g., about 5 to 6 days), which allows for dosing at longer intervals (e.g., weekly dosing, or even biweekly dosing).

[0019] Without intending to be bound by any particular theory, the extended half-life GLP-1/GIP coagonist peptides demonstrate similar or superior glucose-lowering and weight-reduction effects compared to current GLP-1 therapies, but with a reduced incidence of gastrointestinal tolerability problems such as nausea or vomiting.

[0020] The extended half-life GLP-1 GIP coagonist peptides have exhibited glucose normalization and weight lowering properties in a variety of animal models. In lean mice and rats, such peptides provide improved tolerance to a glucose challenge. In diet-induced obese mice, such peptides reduced both food intake and body weight gain.

[0021] In Phase I, randomized, placebo-controlled, sequential, single-ascending dose studies of the extended half-life GLP-1/GIP coagonist peptide (see Example 1), single doses of the extended half-life GLP-1/GIP coagonist peptide were administered to healthy male volunteers, starting at 0.1 mg and ranging up to 32 mg, administered by subcutaneous (SC) injection. Each of the doses was well tolerated and not associated with adverse events, clinically important laboratory findings, or ECG findings.

[0022] In a Phase I, randomized, placebo-controlled, positive-controlled, two-part study (see Example 2), healthy male and female subjects in Part 1 of the study received a subcutaneous (SC) injection of placebo and an SC injection of BYETTA® (Amylin Pharmaceuticals) as a positive control. Subjects in Part 2 of the study received an SC injection of placebo and an SC injection of the extended half-life GLP-1/GIP coagonist peptide. Doses of 4 mg, 8 mg, and 16 mg of the extended half-life GLP-1/GIP coagonist peptide increased insulin secretion in a dose-dependent manner during a graded glucose infusion without an effect on gastric emptying.

Definitions

[0023] As used herein, the term "peptide" encompasses a sequence of 3 or more amino acids and typically less than 100, or less than 50 amino acids, wherein the amino acids are naturally occurring or non-naturally occurring amino acids. Non-naturally occurring amino acids refer to amino acids that do not naturally occur in vivo but which, nevertheless, can be incorporated into the peptide structures described herein.

[0024] As used herein, the term “GLP-1/GIP coagonist peptide” refers to a peptide that exhibits activity at both the GLP-1 and GIP receptors. Such a peptide may optionally have no detectable or relatively low glucagon activity (e.g., at least 10-fold lower percentage potency at the glucagon receptor compared to the GLP-1 or GIP receptors, or at least 100-fold lower potency).

[0025] As used herein, the term “extended half-life” refers to a peptide having a prolonged duration of action and/or a decreased clearance rate. For example, a prolonged duration of action can be due to resistance to dipeptidase IV cleavage and/or conjugation to a heterologous moiety that prolongs half-life in circulation (e.g., polyethylene glycol (PEG), albumin, or an Fc region or fragment thereof).

[0026] As used herein the general term "polyethylene glycol” or "PEG," refers to mixtures of condensation polymers of ethylene oxide and water, in a branched or straight chain, represented by the general formula $H(OCH_2CH_2)_nOH$, wherein n is at least 9.

[0027] As used herein the term "pegylated" and like terms refers to a compound that has been modified from its native state by linking one or more polyethylene glycol moieties to the compound. A "pegylated extended half-life GLP-1/GIP coagonist peptide" is an extended half-life GLP-1/GIP coagonist peptide that has one or more PEG chains covalently bound to

the extended half-life GLP-1/GIP coagonist peptide, e.g. at a collective molecular weight of PEG of about 30,000 Daltons to about 60,000 Daltons.

[0028] As used herein an amino acid "modification" refers to a substitution, addition or deletion of an amino acid, and includes substitution with or addition of any of the 20 amino acids commonly found in human proteins, as well as atypical or non-naturally occurring amino acids. Throughout the application, all references to a particular amino acid position by number (e.g. position 28) refer to the amino acid at that position in native glucagon (SEQ ID NO:1) or the corresponding amino acid position in any analogs thereof. For example, a reference herein to "position 28" would mean the corresponding position 27 for a peptide in which the first amino acid of SEQ ID NO: 1 has been deleted. Similarly, a reference herein to "position 28" would mean the corresponding position 29 for a peptide in which one amino acid has been added before the N-terminus of SEQ ID NO: 1. Commercial sources of atypical amino acids include Sigma-Aldrich (Milwaukee, WI), ChemPep Inc. (Miami, FL), and Genzyme Pharmaceuticals (Cambridge, MA). Atypical amino acids may be purchased from commercial suppliers, synthesized de novo, or chemically modified or derivatized from other amino acids.

[0029] As used herein an amino acid "substitution" refers to the replacement of one amino acid residue by a different amino acid residue.

[0030] As used herein, the term "conservative amino acid substitution" is defined herein as the replacement of one amino acid with another amino acid having similar properties, e.g., size, charge, hydrophobicity, hydrophilicity, and/or aromaticity, and includes exchanges within one of the following five groups:

I. Small aliphatic, nonpolar or slightly polar residues:

Ala, Ser, Thr, Pro, Gly;

II. Polar, negatively charged residues and their amides and esters:

Asp, Asn, Glu, Gln, cysteic acid and homocysteic acid;

III. Polar, positively charged residues:

His, Arg, Lys; Ornithine (Orn)

IV. Large, aliphatic, nonpolar residues:

Met, Leu, Ile, Val, Cys, Norleucine (Nle), homocysteine

V. Large, aromatic residues:

Phe, Tyr, Trp, acetyl phenylalanine

[0031] As used herein the term "native glucagon" refers to a peptide consisting of the amino acid sequence of SEQ ID NO: 1, the term "native GIP" refers to a peptide consisting of the amino acid sequence of SEQ ID NO: 4, and the term "native GLP-1" is a generic term that designates GLP-1(7-36) amide (consisting of the amino acid sequence of SEQ ID NO: 3), GLP-1(7-37) acid (consisting of the amino acid sequence of SEQ ID NO: 2) or a mixture of those two compounds. As used herein, a general reference to "glucagon" or "GIP" or "GLP-1" in the absence of any further designation is intended to mean native glucagon or native GIP or native GLP-1, respectively.

[0032] As used herein a general reference to a peptide is intended to encompass peptides that have modified amino and carboxy termini. For example, an amino acid chain comprising an amide group in place of the terminal carboxylic acid is intended to be encompassed by an amino acid sequence designating the standard amino acids.

[0033] As used herein, the term "selectivity" of a molecule for a first receptor relative to a second receptor refers to the following ratio: EC_{50} of the molecule at the second receptor divided by the EC_{50} of the molecule at the first receptor. For example, a molecule that has an EC_{50} of 1 nM at a first receptor and an EC_{50} of 100 nM at a second receptor has 100-fold selectivity for the first receptor relative to the second receptor.

[0034] As used herein,GLP-1 percentage potency is the EC_{50} of GLP-1 divided by the EC_{50} of the extended half-life GLP-1/GIP coagonist peptide, times 100%.

[0035] As used herein GIP percentage potency is the EC_{50} of GIP divided by the EC_{50} of the extended half-life GLP-1/GIP coagonist peptide, times 100%.

[0036] As used herein glucagon percentage potency is the EC_{50} of glucagon divided by the EC_{50} of the extended half-life GLP-1/GIP coagonist peptide, times 100%.

[0037] As used herein, the term "treating" includes prophylaxis of the specific disorder or condition, or alleviation of the symptoms associated with a specific disorder or condition and/or preventing or eliminating said symptoms. For example, as used herein the term "treating diabetes" will refer in general to altering glucose blood levels in the direction of normal levels and may include increasing or decreasing blood glucose levels depending on a given situation.

[0038] The term, "parenteral" means not through the alimentary canal but by some other route such as subcutaneous, intramuscular, intraspinal, or intravenous.

[0039] As used herein, the term “pharmaceutically acceptable carrier” includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

[0040] As used herein the term "pharmaceutically acceptable salt" refers to salts of compounds that retain the biological activity of the parent compound, and which are not biologically or otherwise undesirable. Many of the compounds disclosed herein are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

[0041] Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines.

[0042] Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid,

cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic acid, salicylic acid, and the like.

GLP-1/GIP Coagonist Peptide Embodiments

[0043] The invention described herein relates to methods of administering an extended half-life GLP-1/GIP coagonist peptide to an adult human in need thereof. GLP-1/GIP coagonist peptides and extended half-life versions of such peptides are exhaustively described in WO 2010/011439, hereby incorporated by reference in its entirety.

[0044] In some embodiments, the extended half-life GLP-1/GIP coagonist peptide is or comprises any of (a) SEQ ID NOs: 5-94, 99-169, and 173-262, which correspond to SEQ ID NOs: 5-94, 99-169, and 173-262 in WO 2010/011439, (b) any of SEQ ID NOs: 263-277, which correspond to SEQ ID NOs 19-28 and 33-36 in U.S. Provisional Patent Application Serial No. 61/29,8812, incorporated herein by reference, or (c) any of SEQ ID NOs: 178-413, (d) any analogs of any of the foregoing peptides having one or more amino acid modifications, including up to 2, 3, 4, 5, 6, 7, 8, 9 or 10 further modifications (including, e.g., conservative substitutions), or (e) pegylated derivatives of any of the foregoing peptides, provided the analog retains the desired activity (e.g. at least 1%, 10%, 20%, 30%, 40% or 50%) of the parent peptide at the GLP-1 and GIP receptors. WO 2010/011439 also provides some guidance regarding amino acids which may be altered; in addition, the invention contemplates peptides with additions, deletions, non-conservative substitutions and/or non-conservative substitutions at one or more of positions 1, 2, 5, 7, 8, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 24, 27, 28 or 29. The peptides and analogs may comprise additional heterologous moieties including one or more additional amino acid sequences (e.g. as a fusion protein) or one or more PEG chains to result in pegylated derivatives of any of SEQ ID NOs: 1-413. In some exemplary embodiments, the extended half-life GLP-1/GIP coagonist peptide is any of SEQ ID NOs: 75, 99-103, 140, 153, 166, and 261. It is understood that analogs are prepared by de novo synthesis rather than by preparing a parent peptide that is subsequently modified, although in some instances, e.g. pegylation, the parent peptide may be prepared first then pegylated.

[0045] In some embodiments, the EC_{50} of the extended half-life GLP-1/GIP coagonist peptide at the GIP receptor is within about 20-fold, about 15-fold, or preferably within about 10-fold, 9-fold, 8-fold, 7-fold, 6-fold, 5-fold, 4-fold, 3-fold, or about 2-fold different (higher or lower) from its EC_{50} at the GLP-1 receptor. In some embodiments, the extended half-life

GLP-1/GIP coagonist peptide exhibits a GLP-1 percentage potency within about 20-fold, about 15-fold, or preferably within about 10-fold, 9-fold, 8-fold, 7-fold, 6-fold, 5-fold, 4-fold, 3-fold, or about 2-fold different (higher or lower) from the GIP percentage potency. In exemplary embodiments (e.g., in any of the embodiments herein), the extended half-life GLP-1/GIP coagonist peptide exhibits a GIP percentage potency of at least about 1%, at least about 5%, or at least about 10%. In exemplary embodiments (e.g., in any of the embodiments herein), the extended half-life GLP-1/GIP coagonist peptide exhibits a GLP-1 percentage potency of at least about 1%. In some embodiments, the extended half-life GLP-1/GIP coagonist peptide has about 10% or less of the activity of native glucagon at the glucagon receptor, e.g. about 1-10%, or about 0.1-10%, or greater than about 0.1% but less than about 10%. In any of the embodiments herein, for example, the selectivity of the extended half-life GLP-1/GIP coagonist peptide for the GLP-1 receptor, compared to the GIP receptor, is less than 100-fold, or the ratio of the GLP-1 percentage potency divided by the GIP percentage potency is less than 100, less than about 20 , 15, 10, 9, 8, 7, 6 or 5.

[0046] The extended half-life GLP-1/GIP coagonist peptides have a prolonged duration of action, e.g. due to a prolonged half-life in circulation (e.g., about 5 to 6 days), which allows for dosing at longer intervals (e.g., weekly dosing, biweekly dosing). In some embodiments, the extended half-life GLP-1/GIP coagonist peptides used according to the invention have a half-life ranging from about 3 to about 15 days, e.g. about 3-5, 3-6, 3-7, 3-8, 3-9, 3-10, 3-11, 3-12, 3-13, 3-14, 3-15, 4-5, 4-6, 4-7, 4-8, 4-9, 4-10, 4-11, 4-12, 4-13, 4-14, 4-15, 5-6, 5-7, 5-8, 5-9, 5-10, 5-11, 5-12, 5-12, 5-14, 5-15, 6-7, 6-8, 6-9, 6-10, 6-11, 6-12, 6-13, 6-14, or 6-15 days. In some embodiments, the extended half-life GLP-1/GIP coagonist peptide undergoes complete clearance within about 10 days to about 30 days, for example about 20 days.

[0047] For example, a prolonged duration of action can be due to resistance or reduced susceptibility to dipeptidase IV cleavage and/or conjugation to a heterologous moiety that prolongs half-life in circulation. Resistance or reduced susceptibility to dipeptidase IV cleavage is provided, e.g., by amino acid modifications (to a "DPP-IV protective amino acid") at positions 1 and/or 2. Examples of suitable substitutions include: at position 1, D-histidine, alpha, alpha-dimethyl imidiazole acetic acid (DMIA), N-methyl histidine, alpha-methyl histidine, imidazole acetic acid, desaminohistidine, hydroxyl-histidine, acetyl-histidine or homo-histidine; or at position 2, D-serine, D-alanine, valine, glycine, N-methyl serine, or alpha-aminoisobutyric acid (AIB).

[0048] Examples of heterologous moieties that prolong duration of action include, but are not limited to, acyl or alkyl groups; dipeptide prodrug moieties (linked via, e.g., amide or ester bonds) or other prodrug moieties; polyethylene glycol (PEG) or other hydrophilic polymers; albumin or other plasma proteins; an Fc region of an immunoglobulin or a fragment thereof; rPEG. Such components are known in the art. For example, acylation or alkylation, hydrophilic polymers, albumin or other plasma proteins, and Fc regions of immunoglobulins or fragments thereof are described in Int'l Pub. No. WO 2010/011439, hereby incorporated by reference in its entirety. Ester prodrug moieties are described in Int'l Pub. No. WO2009/099763, hereby incorporated by reference in its entirety, and amide prodrug moieties are described in Int'l Pub. No. WO 2010/071807, hereby incorporated by reference in its entirety. Recombinant PEG, or rPEG, is described in Int'l Pub. No. WO2009/023270.

[0049] In some embodiments, the extended half-life GLP-1/GIP coagonist peptide comprises an amino acid that is covalently linked to a hydrophilic polymer at any of amino acid positions 16, 17, 20, 21, 24, or 29, after position 29 at an added amino acid (e.g., position 30) within a C-terminal extension (e.g., 30, 31, 32, 33, 34, 35, 36, 37, 38, 39), or at the C-terminal amino acid (e.g., 40, 41, 42, or higher), as described in WO 2010/011439. In exemplary embodiments, this hydrophilic polymer is covalently linked to a Lys, Cys, Orn, homocysteine, or acetyl-phenylalanine residue at any of these positions. Exemplary hydrophilic polymers include one or more polyethylene glycol (PEG) chains, for example, of a collective molecular weight of about or about 20,000 Daltons to about 40,000 Daltons, as described in WO 2010/011439. In some embodiments, the polyethylene glycol polymer has a molecular weight of about 30,000 Daltons to about 60,000 Daltons, or about 30,000 Daltons to about 50,000 Daltons (e.g., 40, 000 Daltons).

[0050] Heterologous moieties

[0051] As used herein, the term "heterologous moiety" is synonymous with the term "conjugate moiety" and refers to any molecule (chemical or biochemical, naturally-occurring or non-coded) which is different from the peptide to which it is attached. In some embodiments the heterologous moiety is another peptide or protein. In some embodiments, the heterologous moiety is a plasma protein, e.g., albumin, transferrin, fibrinogen and globulins. In some embodiments the plasma protein is albumin or transferrin.

[0052] In some embodiments, the heterologous moiety is an Fc portion, or fragment or modified analog thereof, of an immunoglobulins (Ig), e.g., IgG, IgA, IgE, IgD or IgM, preferably a fragment that retains the FcRn binding site. The Fc region is a C-terminal region of an Ig heavy chain, which is responsible for binding to Fc receptors (FcRn) that carry out activities such as recycling immunoglobulins and returning them to circulation (which results in prolonged half-life). For example, according to some definitions the human IgG heavy chain Fc region stretches from Cys226 to the C-terminus of the heavy chain. The region of the Fc portion of IgG that binds to the FcRn receptor has been described based on X-ray crystallography (Burmeister et al. 1994, Nature 372:379). The major contact area of the Fc with the FcRn is near the junction of the CH2 and CH3 domains and include amino acid residues 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. Some conjugate moieties may or may not include binding site(s) for FcγR, which are responsible for ADCC and CDC, or may include modifications designed to reduce ADCC or CDC.

[0053] In some embodiments, the heterologous moiety is a polymer. In some embodiments, the polymer is selected from the group consisting of: polyamides, polycarbonates, polyalkylenes and derivatives thereof including, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polymers of acrylic and methacrylic esters, including poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate), polyvinyl polymers including polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, poly(vinyl acetate), and polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, celluloses including alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, and cellulose sulphate sodium salt, polypropylene, polyethylenes including poly(ethylene glycol), poly(ethylene oxide), and poly(ethylene terephthalate), and polystyrene.

[0054] In some aspects, the polymer is a biodegradable polymer, including a synthetic biodegradable polymer (e.g., polymers of lactic acid and glycolic acid, polyanhydrides,

poly(ortho)esters, polyurethanes, poly(butic acid), poly(valeric acid), and poly(lactide-cocaprolactone)), and a natural biodegradable polymer (e.g., alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins (e.g., zein and other prolamines and hydrophobic proteins)), as well as any copolymer or mixture thereof.

[0055] In some aspects, the polymer is a bioadhesive polymer, such as a bioerodible hydrogel described by H. S. Sawhney, C. P. Pathak and J. A. Hubbell in Macromolecules, 1993, 26, 581-587, the teachings of which are incorporated herein, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

[0056] In some embodiments, the polymer is a water-soluble polymer or a hydrophilic polymer. Suitable water-soluble polymers are known in the art and include, for example, polyvinylpyrrolidone, hydroxypropyl cellulose (HPC; Klucel), hydroxypropyl methylcellulose (HPMC; Methocel), nitrocellulose, hydroxypropyl ethylcellulose, hydroxypropyl butylcellulose, hydroxypropyl pentylcellulose, methyl cellulose, ethylcellulose (Ethocel), hydroxyethyl cellulose, various alkyl celluloses and hydroxyalkyl celluloses, various cellulose ethers, cellulose acetate, carboxymethyl cellulose, sodium carboxymethyl cellulose, calcium carboxymethyl cellulose, vinyl acetate/crotonic acid copolymers, poly-hydroxyalkyl methacrylate, hydroxymethyl methacrylate, methacrylic acid copolymers, polymethacrylic acid, polymethylmethacrylate, maleic anhydride/methyl vinyl ether copolymers, poly vinyl alcohol, sodium and calcium polyacrylic acid, polyacrylic acid, acidic carboxy polymers, carboxypolymethylene, carboxyvinyl polymers, polyoxyethylene polyoxypropylene copolymer, polymethylvinylether co-maleic anhydride, carboxymethylamide, potassium methacrylate divinylbenzene co-polymer, polyoxyethyleneglycols, polyethylene oxide, and derivatives, salts, and combinations thereof.

[0057] In some embodiments, the heterologous moiety is a carbohydrate. In some embodiments, the carbohydrate is a monosaccharide (e.g., glucose, galactose, fructose), a disaccharide (e.g., sucrose, lactose, maltose), an oligosaccharide (e.g., raffinose, stachyose), a

polysaccharide (a starch, amylase, amylopectin, cellulose, chitin, callose, laminarin, xylan, mannan, fucoidan, galactomannan.

[0058] In some embodiments, the heterologous moiety is a lipid. The lipid, in some embodiments, is a fatty acid, eicosanoid, prostaglandin, leukotriene, thromboxane, N-acyl ethanolamine), glycerolipid (e.g., mono-, di-, tri-substituted glycerols), glycerophospholipid (e.g., phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine), sphingolipid (e.g., sphingosine, ceramide), sterol lipid (e.g., steroid, cholesterol), prenol lipid, saccharolipid, or a polyketide, oil, wax, cholesterol, sterol, fat-soluble vitamin, monoglyceride, diglyceride, triglyceride, a phospholipid.

[0059] Hydrophilic polymers

[0060] Suitable hydrophilic polymers that confer an increased hydrodynamic radius and increased serum half-life include polyethylene glycol (PEG), polypropylene glycol, polyoxyethylated polyols (e.g., POG), polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), polyoxyalkylenes, polyethylene glycol propionaldehyde, copolymers of ethylene glycol/propylene glycol, monomethoxy-polyethylene glycol, mono-(C1-C10) alkoxy- or aryloxy-polyethylene glycol, carboxymethylcellulose, polyacetals, polyvinyl alcohol (PVA), polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, poly (.beta.-amino acids) (either homopolymers or random copolymers), poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers (PPG) and other polyakylene oxides, polypropylene oxide/ethylene oxide copolymers, colonic acids or other polysaccharide polymers, Ficoll or dextran and mixtures thereof. Dextrans are polysaccharide polymers of glucose subunits, predominantly linked by α1-6 linkages. Dextran is available in many molecular weight ranges, e.g., about 1 kD to about 100 kD, or from about 5, 10, 15 or 20 kD to about 20, 30, 40, 50, 60, 70, 80 or 90 kD.

[0061] The one or more hydrophilic moieties attached to the peptide, e.g., polyethylene glycol chain, in accordance with some embodiments has a collective molecular weight selected from the range of about 20,000 Daltons to about 60,000 Daltons. In some embodiments the polyethylene glycol chain has a molecular weight selected from the range of about 500 to about 5,000 Daltons, or about 1,000 to about 5,000 Daltons. In another embodiment the hydrophilic moiety, e.g., polyethylene glycol chain, has a molecular weight of about 20,000 to about 60,000 Daltons. In yet other exemplary embodiments the hydrophilic moiety, e.g. polyethylene glycol chain, has a collective molecular weight of about

30,000 to about 50,000 Daltons (e.g., 40,000 Daltons). For example, two 20,000 Dalton PEG chains can be attached to the same or different positions of the peptide to achieve a collective molecular weight of 40,000 Daltons.

[0062] Linear or branched hydrophilic polymers are contemplated. Resulting preparations of conjugates may be essentially monodisperse or polydisperse, and may have about 0.5, 0.7, 1, 1.2, 1.5 or 2 or more polymer moieties per peptide.

[0063] In some embodiments, the native amino acid of the peptide is substituted with an amino acid having a side chain suitable for crosslinking with hydrophilic moieties, to facilitate linkage of the hydrophilic moiety to the peptide. Exemplary amino acids include Cys, Lys, Orn, homo-Cys, or acetyl phenylalanine (Ac-Phe). In other embodiments, an amino acid modified to comprise a hydrophilic group is added to the peptide at the C-terminus.

[0064] Accessory peptides capable of forming an extended conformation similar to chemical PEG (e.g., a recombinant PEG (rPEG) molecule), are described in International Patent Application Publication No. WO2009/023270 and U.S. Patent Application Publication No. US2008/0286808. The rPEG molecule is not polyethylene glycol. The rPEG molecule in some aspects is a polypeptide comprising one or more of glycine, serine, glutamic acid, aspartic acid, alanine, or proline. In some aspects, the rPEG is a homopolymer, e.g., poly-glycine, poly-serine, poly-glutamic acid, poly-aspartic acid, poly-alanine, or poly-proline. In other embodiments, the rPEG comprises two types of amino acids repeated, e.g., poly(Gly-Ser), poly(Gly-Glu), poly(Gly-Ala), poly(Gly-Asp), poly(Gly-Pro), poly(Ser-Glu), etc. In some aspects, the rPEG comprises three different types of amino acids, e.g., poly(Gly-Ser-Glu). In specific aspects, the rPEG increases the half-life of the extended half-life GLP-1/GIP coagonist peptide. In some aspects, the rPEG comprises a net positive or net negative charge. The rPEG in some aspects lacks secondary structure. In some embodiments, the rPEG is greater than or equal to 10 amino acids in length and in some embodiments is about 40 to about 50 amino acids in length. The accessory peptide in some aspects is fused to the N- or C- terminus of the peptide of the invention through a peptide bond or a proteinase cleavage site. The rPEG in some aspects comprises an affinity tag or is linked to a PEG that is greater than 5 kDa.

[0065] Acylation or alkylation

[0066] In some embodiments, the extended half-life GLP-1/GIP coagonist peptides comprise an amino acid that comprises a non-native acyl or alkyl group (referred to herein as an "acylated amino acid" or "alkylated amino acid" respectively, regardless of how it is prepared, e.g., by incorporation of a previously-acylated/alkylated amino acid into the peptide, or acylation/alkylation of the peptide after synthesis). The acyl or alkyl group can be covalently linked directly to an amino acid of the peptide , or indirectly to an amino acid of the peptide via a spacer. The peptide may be acylated at the same amino acid position where a hydrophilic polymer is linked, or at a different amino acid position.

[0067] The acyl or alkyl group may be of any size, can comprise any length of carbon chain, and can be linear or branched. In some exemplary embodiments of the invention, the acyl group is a C4 to C30 fatty acid. For example, the acyl group can be any of a C4 fatty acid, C6 fatty acid, C8 fatty acid, C10 fatty acid, C12 fatty acid, C14 fatty acid, C16 fatty acid, C18 fatty acid, C20 fatty acid, C22 fatty acid, C24 fatty acid, C26 fatty acid, C28 fatty acid, or a C30 fatty acid. In some embodiments, the acyl group is a C8 to C20 fatty acid, e.g., a C14 fatty acid or a C16 fatty acid.

[0068] In other exemplary embodiments, the alkyl group is a C4 to C30 alkyl. For example, the alkyl group can be any of a C4 alkyl, C6 alkyl, C8 alkyl, C10 alkyl, C12 alkyl, C14 alkyl, C16 alkyl, C18 alkyl, C20 alkyl, C22 alkyl, C24 alkyl, C26 alkyl, C28 alkyl, or a C30 alkyl. In some embodiments, the alkyl group is a C8 to C20 alkyl, e.g., a C14 alkyl or a C16 alkyl.

[0069] In some embodiments, the acyl group is a bile acid, including, but not limited to, cholic acid, chenodeoxycholic acid, deoxycholic acid, lithocholic acid, taurocholic acid, glycocholic acid, and cholesterol acid.

[0070] Therapeutic uses of extended half-life GLP-1/GIP coagonist peptides

[0071] Extended half-life GLP-1/GIP coagonist peptides are useful for treating obesity, inducing weight loss (e.g., as measured by reduction in body weight), normalizing body fat distribution, preventing or reducing an increase in body weight, and/or reducing appetite, as described in WO 2010/011439, incorporated by reference herein in its entirety.

[0072] In one aspect, the invention relates to a method of reducing weight gain or inducing weight loss in an adult human in need thereof by administering an extended half-life GLP-1/GLP coagonist peptide to the adult human at a weekly dosage of about 1 mg to about 40 mg. Methods for reducing weight gain or inducing weight loss are expected to be useful to

treat excess body weight and obesity due to various causes, including drug-induced obesity, and reducing complications associated with obesity including vascular disease (coronary artery disease, myocardial infarction, stroke, peripheral vascular disease, ischemia reperfusion, etc.), hypertension, onset of diabetes type II, hyperlipidemia and musculoskeletal diseases. Such methods are also expected to be particularly useful for obesity in patients with other risk factors for cardiovascular disease, such as patients with insulin resistance or diabetes type II, hypertension, hyperlipidemia, or combinations of these risk factors.

[0073] In some embodiments of this aspect of the invention, the adult human has any stage of nonalcoholic fatty liver disease (NAFLD) or any stage of alcoholic liver disease, or alcohol-induced liver disease. NAFLD refers to a wide spectrum of liver disease ranging from simple fatty liver (steatosis), to nonalcoholic steatohepatitis (NASH), to cirrhosis (irreversible, advanced scarring of the liver). All of the stages of NAFLD have in common the accumulation of fat (fatty infiltration) in the liver cells (hepatocytes). Simple fatty liver is the abnormal accumulation of a certain type of fat (e.g., triglyceride) in the liver cells with no inflammation or scarring. In NASH, the fat accumulation is associated with varying degrees of inflammation (hepatitis) and scarring (fibrosis) of the liver. The inflammatory cells can destroy the liver cells (hepatocellular necrosis). In the terms "steatohepatitis" and "steatonecrosis", steato refers to fatty infiltration, hepatitis refers to inflammation in the liver, and necrosis refers to destroyed liver cells. NASH can ultimately lead to scarring of the liver (fibrosis) and then irreversible, advanced scarring (cirrhosis). Cirrhosis that is caused by NASH is the last and most severe stage in the NAFLD spectrum. (Mendler, Michel, “Fatty Liver: Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH),” ed. Schoenfield, Leslie J., MedicineNet.com, August 29, 2005).

[0074] Alcoholic liver disease encompasses three pathologically distinct liver diseases related to, or caused by, the excessive consumption of alcohol: fatty liver (steatosis), chronic or acute hepatitis, and cirrhosis. Alcoholic hepatitis can range from a mild hepatitis, with abnormal laboratory tests being the only indication of disease, to severe liver dysfunction with complications such as jaundice (yellow skin caused by bilirubin retention), hepatic encephalopathy (neurological dysfunction caused by liver failure), ascites (fluid accumulation in the abdomen), bleeding esophageal varices (varicose veins in the esophagus), abnormal blood clotting and coma. Histologically, alcoholic hepatitis has a characteristic appearance with ballooning degeneration of hepatocytes, inflammation with neutrophils and sometimes Mallory bodies (abnormal aggregations of cellular intermediate filament proteins). Cirrhosis

is characterized anatomically by widespread nodules in the liver combined with fibrosis. (Worman, Howard J., "Alcoholic Liver Disease", Columbia University Medical Center website).

[0075] The treatment methods in this embodiment of the invention include reduction in one, two, three or more of the following: liver fat content, incidence or progression of cirrhosis, incidence of hepatocellular carcinoma, signs of inflammation, such as abnormal hepatic enzyme levels (e.g., aspartate aminotransferase AST and/or alanine aminotransferase ALT, or LDH), elevated serum ferritin, elevated serum bilirubin, and/or signs of fibrosis, e.g. elevated TGF-beta levels. In preferred embodiments, the extended half-life GLP-1/GIP coagonist peptide is used treat patients who have progressed beyond simple fatty liver (steatosis) and exhibit signs of inflammation or hepatitis. Such methods may result, for example, in reduction of AST and/or ALT levels.

[0076] In some embodiments of this aspect of the invention, the adult human has metabolic syndrome. Metabolic Syndrome, also known as metabolic syndrome X, insulin resistance syndrome, or Reaven's syndrome, is a disorder that affects over 50 million Americans. Metabolic Syndrome is typically characterized by a clustering of at least three or more of the following risk factors: (1) abdominal obesity (excessive fat tissue in and around the abdomen), (2) atherogenic dyslipidemia (blood fat disorders including high triglycerides, low HDL cholesterol and high LDL cholesterol that enhance the accumulation of plaque in the artery walls), (3) elevated blood pressure, (4) insulin resistance or glucose intolerance, (5) prothrombotic state (e.g. high fibrinogen or plasminogen activator inhibitor-1 in blood), and (6) pro-inflammatory state (e.g. elevated C-reactive protein in blood). Other risk factors may include aging, hormonal imbalance and genetic predisposition.

[0077] Metabolic Syndrome is associated with an increased risk of coronary heart disease and other disorders related to the accumulation of vascular plaque, such as stroke and peripheral vascular disease, referred to as atherosclerotic cardiovascular disease (ASCVD). Patients with metabolic syndrome may progress from an insulin resistant state in its early stages to full blown type II diabetes with further increasing risk of ASCVD. Without intending to be bound by any particular theory, the relationship between insulin resistance, metabolic syndrome and vascular disease may involve one or more concurrent pathogenic mechanisms including impaired insulin-stimulated vasodilation, insulin resistance-associated reduction in NO availability due to enhanced oxidative stress, and abnormalities in adipocyte-

derived hormones such as adiponectin (Lteif and Mather, Can. J. Cardiol. 20 (suppl. B):66B-76B (2004)).

[0078] According to the 2001 National Cholesterol Education Program Adult Treatment Panel (ATP III), any three of the following traits in the same individual meet the criteria for metabolic syndrome: (a) abdominal obesity (a waist circumference over 102 cm in men and over 88 cm in women); (b) serum triglycerides (150 mg/dL or above); (c) HDL cholesterol (40 mg/dL or lower in men and 50 mg/dL or lower in women); (d) blood pressure (130/85 or more); and (e) fasting blood glucose (110 mg/dL or above). According to the World Health Organization (WHO), an individual having high insulin levels (an elevated fasting blood glucose or an elevated post meal glucose alone) with at least two of the following criteria meets the criteria for metabolic syndrome: (a) abdominal obesity (waist to hip ratio of greater than 0.9, a body mass index of at least 30 kg/m^2, or a waist measurement over 37 inches); (b) cholesterol panel showing a triglyceride level of at least 150 mg/dL or an HDL cholesterol lower than 35 mg/dL; (c) blood pressure of 140/90 or more, or on treatment for high blood pressure). (Mathur, Ruchi, "Metabolic Syndrome," ed. Shiel, Jr., William C., MedicineNet.com, May 11, 2009).

[0079] For purposes herein, if an individual meets the criteria of either or both of the criteria set forth by the 2001 National Cholesterol Education Program Adult Treatment Panel or the WHO, that individual is considered as afflicted with metabolic syndrome.

[0080] In another aspect, the invention relates to a method of treating hyperglycemia, reducing blood glucose levels, or normalizing blood glucose levels (e.g., blood glucose level is returned to normal, such as lowering blood glucose level if it is higher than normal) in an adult human in need thereof by administering an extended half-life GLP-1/GLP coagonist peptide to the adult human in a weekly dosage of about 1 mg to about 40 mg. Such methods for treating hyperglycemia are expected to be useful for a variety of types of hyperglycemia, including diabetes (e.g., diabetes mellitus type I, diabetes mellitus type II, or gestational diabetes), either insulin-dependent or non-insulin-dependent, and reducing complications of diabetes including nephropathy, retinopathy and vascular disease. Thus, in some embodiments the adult human has diabetes mellitus type I, diabetes mellitus type II, or gestational diabetes.

[0081] In some embodiments, the administration of the extended half-life GLP-1/GIP coagonist peptide results in a biological response selected from the group consisting of an

increase in insulin level, a decrease in glucose level, an increase in C-peptide level, a decrease in HbA_{1c} level, a decrease in fructosamine level, and combinations thereof. An increase in insulin level would indicate that administration of the extended half-life GLP-1/GIP coagonist peptide effectively is treating diabetes; and a decrease in glucose level would indicate that administration of the extended half-life GLP-1/GIP coagonist peptide is acting to reduce the levels of blood sugar, treating hyperglycemia.

[0082] HbA_{1c} levels depend on blood glucose concentration (i.e., the higher the glucose concentration in blood, the higher the level of HbA_{1c}), but are not influenced by daily fluctuations in the blood glucose concentration. Instead, they represent the average blood glucose level of approximately the past 4 weeks, strongly weighted toward the most recent 2 weeks. A decrease in HbA_{1c} level would indicate that administration of the extended half-life GLP-1/GIP coagonist peptide is reducing the average blood glucose level over the long term.

[0083] C-peptide serves as a linker between the A- and B- chains of insulin and facilitates the efficient assembly, folding, and processing of insulin in the endoplasmic reticulum. High levels of C-peptide generally indicate high levels of endogenous insulin production, while low levels of C-peptide generally indicate low levels of insulin production. An increase in C-peptide level indicates that administration of the extended half-life GLP-1/GIP coagonist peptide is increasing the producing of insulin.

[0084] Fructosamine can be used to identify the plasma glucose concentration. In general, the higher the fructosamine concentration the higher the average blood glucose level. Normal fructosamine levels may indicate that a patient is either not diabetic or that the patient has good diabetic control. An increase in fructosamine levels indicates that the patient's average glucose over the last 2 to 3 weeks has been elevated. A decrease in fructosamine level would indicate that administration of the extended half-life GLP-1/GIP coagonist peptide is reducing the average blood glucose level.

[0085] In some embodiments, the extended half-life GLP-1/GIP coagonist peptides can be administered alone or in combination with an effective amount of one or more second therapeutic agents. In some embodiments, the second therapeutic agent is an anti-diabetic agent, an anti-obesity agent, or a mixture thereof. Anti-diabetic agents known in the art or under investigation include insulin, sulfonylureas, such as tolbutamide (Orinase), acetohexamide (Dymelor), tolazamide (Tolinase), chlorpropamide (Diabinese), glipizide (Glucotrol), glyburide (Diabeta, Micronase, Glynase), glimepiride (Amaryl), or gliclazide

(Diamicron); meglitinides, such as repaglinide (Prandin) or nateglinide (Starlix); biguanides such as metformin (Glucophage) or phenformin; thiazolidinediones such as rosiglitazone (Avandia), pioglitazone (Actos), or troglitazone (Rezulin), or other PPARγ inhibitors; alpha glucosidase inhibitors that inhibit carbohydrate digestion, such as miglitol (Glyset), acarbose (Precose/Glucobay); exenatide (Byetta) or pramlintide; Dipeptidyl peptidase-4 (DPP-4) inhibitors such as vildagliptin or sitagliptin; SGLT (sodium-dependent glucose transporter 1) inhibitors; glucokinase activators (GKA); glucagon receptor antagonists (GRA); or FBPase (fructose 1,6-bisphosphatase) inhibitors. For example, the extended half-life GLP-1/GIP coagonist peptides can be administered with insulin.

[0086] Anti-obesity agents known in the art or under investigation include, Leptin and Fibroblast Growth Factor 21 (FGF-21), appetite suppressants, such as phenethylamine type stimulants, phentermine (optionally with fenfluramine or dexfenfluramine), diethylpropion (Tenuate®), phendimetrazine (Prelu-2®, Bontril®), benzphetamine (Didrex®), sibutramine (Meridia®, Reductil®); rimonabant (Acomplia®), other cannabinoid receptor antagonists; oxyntomodulin; fluoxetine hydrochloride (Prozac); Qnexa (topiramate and phentermine), Excalia (bupropion and zonisamide) or Contrave (bupropion and naltrexone); or lipase inhibitors, similar to xenical (Orlistat) or Cetilistat (also known as ATL-962), or GT 389-255.

[0087] Dosing and administration

[0088] The extended half-life GLP-1/GLP coagonist peptide can be administered in a weekly dosage (a total weekly dosage) of about 1 mg to about 40 mg. For example, the extended half-life GLP-1/GLP coagonist peptide can be administered in a weekly dosage of about 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 11 mg, about 12 mg, about 13 mg, about 14 mg, about 15 mg, about 16 mg, about 17 mg, about 18 mg, about 19 mg, about 20 mg, about 21 mg, about 22 mg, about 23 mg, about 24 mg, about 25 mg, about 26 mg, about 27 mg, about 28 mg, about 29 mg, about 30 mg, about 31 mg, about 32 mg, about 33 mg, about 34 mg, about 35 mg, about 36 mg, about 37 mg, about 38 mg, about 39 mg, and about 40 mg. In some embodiments, the extended half-life GLP-1/GLP coagonist peptide can be administered in a weekly dosage of about 4 mg to about 30 mg. For example, the extended half-life GLP-1/GLP coagonist peptide can be administered in a weekly dosage of about 4 mg, about 12 mg, about 20 mg, or about 30 mg, or from about 4 mg to about 20 mg, or from about 4 mg to about 12 mg, or from about 6 mg to about 30 mg, or from about 6 mg to about 20 mg, or from about 6 mg to about 12 mg, or from about 8 mg to about 30 mg, or from about 8 mg to

about 20 mg, or from about 12 mg to about 30 mg, or from about 12 mg to about 25 mg, or from about 12 mg to about 20 mg, or from about 10 mg to about 30 mg, or form about 10 mg to about 25 mg, or from about 10 mg to about 20 mg. In some embodiments, the extended half-life GLP-1/GLP coagonist peptide can be administered in a weekly dosage of about 2 mg to about 10 mg. For example, the extended half-life GLP-1/GLP coagonist peptide can be administered in a weekly dosage of about 2 mg, about 4 mg, about 6 mg, about 8 mg, or about 10 mg, or from about 2 mg to about 8 mg, or from about 2 mg to about 6 mg, or from about 2 mg to about 4 mg, or from about 4 mg to about 10 mg, or from about 4 mg to about 8 mg, or from about 4 mg to about 6 mg.

[0089] The doses given are for an extended half-life GLP-1/GLP coagonist peptide of molecular weight ranging from about 35 kD to 65 kD, or about 35 kD to about 55 kD, for example about 45 kD. The invention contemplates equivalent molar dosing for peptides of different molecular weight. Thus, the invention contemplates a weekly dose of about 23 nmol to about 930 nmol, or a weekly dose of about 93 nmol to about 465 nmol. The appropriate mg weekly dosage amount is easily calculated. For example, a 12 mg dose for a peptide of about 40-50 kD molecular weight is the molar equivalent of a 6 mg dose for a peptide of about 20-25 kD molecular weight.

[0090] The extended half-life GLP-1/GLP coagonist peptide can be administered to said adult human in any frequency that results in the desired total weekly dosage amount (e.g., every two weeks, every 10 days, once per week, twice per week, three times per week, four times per week, five times per week, six times per week, or daily). If the desired weekly dosage is about 4 mg, for example, the extended half-life GLP-1/GIP coagonist peptide can be administered as 8 mg every two weeks, 4 mg once per week, or 2 mg twice per week. If the desired weekly dosage is about 12 mg, for example, the extended half-life GLP-1/GIP coagonist peptide can be administered as 24 mg every two weeks, 12 mg once per week, or 6 mg twice per week. If the desired weekly dosage is about 20 mg, for example, the extended half-life GLP-1/GIP coagonist peptide can be administered as 40 mg every two weeks, 20 mg once per week, or 10 mg twice per week. If the desired weekly dosage is about 30 mg, for example, the extended half-life GLP-1/GIP coagonist peptide can be administered as 60 mg every two weeks, 30 mg once per week, or 15 mg twice per week.

[0091] Equivalent reduced dosages (proportionately reduced for body surface area or body weight) may be administered to humans of lower than average body weight, teens or children. For example, a total weekly dosage of 12 mg adjusted for a human of about half the body

surface area of an average adult male is a total weekly dosage of 6 mg. As another example, 12 mg weekly for an average 70 kg human is equivalent to about 0.17mg/kg per week; 20 mg weekly for an average 70 kg human is equivalent to about 0.28 mg/kg per week. Thus, for example, the invention contemplates administration of extended half-life GLP-1/GIP coagonist peptide at about 0.029 mg/kg to about 0.143 mg/kg (i.e., 2 mg to 10 mg for a 70 kg human), or about 0.057 mg/kg to about 0.28 mg/kg per week (i.e., 4 mg to 20 mg for a 70 kg human), or about 0.014 mg/kg to about 0.5 mg/kg per week (i.e., 1 mg to 35 mg for a 70 kg human).

[0092] Pharmaceutical formulations and routes of administration

[0093] The extended half-life GLP-1/GIP coagonist peptide can be administered in a pharmaceutical composition, optionally comprising an acceptable diluent, a carrier, an excipient, or mixtures thereof. In some embodiments, the pharmaceutical composition is sterile and has a purity level of, for example, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99%.

[0094] In some embodiments, the pharmaceutical composition comprises the extended half-life GLP-1/GIP coagonist in a concentration of about 1 mg/mL to about 50 mg/mL (e.g., about 1 mg/mL, about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 11 mg/mL, about 12 mg/mL, about 13 mg/mL, about 14 mg/mL about 15 mg/mL, about 16 mg/mL, about 17 mg/mL, about 18 mg/mL, about 19 mg/mL, about 20 mg/mL, about 21 mg/mL, about 22 mg/mL, about 23 mg/mL, about 24 mg/mL, about 25 mg/mL, about 26 mg/mL, about 27 mg/mL, about 28 mg/mL, about 29 mg/mL, about 30 mg/mL, about 31 mg/mL, about 32 mg/mL, about 33 mg/mL, about 34 mg/mL about 35 mg/mL, about 36 mg/mL, about 37 mg/mL, about 38 mg/mL, about 39 mg/mL, about 40 mg/mL, about 41 mg/mL, about 42 mg/mL, about 43 mg/mL, about 44 mg/mL, about 45 mg/mL, about 46 mg/mL, about 47 mg/mL, about 48 mg/mL, about 49 mg/mL, and about 50 mg/mL).

[0095] In one embodiment the pharmaceutical compositions comprise aqueous solutions that are sterilized and optionally stored within various containers. In some embodiments, the extended half-life GLP-1/GIP coagonist peptides can be used to prepare pre-formulated solutions ready for injection. In other embodiments the pharmaceutical compositions comprise a lyophilized powder. The pharmaceutical composition can be further packaged as

part of a kit that includes a disposable device for administering the extended half-life GLP-1/GIP coagonist peptides to a patient. The containers or kits may be labeled for storage at ambient room temperature or at refrigerated temperature.

[0096] In one embodiment the kit is provided with a device for administering the extended half-life GLP-1/GIP coagonist peptides to a patient, e.g. syringe needle, pen device, jet injector or other needle-free injector. The kit may alternatively or in addition include one or more containers, e.g., vials, tubes, bottles, single or multi-chambered pre-filled syringes, cartridges, infusion pumps (external or implantable), jet injectors, pre-filled pen devices and the like, optionally containing the extended half-life GLP-1/GIP coagonist peptides in a lyophilized form or in an aqueous solution. Preferably, the kits will also include instructions for use. In some embodiments the device of the kit is an aerosol dispensing device, wherein the extended half-life GLP-1/GIP coagonist peptides are prepackaged within the aerosol device. In another embodiment the kit comprises a syringe and a needle, and in one embodiment the sterile extended half-life GLP-1/GIP coagonist peptides are prepackaged within the syringe.

[0097] The extended half-life GLP-1/GIP coagonist peptides can be administered to a patient using any standard route of administration, including parenterally, such as intravenously, intraperitoneally, subcutaneously or intramuscularly, intrathecally, transdermally, rectally, orally, nasally or by inhalation. Parenteral injection or extended infusion, e.g. over a period of 1, 2, 3, 4, 5, 6, 7, 8, 12 or 24 hours is possible. In some embodiments, a pharmaceutical composition comprising the extended half-life GLP-1/GIP coagonist peptides is administered via parenteral injection, for example by subcutaneous injection, intravenous injection, or intramuscular injection.

[0098] In some embodiments, the pharmaceutical composition comprises the extended half-life GLP-1/GIP coagonist peptide of the present disclosure, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, and/or one or more pharmaceutically acceptable ingredients. Remington's Pharmaceutical Sciences, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), which is incorporated by reference in its entirety, discloses various components used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional agent is incompatible with the pharmaceutical compositions, its use in pharmaceutical compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

[0099] In some embodiments, the pharmaceutically acceptable ingredient is selected from the group consisting of a sugar (e.g., glucose, sucrose, trehalose, lactose, fructose, maltose, dextran, glycerin, dextran, mellibiose, melezitose, raffinose, mannotriose, stachyose, maltose, lactulose, maltulose, or iso-maltulose, or combinations of these sugars), a sugar alcohol (e.g., glycol, glycerol, erythritol, threitol, arabitol, xylitol, ribitol, mannitol, sorbitol, dulcitol, iditol, isomalt, maltitol, lactitol, or glucitol, or combinations of these sugar alcohols), a salt (e.g., sodium chloride), an emulsifier or surfactant (e.g., polysorbates, such as polyoxyethylene 20 sorbitan monooleate, or other block copolymers of ethylene oxide and propylene oxide), lyoprotectants, and mixtures thereof. For example, excipients such as sugars or sugar alcohols are present, e.g., in a concentration of about 20 mg/mL to about 40 mg/mL, or 25 to 45 mg/mL, such as 35 mg/mL.

[0100] The pharmaceutical composition may be formulated to achieve a physiologically compatible pH, e.g. about pH 4 to about pH 11. In some embodiments, the pH of the pharmaceutical composition is, e.g., about 7 to 10. In other embodiments, the pH of the pharmaceutical composition is, e.g., about 4 to 7, or 4.5 to about 5.5, for example, about 5

[0101] In certain embodiments, the pharmaceutical compositions may comprise buffering agents to achieve a physiological compatible pH. The buffering agents may include any compounds capable of buffering at the desired pH such as, for example, phosphate buffers (e.g. PBS), triethanolamine, Tris, bicine, TAPS, tricine, HEPES, TES, MOPS, PIPES, cacodylate, MES, acetate, citrate, succinate, histidine or other pharmaceutically acceptable buffers. In certain embodiments, the strength of the buffer is at least 0.5 mM, at least 1 mM, at least 5 mM, at least 10 mM, at least 20 mM, at least 30 mM, at least 40 mM, at least 50 mM, at least 60 mM, at least 70 mM, at least 80 mM, at least 90 mM, at least 100 mM, at least 120 mM, at least 150 mM, or at least 200 mM. In some embodiments, the strength of the buffer is no more than 300 mM (e.g. at most 200 mM, at most 100 mM, at most 90 mM, at most 80 mM, at most 70 mM, at most 60 mM, at most 50 mM, at most 40 mM, at most 30 mM, at most 20 mM, at most 10 mM, at most 5 mM, at most 1 mM). For example, the buffer concentration can be about 2 mM to about 100 mM, or about 10 mM to about 50 mM.

[0102] It is understood that all description of therapeutic methods, pharmaceutical compositions, kits and other similar embodiments described herein contemplate that reference to the peptides include all pharmaceutically acceptable salts, esters, conjugates or prodrugs thereof. Specific embodiments of the invention are further described in the following, nonlimiting examples which illustrate various features. The examples should not

be construed as limiting the scope of the invention, as many variations of these embodiments may be practiced and are understood in view of the entire disclosure herein.

[0103] EXEMPLARY EMBODIMENTS

[0104] A. Exemplary embodiments

[0105] In exemplary embodiments, the extended half-life GLP-1/GIP coagonist peptide is an analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, with the following features:

(a) an amino acid modification at position 1 that confers GIP agonist activity,

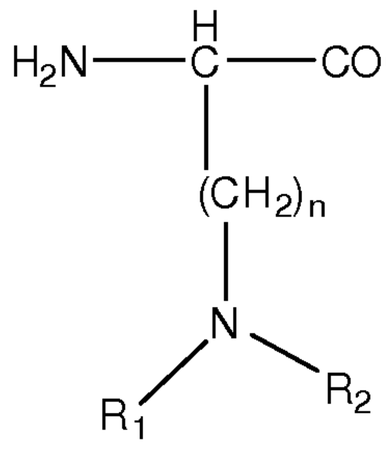
(b) a modification selected from the group consisting of:

(i) a lactam bridge between the side chains of amino acids at positions i and i+4 or between the side chains of amino acids at positions j and j+3, wherein i is 12, 13, 16, 17, 20 or 24, and wherein j is 17, and

(ii) one, two, three, or all of the amino acids at positions 16, 20, 21, and 24 of the analog is substituted with an α,α-disubstituted amino acid, and

(c) 1-10 (e.g., up to 2, 3, 4, 5, 6, 7, 8, 9 or 10) further amino acid modifications; that retains the desired GIP and GLP-1 activity.

[0106] In any of the exemplary embodiments in this section, the amino acid at position 1 is an amino acid lacking an imidazole side chain, optionally a large, aromatic amino acid (e.g., Tyr). In any of the exemplary embodiments, the peptide can have a lactam bridge between the amino acids at positions 16 and 20 (e.g. between Glu and Lys). In any of the exemplary embodiments, the α,α-disubstituted amino acid may be AIB and optionally the amino acid at position 16 is a positive-charged amino acid. In any of the exemplary embodiments, the positive-charged amino acid is an amino acid of Formula IV:



[Formula IV],

wherein n is 1 to 7, wherein each of R1 and R2 is independently selected from the group consisting of H, C_1-C_{18} alkyl, (C_1-C_{18} alkyl)OH, (C_1-C_{18} alkyl)NH_2, (C_1-C_{18} alkyl)SH, (C_0-C_4

alkyl)(C_3-C_6)cycloalkyl, (C_0-C_4 alkyl)(C_2-C_5 heterocyclic), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, and (C_1-C_4 alkyl)(C_3-C_9 heteroaryl), wherein R_7 is H or OH, and the side chain of the amino acid of Formula IV comprises a free amino group; optionally homoLys, Lys, Orn, or 2,4-diaminobutyric acid (Dab).

[0107] In some exemplary embodiments, the extended half-life GLP-1/GIP coagonist peptide is an analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, with the following features: an acylated amino acid at position 10, or an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29 comprising an acylated amino acid; further optionally with (i) an amino acid modification at position 1 that confers GIP agonist activity and (ii) at least one or both of: (A) a lactam bridge between the side chains of amino acids at positions i and i+4 or between the side chains of amino acids at positions j and j+3, wherein i is 12, 13, 16, 17, 20 or 24, and wherein j is 17; and (B) one, two, three, or all of the amino acids at positions 16, 20, 21, and 24 of the analog is substituted with an α,α-disubstituted amino acid; and (iii) up to ten (e.g., up to 2, 3, 4, 5, 6, 7, 8, 9 or 10) further amino acid modifications. Optionally the amino acid at position 16 is a positive-charged amino acid.

[0108] In any of the exemplary embodiments, the peptide also comprises amino acid modifications at one, two or all of positions 27, 28 and 29, e.g. wherein (a) the Met at position 27 is substituted with a large aliphatic amino acid, optionally Leu, (b) the Asn at position 28 is substituted with a small aliphatic amino acid, optionally Ala, (c) the Thr at position 29 is substituted with a small aliphatic amino acid, optionally Gly, or (d) a combination of two or all of (a), (b), and (c). The amino acid at position 2 is optionally a DPP-IV protective amino acid, e.g. an alpha, alpha disubstituted amino acid (e.g., AIB). In any of the exemplary embodiments, the peptide has a C-terminal amide or ester. In any of the exemplary embodiments, the peptide comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29, e.g. GPSSGAPPPS; optionally 1-6 amino acids of the extension are positive-charged amino acids.

[0109] B. Exemplary embodiments

[0110] In some exemplary embodiments, the extended half-life GLP-1/GIP coagonist peptide is an analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, with the following features: an acylated amino acid at position 10, or an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29 comprising an acylated amino acid; optionally with (i) an amino acid modification at position 1 that confers GIP agonist activity

and (ii) at least one or both of: (A) a lactam bridge between the side chains of amino acids at positions i and i+4 or between the side chains of amino acids at positions j and j+3, wherein i is 12, 13, 16, 17, 20 or 24, and wherein j is 17; and (B) one, two, three, or all of the amino acids at positions 16, 20, 21, and 24 of the analog is substituted with an α,α-disubstituted amino acid; and (iii) up to ten (e.g., up to 2, 3, 4, 5, 6, 7, 8, 9 or 10) further amino acid modifications; that retains the desired GIP and GLP-1 activity.. Optionally the amino acid at position 16 is a positive-charged amino acid.

[0111] In any of the exemplary embodiments, the peptide also comprises amino acid modifications at one, two or all of positions 27, 28 and 29, e.g. wherein (a) the Met at position 27 is substituted with a large aliphatic amino acid, optionally Leu, (b) the Asn at position 28 is substituted with a small aliphatic amino acid, optionally Ala, (c) the Thr at position 29 is substituted with a small aliphatic amino acid, optionally Gly, or (d) a combination of two or all of (a), (b), and (c). The amino acid at position 2 is optionally a DPP-IV protective amino acid, e.g. an alpha, alpha disubstituted amino acid (e.g., AIB). In any of the exemplary embodiments, the peptide has a C-terminal amide or ester. In any of the exemplary embodiments, the peptide comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29, e.g. GPSSGAPPPS; optionally 1-6 amino acids of the extension are positive-charged amino acids.

[0112] In some exemplary embodiments, the extended half-life GLP-1/GIP coagonist peptide is an analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, with the following features: (a) an amino acid modification at position 1 that confers GIP agonist activity, e.g. is a substitution of His with an amino acid lacking an imidazole side chain (b) a lactam bridge between the side chains of amino acids at positions i and i+4 or between the side chains of amino acids at positions j and j+3, wherein i is 12, 13, 16, 17, 20 or 24, and wherein j is 17, (c) amino acid modifications at one, two or all of positions 27, 28 and 29, and (d) 1-9 (e.g., up to 2, 3, 4, 5, 6, 7, 8, 9 or 10) further amino acid modifications; that retains the desired GIP and GLP-1 activity.

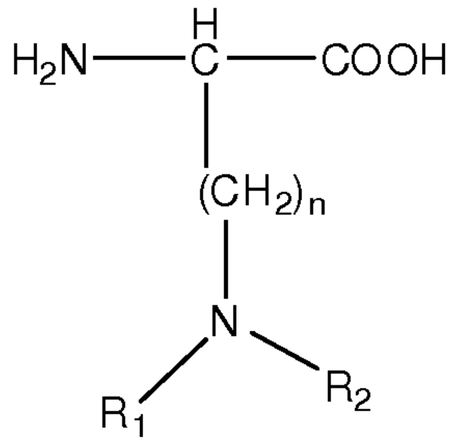
[0113] In any of the exemplary embodiments in this section, the amino acid at position 1 is an amino acid lacking an imidazole side chain, optionally a large, aromatic amino acid (e.g., Tyr). In any of the exemplary embodiments, the peptide can have a lactam bridge between the amino acids at positions 16 and 20 (e.g. between Glu and Lys). In any of the exemplary embodiments, the peptide also comprises (a) the Met at position 27 is substituted with a large aliphatic amino acid, optionally Leu, (b) the Asn at position 28 is substituted with a small

aliphatic amino acid, optionally Ala, (c) the Thr at position 29 is substituted with a small aliphatic amino acid, optionally Gly. The amino acid at position 2 is optionally a DPP-IV protective amino acid, e.g. an alpha, alpha disubstituted amino acid (e.g., AIB). In any of the exemplary embodiments, the peptide has a C-terminal amide or ester. In any of the exemplary embodiments, the peptide comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29, e.g. GPSSGAPPPS; optionally 1-6 amino acids of the extension are positive-charged amino acids.

[0114] In some exemplary embodiments, the extended half-life GLP-1/GIP coagonist peptide is an analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, with the following features: (a) an amino acid modification at position 1 that confers GIP agonist activity, (b) one, two, three, or all of the amino acids at positions 16, 20, 21, and 24 of the analog is substituted with an α,α-disubstituted amino acid, e.g., AIB, (c) amino acid modifications at one, two or all of positions 27, 28 and 29, and (d) 1-9 (e.g., up to 2, 3, 4, 5, 6, 7, 8, 9) further amino acid modifications; that retains the desired GIP and GLP-1 activity.

[0115] In any of the exemplary embodiments in this section, the amino acid at position 1 is an amino acid lacking an imidazole side chain, optionally a large, aromatic amino acid (e.g., Tyr). In any of the exemplary embodiments, the peptide can have a lactam bridge between the amino acids at positions 16 and 20 (e.g. between Glu and Lys). In any of the exemplary embodiments, the peptide also comprises (a) the Met at position 27 is substituted with a large aliphatic amino acid, optionally Leu, (b) the Asn at position 28 is substituted with a small aliphatic amino acid, optionally Ala, (c) the Thr at position 29 is substituted with a small aliphatic amino acid, optionally Gly. The amino acid at position 2 is optionally a DPP-IV protective amino acid, e.g. an alpha, alpha disubstituted amino acid (e.g., AIB). In any of the exemplary embodiments, the peptide has a C-terminal amide or ester. In any of the exemplary embodiments, the peptide comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29, e.g. GPSSGAPPPS; optionally 1-6 amino acids of the extension are positive-charged amino acids.

[0116] In some exemplary embodiments, the extended half-life GLP-1/GIP coagonist peptide is an analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, with the following features: (a) an amino acid modification at position 1 that confers GIP agonist activity, (b) at position 16 an amino acid of Formula IV:



[Formula IV],

wherein n is 1 to 7, wherein each of R1 and R2 is independently selected from the group consisting of H, C_1-C_{18} alkyl, (C_1-C_{18} alkyl)OH, (C_1-C_{18} alkyl)NH_2, (C_1-C_{18} alkyl)SH, (C_0-C_4 alkyl)(C_3-C_6)cycloalkyl, (C_0-C_4 alkyl)(C_2-C_5 heterocyclic), (C_0-C_4 alkyl)(C_6-C_{10} aryl)R_7, and (C_1-C_4 alkyl)(C_3-C_9 heteroaryl), wherein R_7 is H or OH, and the side chain of the amino acid of Formula IV comprises a free amino group, (c) at position 20 an alpha, alpha-disubstituted amino acid, e.g., AIB, (d) amino acid modifications at one, two or all of positions 27, 28 and 29, and (e) 1-9 (e.g., up to 2, 3, 4, 5, 6, 7, 8, 9) further amino acid modifications; that retains the desired GIP and GLP-1 activity.

[0117] In any of the exemplary embodiments in this section, the amino acid at position 1 is an amino acid lacking an imidazole side chain, optionally a large, aromatic amino acid (e.g., Tyr). In any of the exemplary embodiments, the peptide also comprises (a) the Met at position 27 is substituted with a large aliphatic amino acid, optionally Leu, (b) the Asn at position 28 is substituted with a small aliphatic amino acid, optionally Ala, (c) the Thr at position 29 is substituted with a small aliphatic amino acid, optionally Gly. In any of the exemplary embodiments, the amino acid of Formula IV in (b) is homoLys, Lys, Orn, or 2,4-diaminobutyric acid (Dab). The amino acid at position 2 is optionally a DPP-IV protective amino acid, e.g. an alpha, alpha disubstituted amino acid (e.g., AIB). In any of the exemplary embodiments, the peptide has a C-terminal amide or ester. In any of the exemplary embodiments, the peptide comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29, e.g. GPSSGAPPPS; optionally 1-6 amino acids of the extension are positive-charged amino acids.

[0118] Other optional modifications include

(a) Ser at position 2 substituted with D-Ser, Ala, D-Ala, Gly, N-methyl-Ser, AIB, Val, or α-amino-N-butyric acid;

(a) Tyr at position 10 substituted with Trp, Lys, Orn, Glu, Phe, or Val;

(b) Linkage of an acyl group to a Lys at position 10;

(c) Lys at position 12 substituted with Arg or Ile;

(d) Ser at position 16 substituted with Glu, Gln, homoglutamic acid, homocysteic acid, Thr, Gly, or AIB;

(e) Arg at position 17 substituted with Gln;

(f) Arg at position 18 substituted with Ala, Ser, Thr, or Gly;

(g) Gln at position 20 substituted with Ser, Thr, Ala, Lys, Citrulline, Arg, Orn, or AIB;

(h) Asp at position 21 substituted with Glu, homoglutamic acid, homocysteic acid;

(i) Val at position 23 substituted with Ile;

(j) Gln at position 24 substituted with Asn, Ser, Thr, Ala, or AIB; and

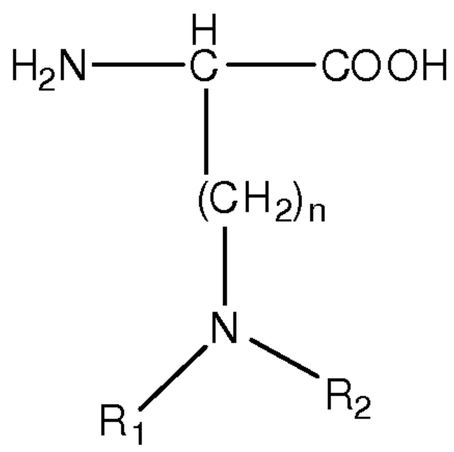
(k) a conservative substitution at any of positions 2, 5, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 24, 27, 28, and 29.

[0119] C. Exemplary Embodiments

[0120] In exemplary embodiments, the extended half-life GLP-1/GIP coagonist peptide is an analog of glucagon (SEQ ID NO: 1) having GIP agonist activity, comprising:

(a) an amino acid comprising an imidazole side chain at position 1,

(b) at position 16, an amino acid of Formula IV:



[Formula IV]

wherein n is 1 to 7, wherein each of R1 and R2 is independently selected from the group consisting of H, C1-C18 alkyl, (C1-C18 alkyl)OH, (C1-C18 alkyl)NH2, (C1-C18 alkyl)SH, (C0-C4 alkyl)(C3-C6)cycloalkyl, (C0-C4 alkyl)(C2-C5 heterocyclic), (C0-C4 alkyl)(C6-C10 aryl)R7, and (C1-C4 alkyl)(C3-C9 heteroaryl), wherein R7 is H or OH, and the side chain of the amino acid of Formula IV comprises a free amino group,

(c) an α,α-disubstituted amino acid at position 20,

(d) up to ten (e.g., up to 2, 3, 4, 5, 6, 7, 8, 9 or 10) additional amino acid modifications relative to SEQ ID NO: 1; that retains the desired GIP and GLP-1 activity.

[0121] In any of the exemplary embodiments in this section, the amino acid at position 1 can be His or a His derivative. In any of the exemplary embodiments, the amino acid of Formula IV in (b) can be homoLys, Lys, Orn, or 2,4-diaminobutyric acid (Dab). In any of the exemplary embodiments, the α,α-disubstituted amino acid comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring; and is optionally AIB. The amino acid at position 2 is optionally a DPP-IV protective amino acid, e.g. an alpha, alpha disubstituted amino acid (e.g., AIB). In any of the exemplary embodiments, the peptide has a C-terminal amide or ester. Optionally the peptide comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29, e.g. GPSSGAPPPS, or a conservatively substituted sequence thereof.

[0122] D. Exemplary embodiments

[0123] In exemplary embodiments, the extended half-life GLP-1/GIP coagonist peptide of the present disclosures is an analog of native glucagon comprising (i) an amino acid comprising an imidazole side chain at position 1, (ii) a DPP-IV protective amino acid at position 2, (iii) an acylated amino acid or alkylated amino acid, optionally at any of positions 9, 10, 12, 16, 20, or 37-43, wherein optionally the acyl or alkyl group is linked to the amino acid via a spacer; (iv) an alpha helix stabilizing amino acid at one or more of positions 16-21, and (v) up to ten (e.g., up to 2, 3, 4, 5, 6, 7, 8, 9 or 10) additional amino acid modifications relative to native glucagon, that retains the desired GIP and GLP-1 activity.

[0124] In any of the exemplary embodiments in this section, the amino acid at position 1 can be His or a His derivative. In any of the exemplary embodiments, the amino acid at position 2 is an α,α-disubstituted amino acid. In any of the exemplary embodiments, the α,α-disubstituted amino acid comprises R1 and R2, each of which is bonded to the alpha carbon, wherein each of R1 and R2 is independently selected from the group consisting of C1-C4 alkyl, optionally substituted with a hydroxyl, amide, thiol, halo, or R1 and R2 together with the alpha carbon to which they are attached form a ring; and is optionally AIB or ACPC. In any of the exemplary embodiments, the glucagon analog comprises an alpha helix stabilizing amino acid such as an alpha, alpha disubstituted amino acid at position 20.

[0125] In any of the exemplary embodiments, the peptide comprises an extension of 1 to 21 amino acids C-terminal to the amino acid at position 29, that comprises an amino acid

sequence which forms a Trp cage structure; optionally GPSSGAPPPS, or a conservatively substituted sequence thereof; and optionally comprising least one charged amino acid.

EXAMPLES

Example 1: Extended half-life GLP-1/GIP coagonist peptide was well tolerated with an extended half-life of about 5 days

[0126] A Phase I, randomized, placebo-controlled, sequential, single-ascending, two period, study was performed in healthy male subjects to evaluate the safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) properties of extended half-life GLP-1/GIP coagonist peptide. Healthy adult male subjects (about 18 to 55 years of age) who were in general good health based on medical history, physical examination, ECG, and routine laboratory tests (e.g., blood, chemistry, hematology, urinalysis, and drug screen) with a body mass index between 20 kg/m^2 and 30 kg/m^2 were selected for the study. The extended half-life GLP-1/GIP coagonist peptide tested in all of the Examples was SEQ ID NO: 153.

[0127] The subjects were randomized into cohorts. Cohort 1, 2, 3, 4, 5, and 6 each consisted of 6 patients that received 0.1 mg, 0.3 mg, 1 mg, 2 mg, 4 mg, and 8 mg, respectively of the extended half-life GLP-1/GIP coagonist peptide and 2 patients that received placebo. Cohorts 1', 2', and 3' each consisted of 6 patients that received 16 mg, 24 mg, and 32 mg, respectively, of the extended half-life GLP-1/GIP coagonist peptide and 2 patients that received placebo.

[0128] Following an overnight fast of at least 10 hours, the subjects received a single dose of the study drug or placebo as a subcutaneous (SC) injection in the abdomen. Blood samples were collected for fasting insulin, glucose, and glucagon levels pre-dose (i.e., within 30 minutes prior to dosing) on Day 1 and on Days 2, 3, 4, 5, 6, and 7. Plasma samples for pharmacokinetic analysis were collected pre-dose and at 0.5, 1, 2, 4, 8, and 12 hours post-dose on Day 1 and on Days 2, 3, 4, 5, 6, 7, 14, 28, and 35. The subjects were monitored through Day 35 for adverse events, weight, and vital signs.

[0129] During the study, plasma $AUC_{0-\tau}$, AUC_{0-inf}, and C_{max} increased with the doses of the study drug and the apparent $t_{1/2}$ was approximately 5 days. The extended half-life GLP-1/GIP coagonist peptide was considered dose proportional for both AUC and C_{max} at doses ranging from 0.1 mg to 32 mg. At the 0.1 mg dose, mean C_{max} was 183 pM, with AUC (AUC_{0-144}) of 12,349 pM·hr. At the 8 mg dose, mean C_{max} was 12,989 pM, with AUC

($AUC_{0\text{-}144}$) of 1,382,376 pM·hr. At the 32 mg dose, mean C_{max} was 54.8 nM, with AUC ($AUC_{0\text{-}last}$) 12,582 nM·hr.

[0130] The time to maximum observed plasma concentration (T_{max}) was higher for subjects dosed with the lowest amount of the extended half-life GLP-1/GIP coagonist peptide (112.5 h at 16 mg), versus those subjects that were dosed at higher concentrations of the extended half-life GLP-1/GIP coagonist peptide (56.0 h at 24 mg; 80.0 h at 32 mg).

[0131] The absolute bioavailability of the extended half-life GLP-1/GIP coagonist peptide was 100% and its accumulation index was 1.7 to 2-fold. Treatment was generally safe and well tolerated.

[0132] No dose-related trends were observed for fasting insulin, glucose, and glucagon levels, as expected in healthy subjects. However, the treatment groups, compared to the placebo groups, tended to have numerically greater reductions from base in mean daily fasting glucose levels by day 7 (placebo: 78.7 mg/dL; 16 mg dose: 76.5 mg/dL; 24 mg dose: 72.7 mg/dL; 32 mg dose: 71.7 mg/dL). Although these reductions were within the normal range for the healthy subjects of the study, the magnitude of decline in patients with diabetes is likely to be clinically important.

Example 2: Extended half-life GLP-1/GIP coagonist peptide increased insulin secretion in a dose-dependent manner

[0133] A Phase I, randomized, placebo-controlled, positive-controlled two-part study was performed in healthy male and female subjects to evaluate the effect of a range of doses on beta cell response to a glucose load and to assess the effect of these doses on gastric emptying. Beta cell function was assessed by calculation of prehepatic insulin secretion in response to a graded glucose infusion. Gastric emptying was assessed by measurement of plasma appearance of ingested acetaminophen.

[0134] Subjects in Part 1 (Cohort 1) received two subcutaneous (SC) injections of placebo, 2 hours apart, in the abdomen on Day 1, followed by two 5 μg subcutaneous (SC) injections of BYETTA (Amylin Pharmaceuticals), 2 hours apart (10 μg total) in the abdomen on Day 2. During Part 2, subjects in Cohort 2 received placebo on Day 1, followed by 8 mg of the extended half-life GLP-1/GIP coagonist peptide on Day 2. Subjects in Cohort 3 received placebo on Day 1, followed by 4 mg of the extended half-life GLP-1/GIP coagonist peptide

on Day 2. Subjects in Cohort 4 received placebo on Day 1, followed by 16 mg of the extended half-life GLP-1/GIP coagonist peptide on Day 2.

Part 1 (Cohort 1)

[0135] On Day 1, following an overnight fast of ≥10 hours, subjects in Part 1 (Cohort 1) received a single SC injection of placebo at -120 minutes. Body weight was measured at -120 minutes to calculate the graded glucose infusion. Blood samples for glucose, C-peptide, insulin, and safety laboratory tests were drawn at -120, -15, and -10 minutes. At -10 minutes, subjects ingested 1000 mg acetaminophen elixir with 240 mL water. At 0 minutes, a step-wise infusion of glucose (20% dextrose) was started, a second SC injection of placebo was administered, and blood samples for glucose, C-peptide, insulin, and safety laboratory tests were drawn. Glucose was infused for a total of 2.5 hours at a rate of 2, 4, 6, 8, and 12 mg/kg/min. Each level of glucose infusion was maintained for 30 minutes. Blood samples for glucose, C-peptide, and insulin were drawn again at 10, 20, and 30 minutes during each 30-minute infusion period. Blood samples for plasma acetaminophen concentration were drawn at -10, 30, 60, 90, 120, 150, 180, 210, 240, 300, and 480 minutes. A 12-lead ECG was conducted at 210 minutes.

[0136] On Day 2, following an overnight fast of ≥10 hours, subjects in Cohort 1 received BYETTA 5 μg by SC injection at -120 minutes. Blood samples for glucose, C-peptide, and insulin were drawn at -120, -15, and -10 minutes. At -10 minutes, subjects ingested 1000 mg acetaminophen elixir with 240 mL water. At 0 minutes, a step-wise infusion of glucose (20% dextrose) was started, the subject received a second SC injection of BYETTA 5 μg, and blood samples for C-peptide, insulin, glucose, and safety laboratory tests were drawn. Glucose was infused for a total of 2.5 hours at a rate of 2, 4, 6, 8, and 12 mg/kg/min. Each level of glucose infusion was maintained for 30 minutes. A blood sample for BYETTA concentration was drawn at 0, 60, and 120 minutes. Blood samples for glucose, C-peptide, and insulin were drawn at 10, 20, and 30 minutes during each 30-minute infusion period. Blood samples for plasma acetaminophen concentration were drawn at -10, 30, 60, 90, 120, 150, 180, 210, 240, 300, and 480 minutes. A 12-lead ECG was conducted at 210 minutes.

[0137] On Day 3, blood samples were collected for safety laboratory tests and a 12-lead ECG was conducted. Subjects in Cohort 1 were released from the investigational site on the evening of Day 3.

Part 2 (Cohort 2 to Cohort 4)

[0138] On Day 1, following an overnight fast of ≥10 hours, subjects in Part 2 (Cohorts 2 to 4) received a single SC injection of placebo at -120 minutes. Body weight was measured at -120 minutes to calculate the graded glucose infusion. Blood samples for glucose, C-peptide, insulin, and safety laboratory tests were drawn at -15 and -10 minutes. At -10 minutes, subjects ingested 1000 mg acetaminophen elixir with 240 mL water. At 0 minutes, a step-wise infusion of glucose (20% dextrose) was started and blood samples for glucose, C-peptide, and insulin were drawn. Glucose was infused for a total of 2.5 hours at a rate of 2, 4, 6, 8, and 12 mg/kg/min. Each level of glucose infusion was maintained for 30 minutes. Blood samples for glucose, C-peptide, and insulin were drawn again at 10, 20, and 30 minutes during each 30-minute infusion period. Blood samples for plasma acetaminophen concentration were drawn at -10, 30, 60, 90, 120, 150, 180, 210, 240, 300, and 480 minutes. A 12-lead ECG was conducted at 210 minutes.

[0139] On Day 2, following an overnight fast of ≥10 hours, a blood sample for glucose, C-peptide, and insulin was drawn at -10 minutes. Subjects received an injection of extended half-life GLP-1/GIP coagonist peptide at 0 minutes. Subjects in Cohort 2 received 8 mg of extended half-life GLP-1/GIP coagonist peptide, subjects in Cohort 3 received 4 mg of extended half-life GLP-1/GIP coagonist peptide, and subjects in Cohort 4 received 16 mg of extended half-life GLP-1/GIP coagonist peptide. At 0 minutes, a blood sample for extended half-life GLP-1/GIP coagonist peptide concentration, C-peptide, insulin, glucose, and safety laboratory tests was drawn. A 12-lead ECG was conducted at 210 minutes.

[0140] On Day 3 and Day 4, blood samples were collected for safety laboratory tests.

[0141] On Day 5 (72 hours after extended half-life GLP-1/GIP coagonist peptide dosing), following an overnight fast of ≥10 hours, blood samples for glucose, C-peptide, and insulin were drawn at -15 minutes and at -10 minutes. At -10 minutes, subjects ingested 1000 mg acetaminophen elixir with 240 mL water. At 0 minutes, a step-wise infusion of glucose (20% dextrose) was started and blood samples for glucose, C-peptide, insulin, safety laboratory tests, and extended half-life GLP-1/GIP coagonist peptide concentration were drawn. Glucose was infused for a total of 2.5 hours at a rate of 2, 4, 6, 8, and 12 mg/kg/min. Each level of glucose infusion was maintained for 30 minutes. Blood samples for glucose, C-peptide, and insulin were drawn at 10, 20, and 30 minutes during each 30 minute infusion period. Blood samples for plasma acetaminophen concentration were drawn at -10, 30, 60, 90, 120, 150, 180, 210, 240, 300, and 480 minutes. A blood sample for extended half-life

GLP-1/GIP coagonist peptide concentration was drawn at 120 minutes. A 12-lead ECG was conducted at 210 minutes.

[0142] Subjects in Cohort 2 to Cohort 4 remained confined to the investigational site until the evening of Day 7. On Day 6 and Day 7, blood samples were collected for safety laboratory tests. On Day 7, a 12-lead ECG was conducted.

Results

[0143] During Part 1, a glucose dose-dependent increase in insulin secretion rate after treatment with BYETTA was observed with each increase in glucose infusion rate. The treatment comparison of least-squares (LS) mean change in insulin secretion rate between placebo and BYETTA at each glucose infusion rate (0.12 μIU/mL per mg/dL) was statistically significant ($p<0.0001$).

[0144] Treatment with BYETTA was associated with a significant delay of acetaminophen absorption compared to placebo. Subjects had a lower C_{max} and a shift in T_{max} from 30 minutes to 210 minutes after treatment with BYETTA. The result is consistent with published effects of BYETTA on gastric emptying.

[0145] During Part 2, a dose dependent, dose proportional, increase in insulin secretion rate (ISR) was observed after treatment with the extended half-life GLP-1/GIP coagonist peptide in comparison to placebo. At the maximum dose (16 mg) insulin secretion rate was increased by approximately 44% ($p = 0.001$). The treatment comparisons of least squares (LS) mean change in insulin secretion rate at each glucose infusion rate between the placebo and the 16 mg extended half-life GLP-1/GIP coagonist peptide group (0.07 μIU/mL), placebo and 8 mg extended half-life GLP-1/GIP coagonist peptide group (0.04 μIU/mL), placebo and 4 mg extended half-life GLP-1/GIP coagonist peptide group (0.03 μIU/mL), the extended half-life GLP-1/GIP coagonist peptide 8 mg and 16 mg groups (0.03 μIU/mL per mg/dL), the extended half-life GLP-1/GIP coagonist peptide 4 mg and 16 mg groups (0.04 μIU/mL per mg/dL), and the extended half-life GLP-1/GIP coagonist peptide 4 mg and 8 mg groups (0.01 μIU/mL per mg/dL) were statistically significant ($p < 0.001$, $p < 0.001$, and $p < 0.001$, $p<0.0001$, $p<0.0001$, and $p=0.0067$, respectively). The results are illustrated in Figure 1. All extended half-life GLP-1/GIP coagonist peptide dose groups showed higher insulin secretion rates than the placebo group. The extended half-life GLP-1/GIP coagonist peptide 16 mg group showed consistently higher insulin secretion rates than the other 2

extended half-life GLP-1/GIP coagonist peptide treatment groups at the 4 mg/kg/min to 12 mg/kg/min glucose infusion rates.

[0146] In general, all extended half-life GLP-1/GIP coagonist peptide dose groups had lower glucose levels than the placebo group at all glucose infusion rates. In general, lower mean glucose values were observed at each glucose infusion rate in the extended half-life GLP-1/GIP coagonist peptide 16 mg group compared to the other extended half-life GLP-1/GIP coagonist peptide dose groups. For the extended half-life GLP-1/GIP coagonist peptide 16 mg and 8 mg groups, there were no increases in glucose levels between the 8 mg/kg/min and 12 mg/kg/min infusion rates. Figure 2A illustrates the results comparing placebo and the 4 mg dose of extended half-life GLP-1/GIP coagonist peptide. Figure 2B illustrates the results comparing placebo and the 8 mg dose of extended half-life GLP-1/GIP coagonist peptide.

[0147] The treatment comparisons of LS mean change in insulin at each glucose infusion rate between the placebo and extended half-life GLP-1/GIP coagonist peptide 16 mg groups (0.43 μIU/mL per mg/dL), the placebo and extended half-life GLP-1/GIP coagonist peptide 8 mg groups (0.35 μIU/mL per mg/dL), the placebo and extended half-life GLP-1/GIP coagonist peptide 4 mg groups (0.27 μIU/mL per mg/dL), the extended half-life GLP-1/GIP coagonist peptide 8 mg and 16 mg groups (0.08 μIU/mL per mg/dL), the extended half-life GLP-1/GIP coagonist peptide 4 mg and 16 mg groups (0.17 μIU/mL per mg/dL), and the extended half-life GLP-1/GIP coagonist peptide 4 mg and 8 mg groups (0.09 μIU/mL per mg/dL) were statistically significant ($p<0.0001$, $p<0.0001$, $p<0.0001$, $p=0.0316$, $p<0.0001$, and $p=0.0251$, respectively).

[0148] Consistent with the observed increase in insulin secretion rate, a dose dependent, dose proportional, increase in C-peptide level was observed after treatment with the extended half-life GLP-1/GIP coagonist peptide in comparison to placebo (Figure 3). The treatment comparisons of LS mean change in C peptide at each glucose infusion rate between the placebo and the extended half-life GLP-1/GIP coagonist peptide 16 mg groups (0.05 ng/mL per mg/dL), the placebo and extended half-life GLP-1/GIP coagonist peptide 8 mg groups (0.03 ng/mL per mg/dL), the placebo and extended half-life GLP-1/GIP coagonist peptide 4 mg groups (0.02 ng/mL per mg/dL), the extended half-life GLP-1/GIP coagonist peptide 8 mg and 16 mg groups (0.02 ng/mL per mg/dL), the extended half-life GLP-1/GIP coagonist peptide 4 mg and 16 mg groups (0.03 ng/mL per mg/dL), and the extended half-life GLP-

1/GIP coagonist peptide 4 mg and 8 mg groups (0.01 ng/mL per mg/dL) were statistically significant ($p<0.0001$, $p<0.0001$, $p<0.0001$, $p<0.0001$, $p<0.0001$, and $p=0.0055$, respectively). In general, a glucose dose-dependent increase in C-peptide after treatment with extended half-life GLP-1/GIP coagonist peptide was observed with each increase in glucose infusion rate.

[0149] The extended half-life GLP-1/GIP coagonist peptide was not associated with a significant delay in the absorption of acetaminophen. There was a trend toward lower C_{max} at a higher dose of extended half-life GLP-1/GIP coagonist peptide, but the T_{max} was not significantly different from placebo nor was the total acetaminophen absorbed (AUC_{0-4hr}) not significantly different from placebo. Figure 4 compares the (AUC_{0-4hr}) of BYETTA and extended half-life GLP-1/GIP coagonist peptide at 4 mg, 8 mg, and 16 mg doses. The extended half-life GLP-1/GIP coagonist peptide does not delay gastric emptying at doses that increase insulin secretion in response to a glucose infusion.

[0150] Doses of 4 mg, 8 mg, and 16 mg of extended half-life GLP-1/GIP coagonist peptide increased insulin secretion in a dose-dependent manner during a graded glucose infusion without an effect on gastric emptying. Patients administered the 4 mg dose of extended half-life GLP-1/GIP coagonist peptide exhibited on average an increased insulin secretion rate, an increased C-peptide level, and a lower glucose level in response to glucose infusion.

Example 3: Effect of extended half-life GLP-1/GIP coagonist peptide in patients with type II diabetes mellitus

[0151] A randomized, placebo-controlled, sequential, multiple-ascending dose study is performed on male and female patients (18 to 70 years of age) diagnosed with type 2 diabetes and on stable metformin monotherapy (i.e., at the same dose for at least about 2 months prior to the study), with an HbA_{1c} level of at least about 6.5% and no more than 10.5%. Other criteria include fasting glucose levels of 110 mg/dL to 200 mg/dL, body mass index of 27 kg/m^2 to 40 kg/m^2, systolic blood pressure less than 155 mmHg, diastolic blood pressure less than 95 mmHg and no history of significant other disease or complications.

[0152] The patients are randomized into 4 cohorts. Cohort 1 consists of 8 patients that receive 4 mg of the extended half-life GLP-1/GIP coagonist peptide and 2 patients that receive placebo. Cohort 2 consists of 8 patients that receive 12 mg of the extended half-life GLP-1/GIP coagonist peptide and 2 patients that receive placebo. Cohort 3 consists of 8 patients that receive 20 mg of the extended half-life GLP-1/GIP coagonist peptide and 2

patients that receive placebo. Cohort 4 consists of 8 patients that receive 30 mg of the extended half-life GLP-1/GIP coagonist peptide and 2 patients that receive placebo.

[0153] The patients receive a total of 6 once per week SC doses of the study drug on Days 1, 8, 15, 22, 29, and 36. A meal tolerance test is performed on Days 1, 4, 36, 43, and 50. On these days, following an overnight fast (i.e., 10 hours), a blood sample is obtained for fasting insulin, glucose, glucagon, and C-peptide. Patients ingest a Sustacal breakfast meal (16 oz) over about 10 minutes. Blood samples for insulin, glucose, glucagon, and C-peptide are again obtained 10 minutes after the start of the Sustacal ingestion, continuing over a 3-hour period after the end of the Sustacal ingestion. Following the meal tolerance test, patients receive a single SC injection of study drug (except on Days 4 and 43). Serial timed blood samples for plasma PK are obtained prior to and after injection of the study drug.

[0154] Home capillary glucose values are recorded at least 6 times per week. Throughout the study, analytes are measured periodically, including chemistry analytes (e.g., alkaline phosphatase, amylase, sodium, potassium, total protein, calcium, chloride, bicarbonate, glucose, creatine phosphokinase, lactate dehydrogenase, alanine aminotransferase, albumin, aspartate aminotransferase, total/direct/indirect bilirubin, blood urea nitrogen, creatinin), hematology analytes (e.g., hemoglobin, hematocrit, red blood cell count, white blood cell count and differential, mean corpuscular hemoglobin concentration, reticulocyte counts, mean corpuscular volume, mean corpuscular hemoglobin, platelet counts), lipids (e.g., low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, total cholesterol, triglycerides), coagulation (e.g., activated partial thromboplastin time, prothrombin time, international normalized ratio), analytes obtain in a urinalysis (e.g,. pH, specific gravity, protein, glucose, leukocyte esterase, bilirubin, blood, nitrate, ketones), and follicle-stimulating hormone.

[0155] Pharmacokinetic variables are assessed. In addition, the pharmacodynamic variables that are assessed include insulin, glucose, glucagon, C-peptide, HbA_{1c}, and fructosamine. The administration of the extended half-life GLP-1/GIP coagonist peptide is expected to result in a biological response in one or more of these parameters. For example, an increase in insulin level, a decrease in glucose level, an increase in C-peptide level, a decrease in HbA1c level, a decrease in fructosamine level, and combinations thereof may be observed.

[0156] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0157] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted.

[0158] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range and each endpoint, unless otherwise indicated herein, and each separate value and endpoint is incorporated into the specification as if it were individually recited herein.

[0159] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0160] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

What is Claimed:

1. A method of reducing weight gain or inducing weight loss comprising administering to an adult human in need thereof an extended half-life GLP-1/GIP coagonist peptide at a weekly dosage of about 1 mg to about 40 mg.

2. The method of claim 1, wherein the weekly dosage is about 2 mg to about 10 mg.

3. The method of claim 1 or 2, wherein the adult human has excess body weight, diabetes mellitus type II, insulin resistance, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), or metabolic syndrome.

4. A method of treating hyperglycemia, reducing blood glucose levels, or normalizing blood glucose levels comprising administering to an adult human in need thereof an extended half-life GLP-1/GIP coagonist peptide at a weekly dosage of about 1 mg to about 40 mg per week.

5. The method of claim 4, wherein the weekly dosage is about 2 mg to about 10 mg

6. The method of claim 4 or 5, wherein the adult human has diabetes mellitus type I, diabetes mellitus type II, or gestational diabetes.

7. The method of any one of claims 1-6, wherein the extended half-life GLP-1/GIP coagonist peptide is administered once per week.

8. The method of any one of claims 1-6, wherein the extended half-life GLP-1/GIP coagonist peptide is administered twice per week.

9. The method of any one of the preceding claims, wherein the extended half-life GLP-1/GIP coagonist peptide exhibits a GLP-1 percentage potency within about 10-fold of the GIP percentage potency.

10. The method of any one of the preceding claims, wherein the extended half-life GLP-1/GIP coagonist peptide has an EC_{50} at the GLP-1 receptor within about 10-fold of the EC_{50} at the GIP receptor.

11. The method of any one of the preceding claims, wherein the extended half-life GLP-1/GIP coagonist peptide exhibits a GIP percentage potency of at least 1% or a GLP-1 percentage potency of at least 1%.

12. The method of any one of the preceding claims, wherein the extended half-life GLP-1/GIP coagonist peptide exhibits a half-life of about 4 days to about 7 days.

13. The method of any one of the preceding claims, wherein the extended half-life GLP-1/GIP coagonist peptide comprises a polyethylene glycol moiety that has a molecular weight of about 30,000 Daltons to about 60,000 Daltons.

14. The method of claim 13, wherein the polyethylene glycol moiety has a molecular weight of about 40,000 Daltons.

15. The method of any one of the preceding claims, wherein the extended half-life GLP-1/GIP coagonist peptide is any of SEQ ID NOs: 5-94, 99-169, 173-413, or an amino acid sequence that comprises up to 6 amino acid modifications compared to any of SEQ ID NOs: 5-94, 99-169, 173-413.

16. The method of claim 15, wherein the GLP-1/GIP coagonist peptide is any of SEQ ID NOs: 75, 99-103, 140, 153, 166, and 261, or an amino acid sequence that comprises up to 6 amino acid modifications compared to any of SEQ ID NOs: 75, 99-103, 140, 153, 166, and 261.

17. The method of any one of the preceding claims, further comprising administering a second therapeutic agent.

18. The method of claim 17, wherein the second therapeutic agent is an anti-diabetic agent.

19. The method of claim 18, wherein the second therapeutic agent is an anti-obesity agent.

20. The method of claim any one of the preceding claims, wherein the extended half-life GLP-1/GIP coagonist peptide is administered in a pharmaceutical composition.

21. The method of claim 20, wherein the pharmaceutical composition is administered via subcutaneous, intravenous, or intramuscular injection.

22. The method of claim 21, wherein the pharmaceutical composition is administered by subcutaneous injection.

23. The method of claims 1 or 4, wherein administration of the extended half-life GLP-1/GIP coagonist peptide results in an increase in insulin level, a decrease in glucose level, an increase in C-peptide level, a decrease in HbA_{1c} level, or a decrease in fructosamine level, or any combination thereof.

24. The method of any one of claims 20-23, wherein the extended half-life GLP-1/GIP coagonist peptide is administered from a pre-filled syringe.

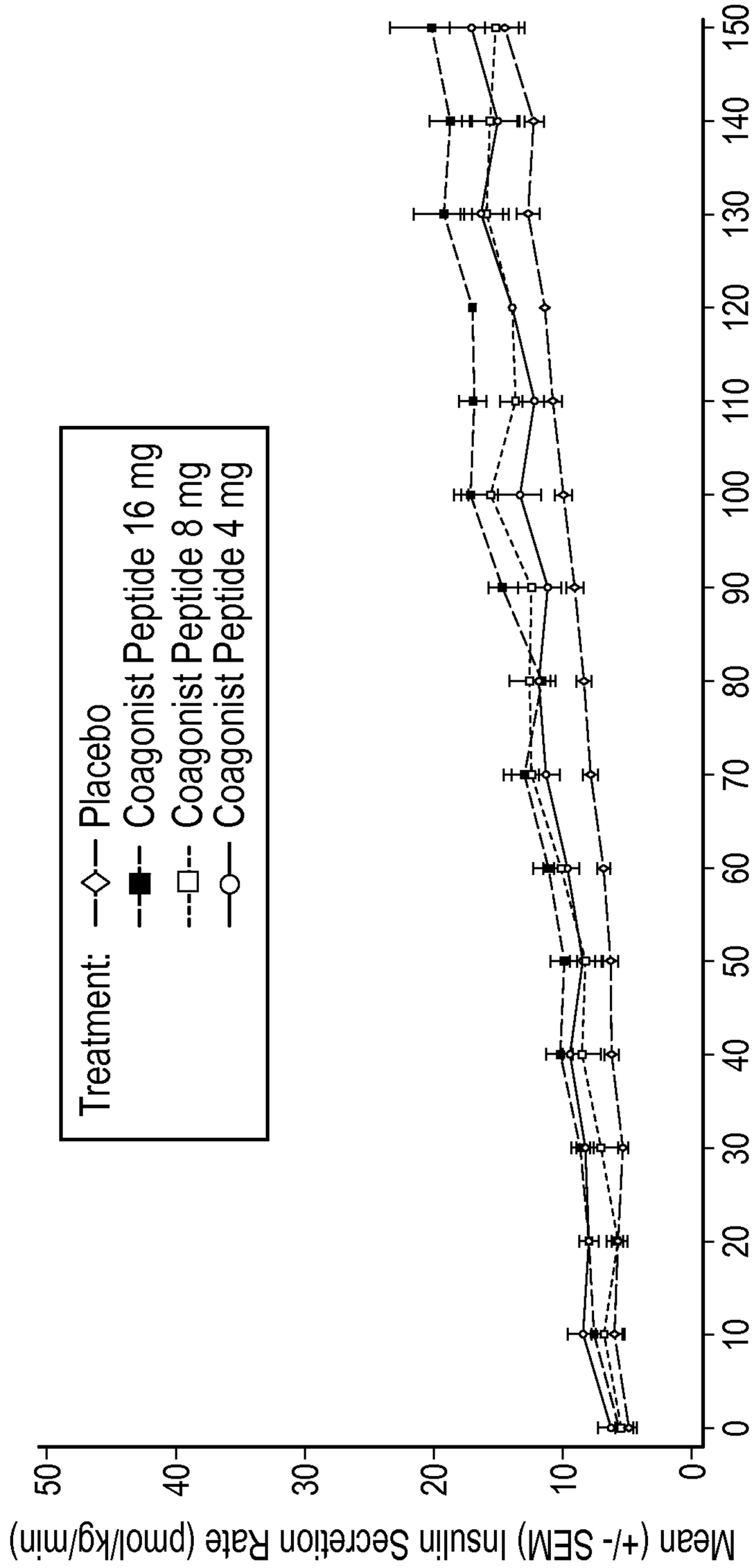

Treatment:
Placebo
Coagonist Peptide 16 mg
Coagonist Peptide 8 mg
Coagonist Peptide 4 mg
Mean (+/- SEM) Insulin Secretion Rate (pmol/kg/min)
0
10
20
30
40
50
Minutes
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150

FIG. 1

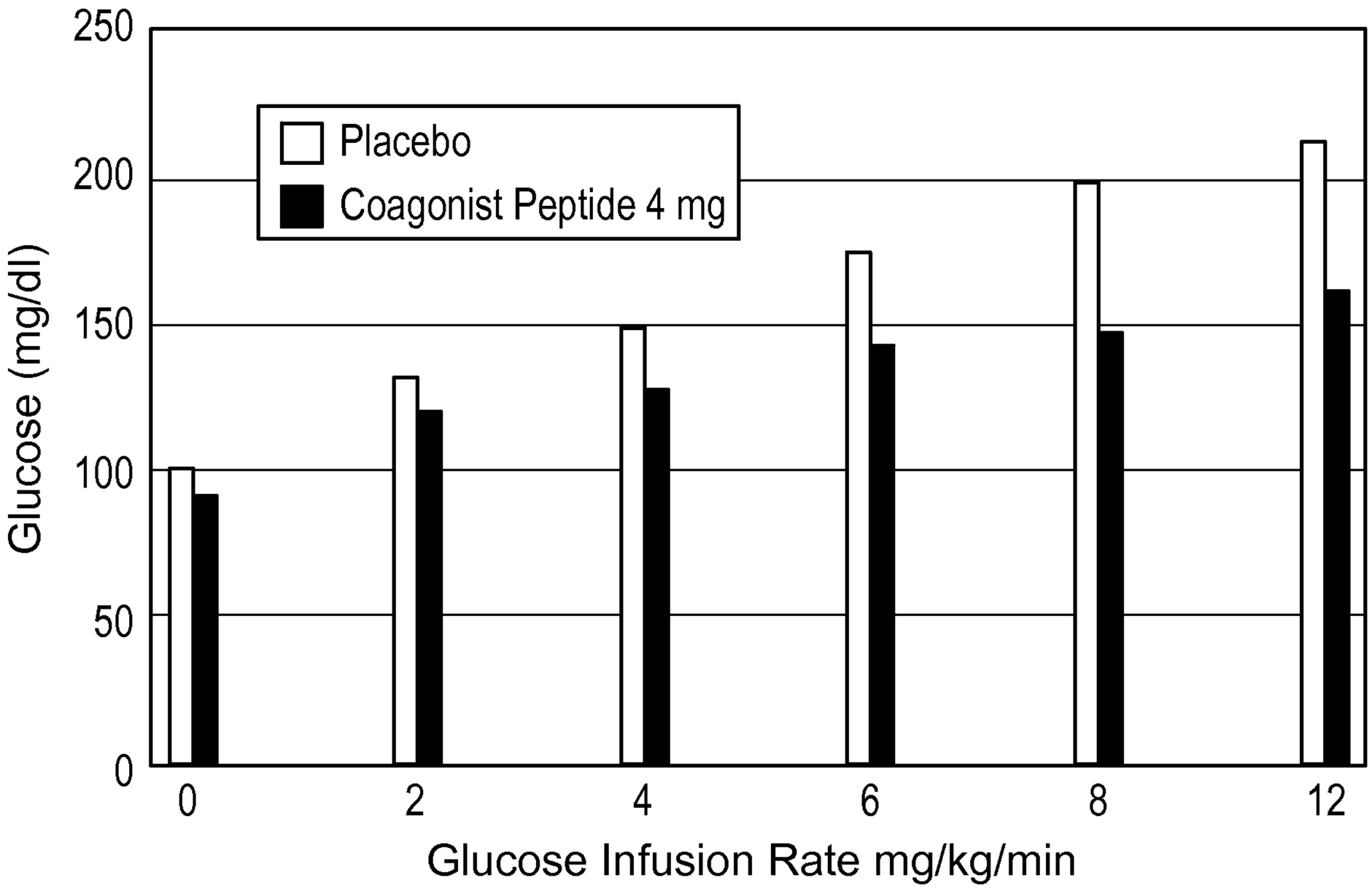

250
200
150
100
50
0
Placebo
Coagonist Peptide 4 mg
Glucose (mg/dl)
0
2
4
6
8
12
Glucose Infusion Rate mg/kg/min

FIG. 2A

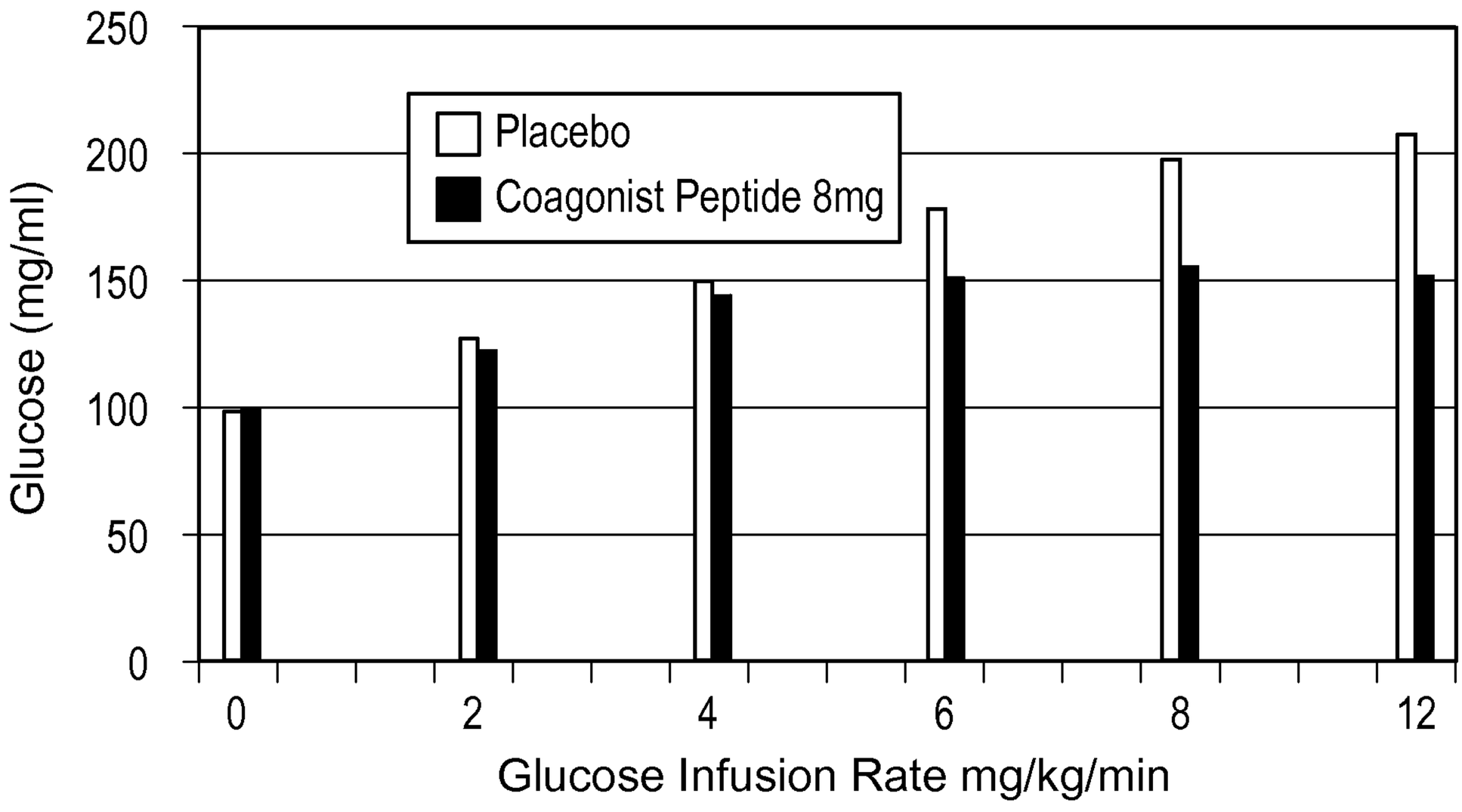

250
200
150
100
50
0
Glucose (mg/ml)
Placebo
Coagonist Peptide 8mg
0
2
4
6
8
12
Glucose Infusion Rate mg/kg/min

FIG. 2B

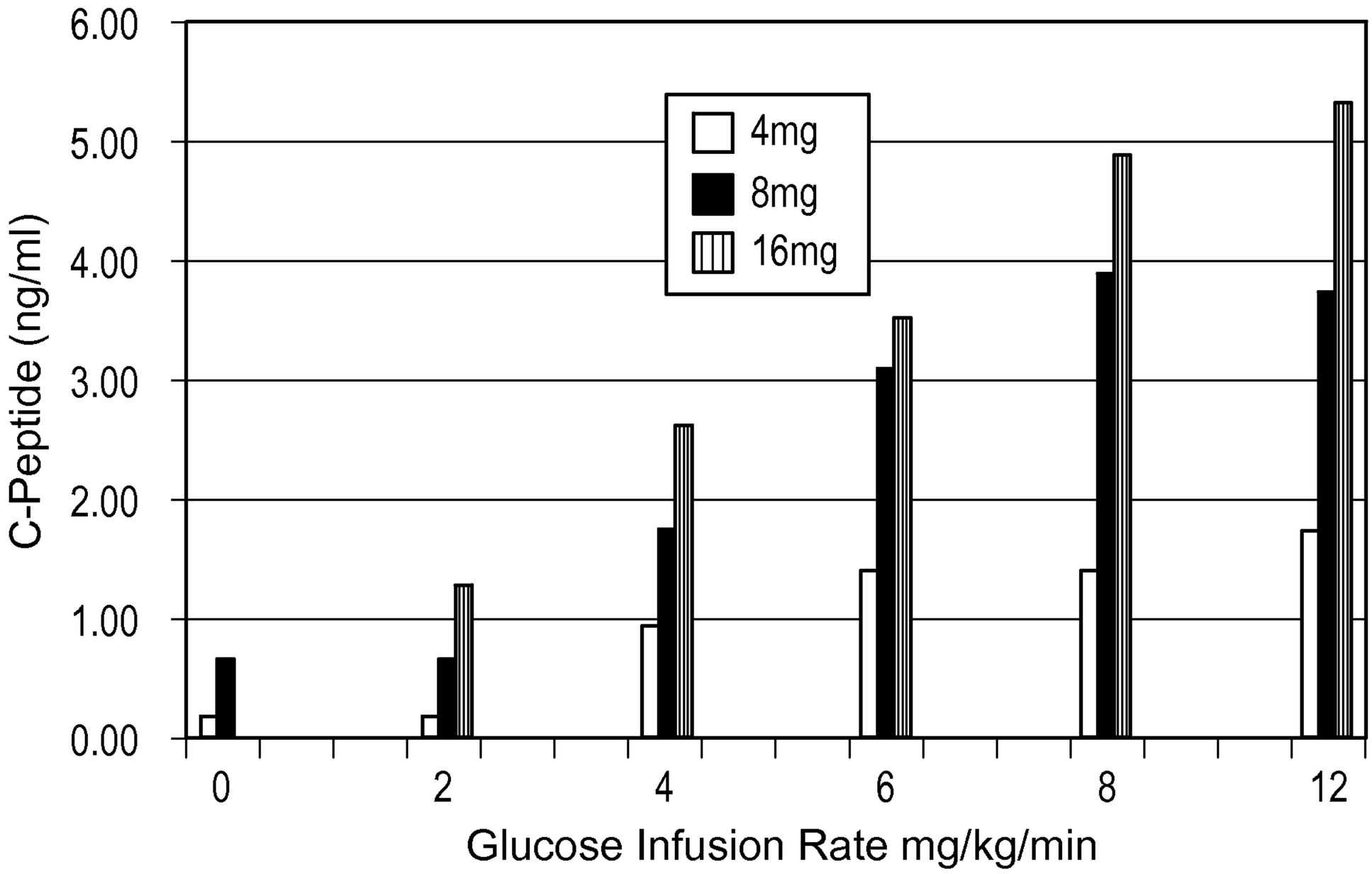

6.00
5.00
4.00
3.00
2.00
1.00
0.00
C-Peptide (ng/ml)
4mg
8mg
16mg
0
2
4
6
8
12
Glucose Infusion Rate mg/kg/min

FIG. 3

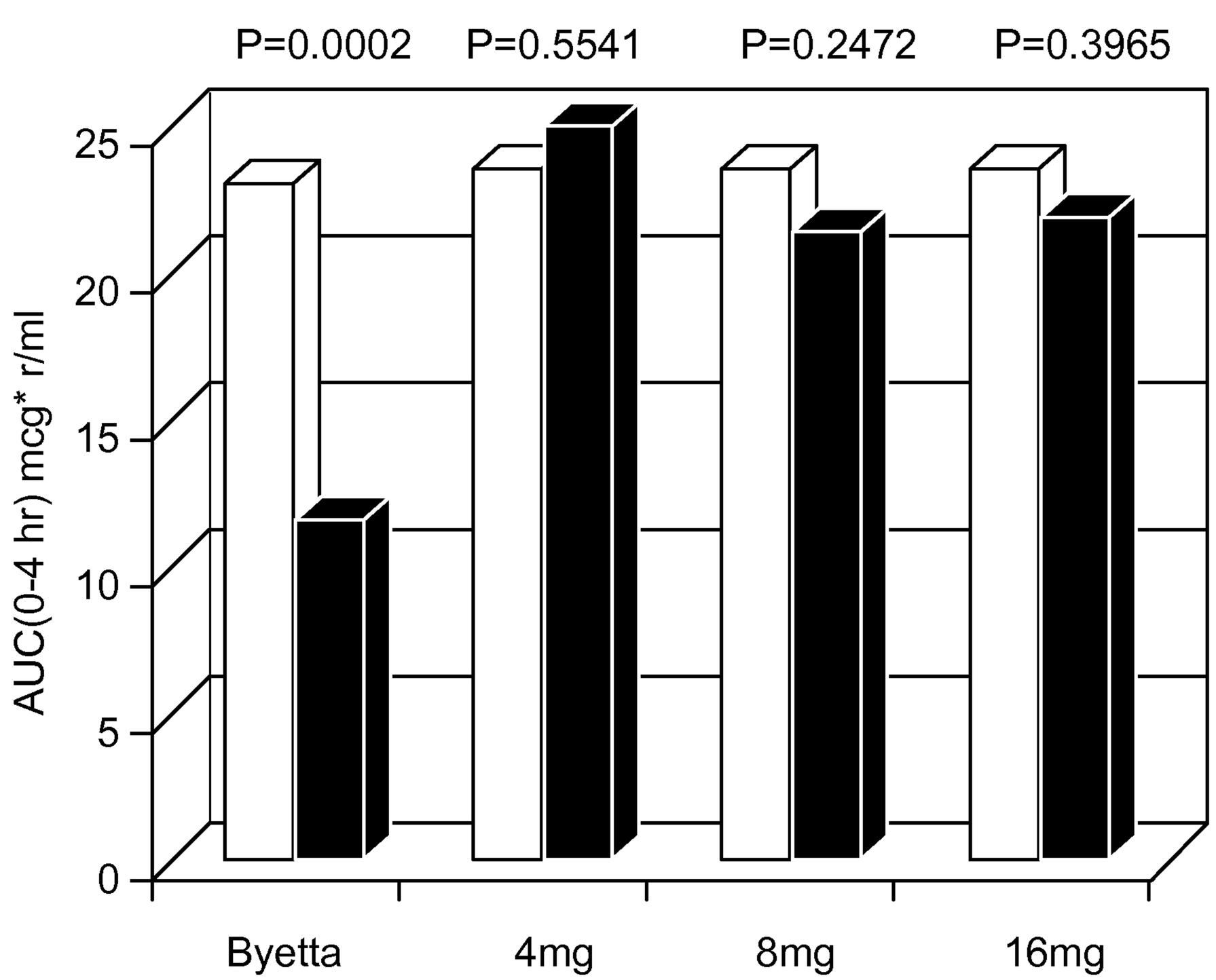

P=0.0002
P=0.5541
P=0.2472
P=0.3965
AUC(0-4 hr) mcg* r/ml
25
20
15
10
5
0
Byetta
4mg
8mg
16mg

FIG. 4