**Abstract**

Described are peptide analogs of glucagon, which have been modified to be resistant to cleavage and inactivation by dipeptidyl peptidase IV (DPP-IV) and to increase in vivo half-life of the peptide analog while enabling the peptide analog to have relatively balanced agonist activity at the glucagon-like peptide 1 (GLP-1) receptor and the glucagon (GCG) receptor, and the use of such GLP-1 receptor/GCG receptor co-agonists for treatment of metabolic disorders such as diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and obesity.
FIG. 1

Cumulative Body Weight

mean ± SEM (g)
FIG. 3

Cumulative Food Intake

Decrease (% Day 0)

Vehicle
SEQ 6, 9 nmol/kg
SEQ 7, 9 nmol/kg
SEQ 8, 9 nmol/kg
SEQ 8, 15 nmol/kg
SEQ 9, 9 nmol/kg
SEQ 9, 25 nmol/kg
SEQ 10, 3 nmol/kg
SEQ 10, 9 nmol/kg
SEQ 11, 3 nmol/kg
SEQ 11, 9 nmol/kg

*p<0.05, **p<0.01, ***p<0.001 vs Vehicle, 1 way ANOVA
CO-AGONISTS OF THE GLUCAGON AND GLP-1 RECEPTORS

CROSS REFERENCE TO RELATED APPLICATIONS


REFERENCE TO SEQUENCE LISTING

The sequence listing of the present application is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name “23848_US_NP_SEQLIST_13OC12015.txt”, creation date of Oct. 13, 2015, and a size of 13.5 Kb. This sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] (1) Field of the Invention

[0004] The present invention relates to peptide analogs of glucagon, which have been modified to be resistant to cleavage and inactivation by dipeptidyl peptidase IV (DPP-IV) and to increase in vivo half-life of the peptide analog while enabling the peptide analog to have relatively balanced agonist activity at the glucagon-like peptide 1 (GLP-1) receptor and the glucagon (GCG) receptor, and the use of such GLP-1 receptor/GCG receptor co-agonists for treatment of metabolic disorders such as diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and obesity.

[0005] (2) Description of Related Art

[0006] Pro-glucagon is a 158 amino acid precursor polypeptide that is processed in different tissues to form a number of different proglucagon-derived peptides, including glucagon, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2) and oxyntomodulin (OXM), that are involved in a wide variety of physiological functions, including glucose homeostasis, insulin secretion, gastric emptying, and intestinal growth, as well as the regulation of food intake. Glucagon is a 29-amino acid peptide that corresponds to amino acids 33 through 61 of pre-proglucagon. GLP-1 (7-36) amide or GLP-1 (7-37) acid are biologically potent forms of GLP-1, that demonstrate essentially equivalent activity at the GLP-1 receptor.

[0007] During hypoglycemia, when blood glucose levels drop below normal, glucagon signals the liver to break down glycogen and release glucose, causing blood glucose levels to rise toward a normal level. Hypoglycemia is a common side effect of insulin therapy in patients with hyperglycemia (elevated blood glucose levels) due to diabetes. Thus, glucagon’s most recognized role in glucose regulation is to counteract the action of insulin and maintain blood glucose levels.

[0008] GLP-1 has different biological activities compared to glucagon. Its actions include stimulation of insulin synthesis and secretion, inhibition of glucagon secretion, and inhibition of food intake. GLP-1 has been shown to reduce hyperglycemia in diabetes. Exendin-4, a peptide from lizard venom that shares about 50% amino acid identity with GLP-1, activates the GLP-1 receptor and likewise has been shown to reduce hyperglycemia in diabetics.

[0009] There is also evidence that GLP-1 and exendin-4 may reduce food intake and promote weight loss, an effect that would be beneficial not only for diabetics but also for patients suffering from obesity. Patients with obesity have a higher risk of diabetes, hypertension, hyperlipidemia, cardiovascular disease, and musculoskeletal diseases.

[0010] Glucagon is a peptide hormone structurally related to GLP-1 that is well recognized for its acute ability to increase blood glucose through stimulation of glycogenolysis and gluconeogenesis (Jiang & Zhang, Am. J. Physiol. Endocrinol. Metab. 284: E671-E678 (2003)). Of lesser appreciation are the chronic effects of glucagon pharmacology characterized by increases in thermogenesis, satiety, lipolysis, fatty acid oxidation, and ketogenesis (Habegger et al., Nat. Rev. Endocrinol. 6: 689-697 (2010)). Repeated administration of glucagon was first reported decades ago to yield improvements in rodent metabolism, accompanied with lower body weight (Saltiel, Am. J. Clin. Nutr. 8: 535-539 (1960)). Nonetheless, the inherent risk of hyperglycemia, especially in insulin resistant states such 12DM, has complicated the translation of these observations to human study.


[0012] In related studies, peripheral administration of OXM dose-dependently inhibited both fast-induced and dark phase food intake, but unlike GLP-1, had no effect on gastric emptying. OXM also reduced levels of fasting ghrelin and increased e-fos immunoreactivity, in the arcuate nucleus (ARC). Repeated seven-day IP administration of OXM caused a reduction in the rate of body weight gain and adiposity in rats (See Dakin et al. Endocrinology, 145: 2687-2695 (2004)).

[0013] Studies of OXM action in mice have demonstrated that although OXM can activate both the glucagon (GCG) and the GLP-1 receptors, the anorectic actions of OXM require only the GLP-1 receptor, as icv OXM inhibits food intake in glucagon receptor knockout mice. However, the anorectic effects of OXM are completely absent in GLP-1 receptor knockout mice. Furthermore, exendin-4, but not OXM, regulates energy expenditure in mice. Hence, OXM appears to be a weak agonist at the GLP-1 receptor, when used in pharmacological concentrations (See Baggio et al., Gastroenterol. 127: 546-58 (2004)). OXM was also found to ameliorate glucose intolerance in mice fed a high fat diet (Dakin et al., Am. J. Physiol. Endocrinol. Metab. 294: E142-E147 (2008)) and increase the intrinsic heart rate in mice independent of the GLP-1 receptor (Bowden et al., Am. J. Physiol. Regul. Integr.
In humans, a single 90-minute intravenous infusion of OXM in normal weight healthy subjects reduced hunger scores and food intake at a buffet meal by about 19%. Cumulative twelve-hour caloric intake was reduced by about 11% with no reports of nausea or changes in food palatability (Cohen et al., J. Clin. Endocrinol. Metab., 88: 4696-4701 (2003); Lykkegaard et al., ADA Scientific Sessions, Abstract #1506-P (2003)). More recently, pre-prandial injections of OXM over a four-week period in obese healthy subjects (BMI about 33) led to a significant reduction of caloric intake on the first day of treatment (about 25%) that was maintained over the course of the study (35% reduction after four weeks) (Wynne et al., Diabetes 54: 2390-2395 (2005)). Robust weight loss was observed at the end of the study in treated subjects (1.9%, placebo-corrected). Plasma levels of OXM were similar to that observed in the infusion study (peak concentration about 950 pM). The absence of any tachyphylaxis and a low incidence of mild and transient nausea (about 3%) despite the relatively high doses necessitated by the poor in vivo stability of OXM (plasma t1/2 12 minutes) renders this hormone one of the few obesity targets with both human validation and an attractive tolerability profile.

OXM has a very short half-life and is rapidly activated by the cell surface dipeptidyl peptidase IV (DPP-IV) (Zhu et al., J. Biol. Chem. 278: 22418-22423 (2002). However, DPP-IV inhibitors are weight-neutral in the clinic, suggesting that supraphysiologic levels of OXM (900-1000 pM) may be required to achieve weight loss in humans. OXM peptide analogs for inducing weight loss in humans have been the object of Published International Application Nos. WO2003/022304, WO2004/062685, WO2006/134340, and WO2010/096052.

Recently, two independent and simultaneous papers reported the use of relatively balanced GLP-1 receptor/GCG receptor co-agonists as being of enhanced efficacy and safety relative to pure GLP1R agonists in the treatment of rodent obesity, with simultaneous improvement in glycemic control (Day et al., Nat. Chem. Biol. 5: 749-757 (2009); Pocai et al., Diabetes 58: 2258-2266 (2009)). Of related significance is work with oxyntomodulin (OXM), an endogenous precursor to glucagon, which is secreted postprandially by L-cells of the jejunum-ileum together with GLP-1 (Holst, Regul. Pept. 93: 45-51 (2000); Drucker, Nat. Clin. Pract. Endocrinol. Metab. 1: 22-31 (2005)).

Glucagon peptide analogs and derivatives modified to have various degrees of activity at the GLP-1 receptor and GCG receptor have been disclosed in Published International Application Nos. WO2008/1010017, WO2009/155258, WO2011/075393, WO2012/174444, and WO2012/174443. Some of the disclosed glucagon peptide analogs were reported therein to have activity at both the GLP-1 receptor and GCG receptor; however, there remains a need for co-agonist peptides that have relatively balanced activity or potency at the GLP-1 receptor and GCG receptor.

BRIEF SUMMARY OF THE INVENTION

The invention provides peptides that are agonists of the glucagon (GCG) receptor and the glucagon-like peptide 1 (GLP-1) receptor and have a relatively balanced activity or potency at both receptors. Native glucagon normally has about 1% of the activity of native GLP-1 at the GLP-1 receptor. However, the GCG receptor/GLP-1 receptor co-agonist peptides of the invention have relatively balanced activity or potency at the GCG receptor and GLP-1 receptor. In particular aspects, the GCG receptor/GLP-1 receptor co-agonist peptides have an EC50 of about 0.02 to about 1.04 nM at the GCG receptor and an EC50 of about 0.03 to about 1.3 nM at the GLP-1 receptor. In particular aspects, the GCG receptor/GLP-1 receptor co-agonist peptides have a ratio of EC50 at the glucagon receptor to EC50 at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12. The peptides herein are useful for the treatment of metabolic disorders, such as but not limited to, diabetes (e.g., type 1 diabetes, type 2 diabetes, or gestational diabetes), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and/or obesity.

In a general embodiment, the GCG receptor/GLP-1 receptor co-agonist peptides of the present invention comprise the amino acid sequence of native human glucagon (SEQ ID NO:1) but in which the L-serine at position 2 is replaced with a D-serine, L-alanine, α-aminoisobutyric acid (Aib), or l-aminocyclobutane carboxylic acid; the tyrosine at position 10 is replaced with (i) L-lysine conjugated to a palmitoyl group by either a gamma-glutamyl acid (γE) spacer or a gamma-glutamyl acid-gamma-glutamyl acid dipeptide (γEγE) spacer; or (ii) para-aminomethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamyl acid-gamma-glutamyl acid dipeptide (γEγE) spacer; and may include up to six additional amino acid substitutions; wherein GCG receptor/GLP-1 receptor co-agonist peptides have a relatively balanced activity at the GCG receptor and GLP-1 receptor, and optionally a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

In particular embodiments, the present invention provides a peptide comprising the amino acid sequence

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\text{SEQ ID NO: 20}
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Wherein X2 is D-serine, L-alanine, α-aminoisobutyric acid (Aib), or L-aminocyclobutane carboxylic acid; X3 is L-phenylalanine, para-fluoro phenylalanine or three β-phenylserine; X10 is L-lysine or para-aminomethyl phenylalanine; X12 is L-lysine or L-leucine; X13 is L-glutamic acid or L-leucine; X14 is L-alanine, Aib, L-glutamic acid, or L-valine; X18 is L-alanine or L-arginine; X27 is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamyl acid (γE) spacer or a gamma-glutamyl acid-gamma-glutamyl acid dipeptide (γEγE) spacer; and, the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.
In particular embodiments, the peptide has the amino acid sequence of SEQ ID NO:7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, or 19.

In particular embodiments, the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC_{50} at the glucagon receptor to EC_{50} at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0×10^{-12}.

In particular embodiments, the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC_{50} at the glucagon receptor to EC_{50} at the GLP-1 receptor of 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.00, 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, or 1.10.

The present invention further provides a composition comprising a peptide having the amino acid sequence

Wherein X_{10} is D-serine, L-alanine, C. α-aminobutyric acid (Aib), or L-amicic acid; X_{11} is L-phenylalanine, para-fluoro phenylalanine or three-jp-phenylserine; X_{12} is L-lysine or para-aminomethyl phenylalanine; X_{13} is L-lysine or L-leucine; X_{14} is L-glutamic acid or L-leucine; X_{15} is L-lysine or L-arginine; X_{16} is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid spacer (γE) or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γE γE) spacer; the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; and, a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

Wherein X_{12} is D-serine, L-alanine, C. α-aminobutyric acid (Aib), or L-amicic acid; X_{13} is L-phenylalanine, para-fluoro phenylalanine or three-jp-phenylserine; X_{14} is L-lysine or para-aminomethyl phenylalanine; X_{15} is L-lysine or L-leucine; X_{16} is L-lysine or L-arginine; X_{17} is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid spacer (γE) or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γE γE) spacer; the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; and, a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

Wherein X_{12} is D-serine, L-alanine, C. α-aminobutyric acid (Aib), or L-amicic acid; X_{13} is L-phenylalanine, para-fluoro phenylalanine or three-jp-phenylserine; X_{14} is L-lysine or para-aminomethyl phenylalanine; X_{15} is L-lysine or L-leucine; X_{16} is L-lysine or L-leucine; X_{17} is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid spacer (γE) or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γE γE) spacer; the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; and, a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.
glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer; and up to six additional amino acid substitutions; wherein the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC_{50} at the glucagon receptor to EC_{50} at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12, and wherein the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxyl group of the peptide, and (ii) a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO.21.

[0036] In particular aspects, the serine at position 16 is replaced with L-alanine, the arginine at position 18 is replaced with L-alanine, the methionine at position 27 is replaced with L-leucine, the asparagine at position 28 is replaced with L-aspartic acid, or combinations thereof.

[0037] The present invention further provides a pharmaceutical composition comprising (i) a peptide having the amino acid sequence of native human glucagon as shown in SEQ ID NO.:1 but in which the L-serine at position 2 is replaced with a D-serine; the tyrosine at position 10 is replaced with (i) L-lysinine conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer or (ii) para-aminomethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer; and up to six additional amino acid substitutions; wherein the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC_{50} at the glucagon receptor to EC_{50} at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12, and wherein the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxyl group of the peptide, and (ii) a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO.21.

[0038] In particular aspects, the serine at position 16 is replaced with L-alanine, the arginine at position 18 is replaced with L-alanine, the methionine at position 27 is replaced with L-leucine, the asparagine at position 28 is replaced with L-aspartic acid, or combinations thereof.

[0039] The present invention further provides a method for treating a patient for metabolic disease comprising administering the patient an effective amount of the peptide of any one of the aforementioned peptides to treat the metabolic disease in the patient.

[0040] The present invention further provides method for treating a patient for metabolic disease comprising administering the patient an effective amount of the composition of the aforementioned compositions to treat the metabolic disease in the patient.

[0041] In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

[0042] In particular aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

[0043] In particular aspects, the patient has more than one metabolic disease, for example, diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.

[0044] The present invention further provides for the use of any one of the aforementioned peptides for manufacture of a medicament for the treatment of metabolic disease.

[0045] The present invention further provides for the use of any one of the aforementioned compositions for manufacture of a medicament for the treatment of metabolic disease.

[0046] In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

[0047] In particular aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

[0048] In particular aspects, the medicament is for treatment of more than one metabolic disease, for example, diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.

[0049] In particular aspects of the compounds disclosed herein the C-terminal protecting group may be an amide or ester. For example, the carboxylic acid of the C-terminal amino acid is replaced with a charge-neutral group, such as an amide or ester.

[0050] Further provided is method for treating a metabolic disease in a patient or individual comprising: administering to the patient or individual an effective amount of any one of the aforementioned compositions comprising a co-agonist peptide and administering to the patient or individual an effective amount of a composition comprising an insulin or insulin analog to treat the metabolic disease in the patient or individual.

[0051] In particular aspects, the composition comprising the co-agonist peptide is administered at a time prior to the time the composition comprising the insulin or insulin analog is administered. In another aspect, the composition comprising the insulin or insulin analog is administered at a time prior to the time the composition comprising the co-agonist peptide is administered. In a further aspect, the composition comprising the co-agonist peptide is administered at the same time as the composition comprising the insulin or insulin analog is administered.

[0052] In particular aspects, the insulin analog is insulin detemir, insulin glargine, insulin levenir, insulin glulisine, or insulin lispro.

[0053] In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

[0054] In particular aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

[0055] In particular aspects, the patient has more than one metabolic disease, for example, diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.

[0056] The present invention further provides a composition comprising any one of the aforementioned peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier.

[0057] The present invention further provides for the use of a composition comprising any one of the aforementioned peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier for the treatment of a metabolic disease.

[0058] In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic
steatohepatitis (NASH), or obesity. In further aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

[0059] The present invention further provides for the use of a composition comprising any one of the aforementioned peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier for the manufacture of a medicament for the treatment of a metabolic disease.

[0060] In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity. In further aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

[0061] In a further embodiment, the GCG receptor/GLP-1 receptor co-agonist peptides of the present invention consist of the amino acid sequence of native human glucagon (SEQ ID NO:1) but in which the L-serine at position 2 is replaced with a D-serine, L-alanine, α-aminoisobutyric acid (Aib), or L-1-amino-cyclobutane carboxylic acid; the tyrosine at position 10 is replaced with (i) L-lysine conjugated to a palmitoyl group by either a gamma-glutamic acid spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide spacer (γE) spacer, or (ii) para-aminomethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamic acid spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide spacer (γE) spacer; and may include up to six additional amino acid substitutions; wherein GCG receptor/GLP-1 receptor co-agonist peptides have a relatively balanced activity at the GCG receptor and GLP-1 receptor, and optionally a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

[0062] In particular embodiments, the present invention provides a peptide consisting of the amino acid sequence

**SEQ ID NO: 20**

Wherein X<sub>5</sub> is D-serine, L-alanine, α-aminoisobutyric acid (Aib), or 1-amino-cyclobutane carboxylic acid; X<sub>6</sub> is L-phenylalanine, para-fluoro phenylalanine or three-b-phe-nylsorine; X<sub>7</sub> is L-lysine or para-aminomethyl phenylalanine; X<sub>8</sub> is L-lysine or L-leucine; X<sub>9</sub> is L-glutamic acid or L-leucine; X<sub>10</sub> is L-alanine, Aib, L-glutamic acid, or L-va-line; X<sub>11</sub> is L-alanine or L-arginine; X<sub>12</sub> is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide spacer (γEγE) spacer; and the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

[0063] In particular embodiments, the peptide has the amino acid sequence of SEQ ID NO:7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, or 19.

[0064] In particular embodiments, the peptide is a co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC<sub>50</sub> at the glucagon receptor to EC<sub>50</sub> at the GLP-1 receptor of 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.00, 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, or 1.10.

[0066] The present invention further provides a composition consisting of a peptide having the amino acid sequence

**SEQ ID NO: 20**

Wherein X<sub>5</sub> is D-serine, L-alanine, α-aminoisobutyric acid (Aib), or 1-amino-cyclobutane carboxylic acid; X<sub>6</sub> is L-phenylalanine, para-fluoro phenylalanine or three-b-phe-nylsorine; X<sub>7</sub> is L-lysine or para-aminomethyl phenylalanine; X<sub>8</sub> is L-lysine or L-leucine; X<sub>9</sub> is L-glutamic acid or L-leucine; X<sub>10</sub> is L-alanine, Aib, L-glutamic acid, or L-va-line; X<sub>11</sub> is L-alanine or L-arginine; X<sub>12</sub> is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid spacer (γE) or a gamma-glutamic acid-gamma-glutamic acid dipeptide spacer (γEγE) spacer; the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; and, a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

[0067] In particular embodiments, the peptide has the amino acid sequence of SEQ ID NO:7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, or 19.

[0068] In particular embodiments, the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC<sub>50</sub> at the glucagon receptor to EC<sub>50</sub> at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.00 to 1.10.

[0069] In particular embodiments, the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC<sub>50</sub> at the glucagon receptor to EC<sub>50</sub> at the GLP-1 receptor of 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.00, 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, or 1.10.

[0070] The present invention further provides a pharmaceutical composition consisting of a peptide having the amino acid sequence

**SEQ ID NO: 20**

Wherein X<sub>5</sub> is D-serine, L-alanine, α-aminoisobutyric acid (Aib), or 1-amino-cyclobutane carboxylic acid; X<sub>6</sub> is L-phenylalanine, para-fluoro phenylalanine or three-b-phe-nylsorine; X<sub>7</sub> is L-lysine or para-aminomethyl phenylalanine; X<sub>8</sub> is L-lysine or L-leucine; X<sub>9</sub> is L-glutamic acid or L-leucine; X<sub>10</sub> is L-alanine, Aib, L-glutamic acid, or L-va-line; X<sub>11</sub> is L-alanine or L-arginine; X<sub>12</sub> is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid spacer (γE) or a gamma-glutamic acid-gamma-glutamic acid dipeptide spacer (γEγE) spacer; the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; and, a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.
[0071] In particular embodiments, the peptide has the amino acid sequence of SEQ ID NO:7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, or 19.

[0072] In particular embodiments, the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC_{25} at the glucagon receptor to EC_{50} at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12.

[0073] In particular embodiments, the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC_{25} at the glucagon receptor to EC_{50} at the GLP-1 receptor of about 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.00, 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, or 1.10.

[0074] The present invention further provides a peptide consisting of the amino acid sequence of native human glucagon having the amino acid sequence shown in SEQ ID NO:1 but in which the L-serine at position 2 is replaced with a D-serine; the tyrosine at position 10 is replaced with (i) L-lysine conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer or (ii) para-aminoethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer; and up to six additional amino acid substituitions; wherein the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC_{25} at the glucagon receptor to EC_{50} at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12, and wherein the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

[0075] In particular aspects, the serine at position 16 is replaced with D-alanine or L-alanine, the arginine at position 18 is replaced with L-alanine, the methionine at position 27 is replaced with L-leucine, and the asparagine at position 29 is replaced with L-leucine, or combinations thereof.

[0076] In particular aspects, the serine at position 16 is replaced with D-alanine or L-alanine, the arginine at position 18 is replaced with L-alanine, the methionine at position 27 is replaced with L-leucine, and the asparagine at position 29 is replaced with L-leucine.

[0077] The present invention further provides a composition consisting of (i) a peptide consisting of the amino acid sequence of native human glucagon as shown in SEQ ID NO:1 but in which the L-serine at position 2 is replaced with a D-serine; the tyrosine at position 10 is replaced with (i) L-lysine conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer or (ii) para-aminoethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer; and up to six additional amino acid substituitions; wherein the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC_{25} at the glucagon receptor to EC_{50} at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12, and wherein the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide, and (ii) a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

[0078] In particular aspects, the serine at position 16 is replaced with L-alanine, the arginine at position 18 is replaced with L-alanine, the methionine at position 27 is replaced with L-leucine, the asparagine at position 28 is replaced with L-aspartic acid, or combinations thereof.

[0079] The present invention further provides a pharmaceutical composition consisting of (i) a peptide consisting of the amino acid sequence of native human glucagon as shown in SEQ ID NO:1 but in which the L-serine at position 2 is replaced with a D-serine; the tyrosine at position 10 is replaced with (i) L-lysine conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer or (ii) para-aminoethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer; and up to six additional amino acid substituitions; wherein the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC_{25} at the glucagon receptor to EC_{50} at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12, and wherein the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide, and (ii) a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

[0080] In particular aspects, the serine at position 16 is replaced with L-alanine, the arginine at position 18 is replaced with L-alanine, the methionine at position 27 is replaced with L-leucine, the asparagine at position 28 is replaced with L-aspartic acid, or combinations thereof.

[0081] The present invention further provides a method for treating a patient for obesity metabolic disease consisting of administering the patient an effective amount of the peptide of any one of the aforementioned peptides to treat the metabolic disease in the patient.

[0082] The present invention further provides method for treating a patient for obesity metabolic disease consisting of administering the patient an effective amount of the composition of the aforementioned compositions to treat the metabolic disease in the patient.

[0083] In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

[0084] In particular aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

[0085] In particular aspects, the patient has more than one metabolic disease, for example, diabetes and NASH, NASH disease, obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.

[0086] The present invention further provides for the use of any one of the aforementioned peptides for manufacture of a medicament for the treatment of obesity metabolic disease.

[0087] The present invention further provides for the use of any one of the aforementioned compositions for manufacture of a medicament for the treatment of obesity metabolic disease.
In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity. In particular aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

In particular aspects, the medicament is for treatment of more than one metabolic disease, for example, diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.

In particular aspects of the compounds disclosed herein the C-terminal protecting group may be an amide or ester. For example, the carboxylic acid of the C-terminal amino acid is replaced with a charge-neutral group, such as an amide or ester.

Further provided is a method for treating a metabolic disease in a patient or individual consisting of: administering to the patient or individual an effective amount of any one of the aforementioned compositions consisting of a co-agonist peptide and administering to the patient or individual an effective amount of a composition consisting of an insulin or insulin analog to treat the metabolic disease in the patient or individual.

In particular aspects, the composition consisting of the co-agonist peptide is administered at a time prior to the time the composition consisting of the insulin or insulin analog is administered. In another aspect, the composition consisting of the insulin or insulin analog is administered at a time prior to the time the composition consisting of the co-agonist peptide is administered. In a further still aspect, the composition consisting of the co-agonist peptide is administered at the same time as the composition consisting of the insulin or insulin analog is administered.

In particular aspects, the insulin analog is insulin detemir, insulin glargine, insulin leemver, insulin glulisine, or insulin lispro.

In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

In particular aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

In particular aspects, the patient has more than one metabolic disease, for example, diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.

The present invention further provides a composition consisting of any one of the aforementioned peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier.

The present invention further provides for the use of a composition consisting of any one of the aforementioned peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier for the treatment of a metabolic disease.

In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity. In further aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

The present invention further provides for the use of a composition consisting of any one of the aforementioned peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier for the manufacture of a medicament for the treatment of a metabolic disease.
include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N'-dibenzythylene diamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethyl morpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydabamine, isopropylamine, lysine, methyglu camine, morpholine, piperazine, piperidine, polyamine resins, pyrrole, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like. The term “pharmacologically acceptable salt” further includes all acceptable salts such as acetate, lactobionate, benzenesulfonate, laurate, benzoate, malate, bicarbonate, maleate, bisulfate, mandelate, bitartrate, mesylate, borate, methy bromide, bromide, methyltinrate, calcium edetate, methylsul fate, camsylate, mucate, carbonate, napsylate, chloride, nitrate, clavulanate, N-methylglucamine, citrate, ammonium salt, dihydrochloride, oleate, edetate, oxalate, edisylate, pamoyte (embonate), esolate, palmitate, esylate, pantothenate, fumarate, phosphate/diphosphate, glucosate, polygalacturonate, gluconate, salicylate, glutamate, stearatate, glycolyl psallicate, sulfate, hexylresorcinolate, subocotate, hydabamine, succinate, hydrobromide, tannate, hydrochlor ide, tartrate, hydroxyphosphate, teoclates, iodide, tosylate, isothionate, triiodide, lactate, panolate, valerate, and the like which can be used as a dosage form for modifying the solubility or hydrolysis characteristics or can be used in sustained release or pro-drug formulations. It will be understood that, as used herein, references to the co-agonist peptides disclosed herein are meant to also include embodiments that comprise a co-agonist peptide or peptides and one or more of the pharmaceutically acceptable salts.

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.

As used herein an “effective” amount or a “therapeutically effective amount” means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. It refers to the amount of a co-agonist peptide or peptides that is nontoxic but sufficient to provide the desired effect. For example one desired effect would be the prevention or treatment of hyperglycemia, e.g., as measured by a change in blood glucose level closer to normal, or treatment of obesity by inducing weight loss and/or preventing weight gain, e.g., as measured by reduction in body weight, or preventing or reducing an increase in body weight, or normalizing body fat distribution. The amount that is “effective” will vary from subject to subject, depending on the age and general condition of the individual, mode of administration, and the like. Thus, it is not always possible to specify an exact “effective amount.” However, an appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially, or simultaneously.

The term, “parenteral” means not through the alimentary canal but by some other route, e.g., subcutaneous, intramuscular, intraspinal, or intravenous.

As used herein, the term “peptide” encompasses a chain of 3 or more amino acids and typically less than 100 amino acids, wherein the amino acids are naturally occurring or coded or non-naturally occurring or non-coded 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. “Non-coded” as used herein refers to an amino acid that is not an L-isomer of any of the following 20 amino acids: Ala, Cys, Asp, Glu, Phe, Gly, His, He, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr. “Coded” as herein refers to an amino acid that is an L-isomer of any of the following 20 amino acids: Ala, Cys, Asp, Glu, Phe, Gly, His, He, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr. In some embodiments, the peptides and variant peptides described herein are about the same length as SEQ ID NO: 1 (which is 29 amino acids in length), e.g. 25-35 amino acids in length. Exemplary lengths include 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids in length.

Typically, polypeptides and proteins have a polymer length that is greater than that of “peptides.”

Similarly, a reference herein to “position 28” would mean the corresponding position 29 for a glucagon analog in which one amino acid has been added before the N-terminus of SEQ ID NO: 1. As used herein an “amino acid modification” refers to (i) a substitution or replacement of an amino acid of SEQ ID NO: 1 with a different amino acid (naturally-occuring or coded or non-coded or non-naturally-occurring amino acid), (ii) an addition of an amino acid (naturally occurring or coded or non-coded or non-naturally-occurring amino acid), to SEQ ID NO: 1 or (iii) a deletion of one or more amino acids of SEQ ID NO: 1.

Amino acid “modification” refers to an insertion, deletion or substitution of one amino acid with another. In some embodiments, the amino acid substitution or replacement is a conservative amino acid substitution, e.g., a conservative substitution of the amino acid at one or more of positions 2, 5, 7, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 24, 27, 28 or 29. As used herein, the term “conservative amino acid substitution” is 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:

1. Small aliphatic, nonpolar or slightly polar residues:
   Ala, Ser, Thr, Pro, Gly;

2. Polar, negative-charged residues and their amides and esters:
   Asp, Asn, Glu, Gln, cysteic acid and homocysteic acid;

3. Polar, positive-charged residues:
   His, Arg, Lys; Ornithine (Orn)

4. Large, aliphatic, nonpolar residues:
   Met, Leu, He, Val, Cys, Norleucine (Nle), homocysteine
V. Large, aromatic residues: Phe, Tyr, Trp, acetyl phenylalanine

[0117] In some embodiments, the amino acid substitution is not a conservative amino acid substitution, e.g., is a non-conservative amino acid substitution.

[0118] As used herein the term “charged amino acid” or “charged residue” refers to an amino acid that comprises a side chain that is negative-charged (i.e., de-protonated) or positive-charged (i.e., protonated) in aqueous solution at physiological pH. For example negative-charged amino acids include aspartic acid, glutamic acid, cysteic acid, homocysteic acid, and homoglutamic acid, whereas positive-charged amino acids include arginine, lysine and histidine. Charged amino acids include the charged amino acids among the 20 coded amino acids, as well as atypical or non-naturally occurring or non-coded amino acids.

[0119] As used herein the term “acidic amino acid” refers to an amino acid that comprises a second acidic moiety (other than the carboxylic acid of the amino acid), including for example, a carboxylic acid or sulfonic acid group.

[0120] As used herein, the term “acylated amino acid” refers to an amino acid comprising an acyl group which is non-native to a naturally-occurring amino acid, regardless of the means by which it is produced (e.g., acylation prior to incorporation into the amino acid into a peptide, or acylation after incorporation into a peptide).

[0121] As used herein the term “alkylated amino acid” refers to an amino acid comprising an alkyl group which is non-native to a naturally-occurring amino acid, regardless of the means by which it is produced. Accordingly, the alkylated amino acids and alkylated amino acids of the present disclosures are non-coded amino acids.

[0122] 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.

[0123] As used herein the term “native glucagon” refers to a peptide consisting of the sequence of SEQ ID NO: 1 and the term “native GLP-1” is a generic term that designates GLP-1 (7-36) amide, GLP-1 (7-37) or a mixture of those two compounds.

[0124] As used herein, “glucagon potency” or “potency compared to native glucagon” of a molecule refers to the inverse ratio of the EC_{50} of the molecule at the glucagon receptor divided by the EC_{50} of native glucagon at glucagon receptor.

[0125] As used herein, “GLP-1 potency” or “potency compared to native GLP-1” of a molecule refers to the inverse ratio of the EC_{50} of the molecule at GLP-1 receptor divided by the EC_{50} of native GLP-1 at GLP-1 receptor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0126] FIG. 1 represents a graph of the cumulative weight change (grams) of diet-induced obese (DIO) mice treated with a vehicle control or a daily dose of a peptide of SEQ ID NO: 6 to 11, as detailed in Example 18.

[0127] FIG. 2 represents a graph of the cumulative body weight change (%) relative to a vehicle treated control group of DIO mice treated with a daily dose of a peptide of SEQ ID NO: 6 to 11, as detailed in Example 19.

[0128] FIG. 3 represents a graph of the cumulative change in food intake (expressed as a percent change of food intake relative to Day 0) of DIO mice treated with a vehicle control or with a dose of a peptide of SEQ ID NO: 6 to 11, as detailed in Example 20.

[0129] FIG. 4 represents a graph of the basal glucose (mg/dL) of DIO mice treated with a vehicle control or with a dose of a peptide of SEQ ID NO: 6 to 11, as detailed in Example 21.

DETAILED DESCRIPTION OF THE INVENTION

[0130] The present disclosures provide peptides and variant peptides that exhibit activity at both the GLP-1 receptor (GLP-1) and at the glucagon (GCG) receptor. In this regard, the present disclosures provide GLP-1 receptor/GCG receptor co-agonist peptides. In exemplary embodiments, the presently disclosed peptides and variant peptides exhibit relatively balanced activity or potency at the GCG receptor and the GLP-1 receptors, as compared to native human glucagon (SEQ ID NO: 1).

[0131] In exemplary embodiments, the peptides and variant peptides described herein exhibit other improvements in properties relative to native glucagon or native GLP-1, such as greater stability, greater solubility, a prolonged half-life in circulation, a delayed onset of action, an extended duration of action, a damped peak (e.g., relatively decreased mean peak plasma concentration), and an improved resistance to proteases, such as DPP-IV.

[0132] Conjugates, fusion proteins and multimers of any of the peptide sequences disclosed herein are also contemplated.

[0133] Glucagon is a peptide hormone structurally related to GLP-1 that is well recognized for its acute ability to increase blood glucose through stimulation of glycogenolysis and gluconeogenesis. While administration of glucagon was first reported over 60 years ago to yield improvements in rodent metabolism, including lowering body weight (Saltz, Am. J. Clin. Nutr. 9: 535-539 (1960)) these results have not been translated into the use of glucagon in therapies for a treatment of obesity in humans, particularly due to the inherent risk of hyperglycemia, especially in insulin-resistant type-2 diabetic patients.

[0134] The use of balanced GCG receptor/GLP-1 receptor co-agonists as being of enhanced efficacy and safety relative to pure GLP-1 receptor agonists in the treatment of rodent obesity, with simultaneous improvement in glycemic control (Day et al., Nat. Chem. Biol. 5: 749-757 (2009); Pocai et al., Diabetes 58: 2258-2266 (2009)). Oxyntomodulin (OXM) is an endogenous precursor to glucagon, which is secreted post-prandially by L-cells of the jejunum-ilium together with GLP-1 and has been shown to be a balanced co-agonist at the GLP-1 receptor and glucagon receptor albeit of relatively low potency (Holst, Regul. Pept. 93: 45-51 (2000); Drucker, J. Nat. Clin. Pract. Endocrinol. Metab. 1: 22-31 (2005); Baldiiserra et al., Regul. Pept. 21: 151-166 (1988); Gros et al., Endocrinol. 133: 631-638 (1993); Pocai et al., op. cit.). A 4-week clinical study in obese subjects demonstrated that repeated subcutaneous administration of OXM was well tolerated and caused significant weight loss, with a concomitant reduction in food intake (Wynne et al., Diabetes 54: 2390-2395 (2005)).

[0135] While Day et al., Peptide Sci. 98: 443-450 (2012) reported the importance of GLP-1 receptor balance in preventing GCG receptor-mediated hyperglycemia in obese mice using a family of co-agonist peptides that vary in murine receptor potency for the two receptors, they do not disclose
co-agonist peptides that have the balanced potency or activity of the co-agonists peptides disclosed herein.

[0136] In contrast, the GCG receptor/GLP-1 receptor co-agonist peptides of the invention have relatively balanced activity or potency at the GCG receptor and GLP-1 receptor. The present invention provides peptides comprising the amino acid sequence of native human glucagon having the amino acid sequence shown in SEID NO:1, but in which the L-serine at position 2 is replaced with a D-serine; the tyrosine at position 10 is replaced with (i) L-lysin conjugated to a palmitoyl group by either a gamma-glutamyl acid (γE) spacer or a gamma-glutamyl-acid-gamma-glutamyl acid dipeptide (γEγE) spacer or (ii) para-aminoethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamyl acid (γE) spacer or a gamma-glutamyl-acid-gamma-glutamyl acid dipeptide (γEγE) spacer; and up to six additional amino acid substitutions; wherein the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC50 at the glucagon receptor to EC50 at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.10, or about 0.95 to about 1.00, or about 1.06, 1.07, 1.08, 1.09, or 1.10. The present invention further provides peptides comprising the amino acid sequence

[0137] The present invention further provides peptides comprising the amino acid sequence

<table>
<thead>
<tr>
<th>SEQ ID NO: 20</th>
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<tbody>
<tr>
<td>Hx1QTX4Sx4X2Y1Lx1X1Rx4QPx2Qx1Lx1D</td>
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</table>

Wherein X1 is D-serine, L-alanine, α-aminoisonobutyric acid (Aib), or L-α-methylcyclohexane carboxylic acid; X2 is L-phenylalanine, para-fluoro phenylalanine or three-[β-phenylserine]; X3 is L-lysine or para-aminoethyl phenylalanine; X4 is L-lysine or L-α-methylcyclohexane carboxylic acid; X5 is L-lysine or L-α-methylcyclohexane carboxylic acid; X6 is L-α-methylcyclohexane carboxylic acid; X7 is L-lysine; methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamyl acid (γE) spacer or a gamma-glutamyl-acid-gamma-glutamyl acid dipeptide (γEγE) spacer; and, the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; with the proviso that the peptide does not have the amino acid sequence of SEID NO:21. In particular aspects, the peptides have a ratio of EC50 at the glucagon receptor to EC50 at the GLP-1 receptor of 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.00, 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, or 1.10.

[0138] The present invention further provides peptides comprising or consisting of the amino acid sequence

[0139] Examples of peptides of the present invention are shown in Table 1.

<table>
<thead>
<tr>
<th>SEQ ID NO: Peptide</th>
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<tbody>
<tr>
<td>6</td>
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<td>19</td>
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</tbody>
</table>

* = D-serine; γ = γ-aminoisonobutyric acid (Aib); K = norleucine (Nle); F = para-aminoethyl phenylalanine; pFF = para-fluoro phenylalanine; γ = γ-aminoisonobutyric acid (Aib); β = threo-[β-phenylserine]; Ac = acetyl; PFP = p-fluorophenylalanine
As shown in the examples, the GCG receptor/ GLP-1 receptor co-agonist peptides of the present invention have an EC$_{50}$ of about 0.02 to about 1.04 nM at the GCG receptor and an EC$_{50}$ of about 0.03 to about 1.3 nM at the GLP-1 receptor. In particular aspects, the GCG receptor/ GLP-1 receptor co-agonist peptides have a ratio of EC$_{50}$ at the glucagon receptor to EC$_{50}$ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12. Thus, the present invention provides peptides with relative balanced agonist activities or potencies against GLP-1 receptor and GCG receptor and which display the optimal therapeutic profile for weight loss while mitigating the hyperglycemic risk associated with GCG receptor activation.

The present invention provides compositions comprising or consisting of one or more peptides comprising the amino acid sequence of native human glucagon having the primary structure sequence shown in SEQ ID NO:1 but in which the L-serine at position 2 is replaced with a D-serine; the tyrosine at position 10 is replaced with (i) L-lysine conjugated to a palmitoyl group by either a gamma-glutamatic acid (γE) spacer or a gamma-glutamatic acid-gamma-glutamatic acid dipeptide (γEγE) spacer or (ii) para-aminomethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamatic acid (γE) spacer or a gamma-glutamatic acid-gamma-glutamatic acid dipeptide (γEγE) spacer; and up to six additional amino acid substitutions; wherein the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC$_{50}$ at the glucagon receptor to EC$_{50}$ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12, and wherein the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

In particular aspects, the serine at position 16 is replaced with D-alanine or L-alanine, the arginine at position 18 is replaced with D-alanine, the methionine at position 27 is replaced with L-leucine, the asparagine at position 29 is replaced with L-leucine, or combinations thereof.

In particular aspects, the serine at position 16 is replaced with D-alanine or L-alanine, the arginine at position 18 is replaced with D-alanine, the methionine at position 27 is replaced with L-leucine, and the asparagine at position 29 is replaced with L-leucine.

The present invention further provides a composition comprising (i) a peptide having or consisting of the amino acid sequence of native human glucagon as shown in SEQ ID NO:1 but in which the L-serine at position 2 is replaced with a D-serine; the tyrosine at position 10 is replaced with (i) L-lysine conjugated to a palmitoyl group by either a gamma-glutamatic acid (γE) spacer or a gamma-glutamatic acid-gamma-glutamatic acid dipeptide (γEγE) spacer or (ii) para-aminomethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamatic acid (γE) spacer or a gamma-glutamatic acid-gamma-glutamatic acid dipeptide (γEγE) spacer; and up to six additional amino acid substitutions; wherein the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC$_{50}$ at the glucagon receptor to EC$_{50}$ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12, and wherein the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; and (ii) a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

The present invention further includes compositions comprising or consisting of one or more of the peptides shown in Table 1 and a pharmaceutically acceptable carrier.
Pharmaceutical Compositions

[0148] Further provided are pharmaceutical compositions comprising a therapeutically effective amount of one or more of the co-agonist peptides disclosed herein for the treatment of a metabolic disorder in an individual. Such disorders include, but are not limited to, obesity, metabolic syndrome or syndrome X, type II diabetes, complications of diabetes such as retinopathy, hypertension, dyslipidemias, cardiovascular disease, gallstones, osteoarthritis, and certain forms of cancers. The obesity-related disorders herein are associated with, caused by, or result from obesity.

[0149] “Obesity” is a condition in which there is an excess of body fat. The operational definition of obesity is based on the Body Mass Index (BMI), calculated as body weight per height in meters squared (kg/m²). “Obesity” refers to a condition whereby an otherwise healthy subject has a Body Mass Index (BMI) greater than or equal to 30 kg/m², or a condition whereby a subject with at least one co-morbidity has a BMI greater than or equal to 27 kg/m². An “obese subject” is an otherwise healthy subject with a Body Mass Index (BMI) greater than or equal to 30 kg/m² or a subject with at least one co-morbidity with a BMI greater than or equal to 27 kg/m². A “subject at risk for obesity” is an otherwise healthy subject with a BMI of 25 kg/m² to less than 30 kg/m² or a subject with at least one co-morbidity with a BMI of 25 kg/m² to less than 27 kg/m².

[0150] The increased risks associated with obesity occur at a lower Body Mass Index (BMI) in Asians. In Asian countries, including Japan, “obesity” refers to a condition whereby a subject with at least one obesity-induced or obesity-related co-morbidity that requires weight reduction or that would be improved by weight reduction, has a BMI greater than or equal to 25 kg/m². In Asian countries, including Japan, an “obese subject” refers to a subject with at least one obesity-induced or obesity-related co-morbidity that requires weight reduction or that would be improved by weight reduction, with a BMI greater than or equal to 25 kg/m². In Asian countries, a “subject at risk of obesity” is a subject with a BMI of greater than 23 kg/m² to less than 25 kg/m².

[0151] As used herein, the term “obesity” is meant to encompass all of the above definitions of obesity.

[0152] Obesity-induced or obesity-related co-morbidities include, but are not limited to, diabetes, non-insulin dependent diabetes mellitus—type 2, impaired glucose tolerance, impaired fasting glucose, insulin resistance syndrome, dyslipidemia, hypertension, hyperuricemia, gout, coronary artery disease, myocardial infarction, angina pectoris, sleep apnea syndrome, Pickwickian syndrome, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), fatty liver, cerebral infarction, cerebral thrombosis, transient ischemic attack, orthopedic disorders, arthritis deformans, lumbodynia, enmeniopathy, and infertility. In particular, co-morbidities include: hypertension, hyperlipidemia, dyslipidemia, glucose intolerance, cardiovascular disease, sleep apnea, diabetes mellitus, and other obesity-related conditions.

[0153] “Treatment” (of obesity and obesity-related disorders) refers to the administration of the compounds of the present invention to reduce or maintain the body weight of an obese subject. One outcome of treatment may be reducing the body weight of an obese subject relative to that subject’s body weight immediately before the administration of the compounds of the present invention. Another outcome of treatment may be preventing body weight regain of body weight previously lost as a result of diet, exercise, or pharmacotherapy. Another outcome of treatment may be decreasing the occurrence of and/or the severity of obesity-related diseases. The treatment may suitably result in a reduction in food or calorie intake by the subject, including a reduction in total food intake, or a reduction of intake of specific components of the diet such as carbohydrates or fats; and/or the inhibition of nutrient absorption; and/or the inhibition of the reduction of metabolic rate; and in weight reduction in patients in need thereof. The treatment may also result in an alteration of metabolic rate, such as an increase in metabolic rate, rather than or in addition to an inhibition of the reduction of metabolic rate; and/or in minimization of the metabolic resistance that normally results from weight loss.

[0154] “Prevention” (of obesity and obesity-related disorders) refers to the administration of the compounds of the present invention to reduce or maintain the body weight of a subject at risk of obesity. One outcome of prevention may be reducing the body weight of a subject at risk of obesity relative to that subject’s body weight immediately before the administration of the compounds of the present invention. Another outcome of prevention may be preventing body weight regain of body weight previously lost as a result of diet, exercise, or pharmacotherapy. Another outcome of prevention may be preventing obesity from occurring if the treatment is administered prior to the onset of obesity in a subject at risk of obesity. Another outcome of prevention may be decreasing the occurrence and/or severity of obesity-related disorders if the treatment is administered prior to the onset of obesity in a subject at risk of obesity. Moreover, if treatment is commenced in already obese subjects, such treatment may prevent the occurrence, progression or severity of obesity-related disorders, such as, but not limited to, arteriosclerosis, type II diabetes, polycystic ovarian disease, cardiovascular diseases, osteoarthritis, dermatological disorders, hypertension, insulin resistance, hypercholesterolemia, hypertriglyceridemia, and cholelithiasis.

[0155] The obesity-related disorders herein are associated with, caused by, or result from obesity. Examples of obesity-related disorders include overeating and bulimia, hypertension, diabetes, elevated plasma insulin concentrations and insulin resistance, dyslipidemias, hyperlipidemia, endometrial, breast, prostate and colon cancer, osteoarthritis, obstructive sleep apnea, cholelithiasis, gallstones, heart disease, abnormal heart rhythms and arrhythmias, myocardial infarction, congestive heart failure, coronary heart disease, sudden death, stroke, polycystic ovarian disease, cranopharyngioma, the Prader-Willi Syndrome, Frahlich’s syndrome, GH-deficient subjects, normal variant short stature, Turner’s syndrome, and other pathological conditions showing reduced metabolic activity or a decrease in resting energy expenditure as a percentage of total fat-free mass, e.g., children with acute lymphoblastic leukemia. Further examples of obesity-related disorders are metabolic syndrome, also known as syndrome X, insulin resistance syndrome, sexual and reproductive dysfunction, such as infertility, hypogonadism in males and hirsutism in females, gastrointestinal motility disorders, such as obesity-related gastro-esophageal reflux, respiratory disorders, such as obesity-hypoventilation syndrome (Pickwickian syndrome), cardiovascular disorders, inflammation, such as systemic inflammation of the vasculature, arteriosclerosis, hypercholesterolemia, hyperuricaemia, lower back pain, gallbladder disease, gout, and kidney cancer. The compounds of the present invention are
also useful for reducing the risk of secondary outcomes of obesity, such as reducing the risk of left ventricular hypertrophy.

[0156] The term “diabetes,” as used herein, includes both insulin-dependent diabetes mellitus (IDDM, also known as type I diabetes) and non-insulin-dependent diabetes mellitus (NIDDM, also known as Type II diabetes). Type I diabetes, or insulin-dependent diabetes, is the result of an absolute deficiency of insulin, the hormone which regulates glucose utilization. Type II diabetes, or insulin-independent diabetes (i.e., non-insulin-dependent diabetes mellitus), often occurs in the face of normal, or even elevated levels of insulin and appears to be the result of the inability of tissues to respond appropriately to insulin. Most of the Type II diabetics are also obese. The compounds of the present invention are useful for treating both Type I and Type II diabetes. The compounds are especially effective for treating Type II diabetes. The compounds of the present invention are also useful for treating and/or preventing gestational diabetes mellitus.

[0157] U.S. Pat. No. 6,852,690, which is incorporated herein by reference in its entirety, discloses methods for enhancing metabolism of nutrients comprising administering to a non-diabetic patient a formulation comprising a nutritively effective amount of one or more nutrients or any combination thereof and one or more insulinotropic peptides. The co-agonist peptides disclosed herein are insulinotropic and can be administered to patients with a disturbed glucose metabolism such as insulin resistance but no overt diabetes, as well as patients who for any reason cannot receive nutrition through the alimentary canal. Such patients include surgery patients, comatose patients, patients in shock, patients with gastrointestinal disease, patients with digestive hormone disease, and the like. In particular, obese patients, atherosclerotic patients, vascular disease patients, patients with gestational diabetes, patients with liver disease such as liver cirrhosis, patients with acromegaly, patients with glucocorticoid excess such as cortisol treatment or Cushings disease, patients with activated counterregulatory hormones such as would occur after trauma, accidents and surgery and the like, patients with hypertriglyceridemia and patients with chronic pancreatitis can be readily and suitably nourished according to the invention without subjecting the patient to hyper- or hyperglycemia. In particular, the administration to such a patient aims to provide a therapy to as rapidly as possible deliver the nutritional and caloric requirements to the patient while maintaining his plasma glucose below the so-called renal threshold of about 160 to 180 milligrams per deciliter of glucose in the blood. Although normal patients not having glucose levels just below the renal threshold can also be treated according to the invention as described above, patients with disturbed glucose metabolism such as hyperglycemic patients whose plasma glucose level is just above the renal threshold also find the therapy suitable for their condition. In particular, such patients who have a degree of hyperglycemia below the renal threshold at intermittent intervals can receive a combination treatment of nutrients plus insulinotropic peptides according to any of the following regimens. Normal patients not suffering from such hyperglycemia can also be treated using the peptide analogs disclosed herein.

[0158] The co-agonist peptides disclosed herein may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such compositions comprise a therapeutically-effective amount of one or more of the co-agonist peptides disclosed herein and a pharmaceutically acceptable carrier. Such a composition may also be comprised of (in addition to the co-agonist peptides disclosed herein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. Compositions comprising the co-agonist peptides disclosed herein can be administered, if desired, in the form of salts provided the salts are pharmaceutically acceptable. Salts may be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry. The co-agonist peptides disclosed herein may be in multimers (for example, heterodimers or homodimers) or complexes with itself or other peptides. As a result, pharmaceutical compositions of the invention may comprise one or more co-agonist peptides disclosed herein in such multimeric or complexed form.

[0159] The pharmacological composition can comprise one or more co-agonist peptides disclosed herein; one or more co-agonist peptides disclosed herein and one or more other agents for treating a metabolic disorder; or the pharmacological composition comprising the one or more co-agonist peptides disclosed herein can be used concurrently with a pharmacological composition comprising an agent for treating a metabolic disorder. Such disorders include, but are not limited to, obesity, metabolic syndrome or syndrome X, type II diabetes, complications of diabetes, hypertension, dyslipidemias, cardiovascular disease, gallstones, osteoarthritis, and certain forms of cancers.

[0160] When the pharmacological composition comprises another agent for treating a metabolic disorder or the treatment includes a second pharmacological composition comprising an agent for treating a metabolic disorder, the agent includes, but are not limited to, cannabinoid (CB1) receptor antagonists, glucagon like peptide 1 (GLP-1) receptor agonists, glucagon receptor antagonists, lipase inhibitors, leptin, tetrahydrofipstatin, 2,4-dinitrophenol, acarbose, sibutramine, phentermine, fat absorption blockers, simvastatin, mevastatin, ezetimibe, atorvastatin, sitagliptin, metformin, orlistat, Qnexa, topiramate, naltrexone, bupropion, phentermine, losartan, losartan with hydrochlorothiazide, and the like.

[0161] Examples of other active ingredients that may be administered separately or in the same pharmaceutical composition in combination with a co-agonist peptide as described herein include, but are not limited to:

[0162] (1) other dipeptidyl peptidase-IV (DPP-4) inhibitors (e.g., sitagliptin, alogliptin, vildagliptin, saxagliptin and omaglipitin);

[0163] (2) insulin sensitizers, including (i) PPARγ agonists, such as the glitazones (e.g., pioglitazone, AMG 131, MBX2044, mirtoglitazone, lodelglinzone, IDR-105, rosiglitazone, and balaglitazone), and other PPAR ligands, including (1) PPARγ/γ dual agonists (e.g., ZY112, ZY111, GFT505, chiglitazar, muraglitazar, aleglitazar, sodelglitazar, and navaglitazar); (2) PPARα agonists such as fenofibric acid derivatives (e.g., gemfibrozil, clofibrate, ciprofibrate, fenofibrate, bezafibrate), (3) selective PPAR γ modulators (SP-Parγ M’s), (e.g., such as those disclosed in US 02/060388, US 02/08188, WO 04/019869, WO 04/02409, WO 04/02408, and WO 04/066963; and (4) PPARα partial agonists; (ii) biguanides, such as metformin and its pharmaceutically acceptable salts, in particular, metformin hydrochloride, and extended-release formulations thereof, such as Glumetza™, Fortamet™, and GlucophageXR™; and (iii) protein tyrosine phosphatase-1B (PTP-1B) inhibitors (e.g., ISIS-113715 and TTP814); (3) insulin or insulin analogs
(e.g., insulin detemir, insulin glulisine, insulin degludec, insulin glargine, insulin lispro and inhalable formulations of each);

[0164] (4) leptin and leptin derivatives and agonists;

[0165] (5) amylin and amylin analogs (e.g., pramlintide);

[0166] (6) sulfonylurea and non-sulfonylurea insulin secretagogues (e.g., tolbutamide, glyburide, glipizide, glimepiride, mitiglinide, meglitinides, nateglinide and repaglinide);

[0167] (7) α-glucosidase inhibitors (e.g., acarbose, voglibose and miglitol);

[0168] (8) glucagon receptor antagonists (e.g., such as those disclosed in WO 98/04528, WO 99/01423, WO 00/39088, and WO 00/60810);

[0169] (9) incretin mimetics, such as GLP-1, GLP-1 analogs, derivatives, and mimetics; and GLP-1 receptor agonists (e.g., dulaglutide, semaglutide, albiglutide, exenatide, liraglutide, lixisenatide, tasaglutide, CJC-1131, and BIM-51077, including intranasal, transdermal, and once-weekly formulations thereof);

[0170] (10) LDL cholesterol lowering agents such as (i) HMG-CoA reductase inhibitors (e.g., simvastatin, lovastatin, pravastatin, cerivastatin, fluvastatin, atorvastatin, pitavastatin and rosuvastatin), (ii) bile acid sequestering agents (e.g., colestipol, colestipide, colesvamal hydrochloride, colestipol, cholestyramine, and dacylaminol oxidoly derivatives of a cross-linked dextran), (iii) inhibitors of cholesterol absorption (e.g., ezetimibe), and (iv) acyl CoA:cholesterol acyltransferase inhibitors, (e.g., avasimibe);

[0171] (11) HDL-raising drugs, (e.g., niacin and nicotinic acid receptor agonists, and extended-release versions thereof; MK-524A, which is a combination of niacin extended-release and the DP-1 antagonist MK-524);

[0172] (12) antiobesity compounds;

[0173] (13) agents intended for use in inflammatory conditions, such as aspirin, non-steroidal anti-inflammatory drugs or NSAIDs, glucocorticoids, and selective cyclooxygenase-2 or COX-2 inhibitors;

[0174] (14) antihyperlipidemic agents, such as ACE inhibitors (e.g., lisinopril, enalapril, ramipril, captopril, quinapril, and tandolapril), A-II receptor blockers (e.g., losartan, candesartan, irbesartan, olmesartan medoxomil, valsartan, telmisartan and eprosartan), renin inhibitors (e.g., aliskiren), beta blockers, and calcium channel blockers;

[0175] (15) glucokinase activators (GKAs) (e.g., AZD6370);

[0176] (16) inhibitors of 11β-hydroxysteroid dehydrogenase type 1, (e.g., such as those disclosed in U.S. Pat. No. 6,730,690, and LY-2523199);

[0177] (17) CETP inhibitors (e.g., anacetrabip, and torcestrabip);

[0178] (18) inhibitors of fructose 1,6-bisphosphatase, (e.g., such as those disclosed in U.S. Pat. Nos. 6,054,587; 6,110,903; 6,284,748; 6,399,782; and 6,489,476);

[0179] (19) inhibitors of acetyl CoA carboxylase 1 or 2 (ACC1 or ACC2);

[0180] (20) AMP-activated Protein Kinase (AMPK) activators;

[0181] (21) other agonists of the G-protein-coupled receptors: (i) GPR-109, (ii) GPR-119 (e.g., MBX2982 and PN8281), and (iii) GPR-40 (e.g., TAK875, 5-(4-[([1(1R,4S)]-2,6-dihydro-1H-indene-1-yl][oxyl]phenyl)isothiazole-3-ol 1-oxide; 5-(4-[3-(2,6-dimethyl-4-(3-methylsulfonf)propoxy)phenyl]phenoxyl]phenoxyl]phenyl)methoxyphenyl]liso, 5-(4-([3-(2-methyl-6-(3-hydroxypropoxy)pyridine-3-yl)]-2-methylenyl) methoxy)phenyl)isothiazole-3-ol 1-oxide, and 5-[4-[3-(3-aminoproxy)-2,6-dimethylphenyl]phenyl)methoxy phenyl]isothiazole-3-ol 1-oxide);

[0182] (22) SSTR3 antagonists (e.g., such as those disclosed in WO 2009/001836);

[0183] (23) neuropeptide U receptor agonists (e.g., such as those disclosed in WO 2009/042053, including, but not limited to, neuropeptide S (NMS));

[0184] (24) SCD inhibitors;

[0185] (25) GPR-105 antagonists (e.g., such as those disclosed in WO 2009/000087);

[0186] (26) SGLT inhibitors (e.g., ASP1941, SGLT-3, empagliflozin, dapagliflozin, canagliflozin, BI-10773, PK-04971729, remogliflozin, TS-071, tofogliflozin, ipragliflozin, and LX-4211);

[0187] (27) inhibitors of acyl coenzyme A:acyltransferase 1 and 2 (DGAT-1 and DGAT-2);

[0188] (28) inhibitors of fatty acid synthase;

[0189] (29) inhibitors of acyl coenzyme A:monoylglcerol acyltransferase 1 and 2 (MGAT-1 and MGAT-2);

[0190] (30) agonists of the TGR5 receptor (also known as GPBAR1, BG37, GPCR19, GPR131, and M-BAR);

[0191] (31) ileal bile acid transporter inhibitors;

[0192] (32) PACAP, PACAP mimetics, and PACAP receptor 3 agonists;

[0193] (33) PPAR agonists;

[0194] (34) protein tyrosine phosphatase-1B (PTP-1B) inhibitors;

[0195] (35) IL-1b antibodies, (e.g., XOMA052 and canakinumab); and

[0196] (36) bromocriptine mesylate and rapid-release formulations thereof.

[0197] Of particular interest are metformin hydrochloride, pioglitazone, rosiglitazone, simvastatin, atorvastatin, or sulfonylurea.

[0198] Antiobesity compounds that can be combined with compounds as disclosed herein include topiramate; zonisamide; naltrexone; phentermine; bupropion; the combination of bupropion and naltrexone; the combination of bupropion and zonisamide; the combination of topiramate and phentermine; fenfluramine; dexfenfluramine; sibutramine; lipase inhibitors, such as orlistat and cetilistat; melanocortin receptor agonists, in particular, melanocortin-4 receptor agonists; CCK-1 agonists; melanin-concentrating hormone (MCH) receptor antagonists; neuropeptide Y or Y2 antagonists (such as MK-0557); CB1 receptor inverse agonists and antagonists (such as rimonabant and tanaanab); P3 adrenergic receptor agonists; ghrelin antagonists; bombesin receptor agonists (such as bombesin receptor subtype-3 agonists); and 5-hydroxytryptamine-2c (5-HT2c) agonists, such as lorcaserin. For a review of anti-obesity compounds that can be combined with compounds of the present invention, see Chaki et al., “Recent advances in feeding suppressing agents: potential therapeutic strategy for the treatment of obesity,” Expert Opin. Ther. Patents, 11: 1677-1692 (2001); Spanswick and Lee, “Emerging antiobesity drugs,” Expert Opin. Emerging Drugs, 8: 217-237 (2003); Fernandez-Lopez, et al., “Pharmacological Approaches for the Treatment of Obesity,” Drugs, 62: 915-944 (2002); and Gadde, et al., “Combination pharmaceutical therapies for obesity,” Exp. Opin. Pharmacother., 10: 921-925 (2009).
In another aspect of the invention, a pharmaceutical composition is disclosed which comprises one or more of the following agents:

(a) a compound as disclosed herein, e.g. a one or co-agonists as disclosed herein wherein each co-agonist may independently be a peptide comprising the amino acid sequence of SEQ ID NO:20 with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21;

(b) one or more compounds selected from the group consisting of:

1. other dipeptidyl peptidase-IV (DPP-4) inhibitors;
2. insulin sensitizers, including (i) PPARγ agonists, such as the glitazones (e.g. AMG 131, MBX2044, methitiglazone, lodiglitazone, IQR-105, pioglitazone, rosiglitazone, and balaglitzone) and other PPAR ligands, (1) PPARα/γ dual agonists, such as ZYH1, YYYY2, chigitazur, GFT505, muraglitazar, aleglitazar, sodelglitazar, and naveglitazar, (2) PPARα agonists, such as fenofibrate derivatives (e.g., gemfibrozil, clofibrate, cipofibrate, fenofibrate and bezafibrate), (3) selective PPARα modulators (SPPARαMs), and (4) PPARγ partial agonists; (ii) biguanides, such as metformin and its pharmaceutically acceptable salts, in particular, metformin hydrochloride, and extended-release formulations thereof, such as Glumetza®, Fortamet®, and GlucophageXR®; (iii) protein tyrosine phosphatase-1B (PTP-1B) inhibitors, such as S1I-13715, and TTP14;
3. sulfonamide and non-sulfonamide insulin secretagogues, (e.g., tolbutamide, glyburide, glipizide, gliimepiride, mitiglizone, and meglitinides, such as nateglinide and repaglinide);
4. α-glucosidase inhibitors (e.g., acarbose, voglibose and miglitol);
5. glucagon receptor antagonists;
6. LDL cholesterol lowering agents such as (i) HMG-CoA reductase inhibitors (e.g., lovastatin, simvastatin, pravastatin, cerivastatin, fluvastatin, atorvastatin, pitavastatin, and rosvastatin), (ii) bile acid sequestrating agents (e.g., colestilan, cholestyramine, colestipole, colesvelam hydrochloride, colestipol, and dialkylaminooxyl derivatives of a cross-linked dextran), (iii) inhibitors of cholesterol absorption, (e.g., ezetimibe), and (iv) acyl CoA: cholesterol acyltransferase inhibitors (e.g., avasimibe);
7. HDL-raising drugs, such as niacin or a salt thereof and extended-release versions thereof; MK-524A, which is a combination of niacin extended-release and the DP-1 antagonist MK-524; and nicotinic acid receptor agonists;
8. antiobesity compounds;
9. agents intended for use in inflammatory conditions, such as aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, and selective cyclooxygenase-2 (COX-2) inhibitors;
10. antihypertensive agents, such as ACE inhibitors (e.g., enalapril, lisinopril, ramipril, captopril, quinapril, and tandolapril), A-II receptor blockers (e.g., losartan, candesartan, irbesartan, olmesartan medoxomil, valsartan, telmisartan, and eprosartan), renin inhibitors (e.g., aliskiren), beta blockers (e.g., calcium channel blockers);
11. glucokinase activators (GKAs) (e.g., AZD6370);
12. inhibitors of 11β-hydroxysteroid dehydrogenase type 1 (e.g., such as those disclosed in U.S. Pat. No. 6,730,690; WO 03/104207; and WO 04/058741);
13. inhibitors of cholesteryl ester transfer protein (CETP), (e.g., torcetrapib and MK-0859);
14. inhibitors of fructose 1,6-bisphosphatase (e.g., such as those disclosed in U.S. Pat. Nos. 6,054,587; 6,110,903; 6,284,748; 6,399,782; and 6,495,476);
15. inhibitors of acetyl CoA carboxylase-1 or 2 (ACC1 or ACC2);
16. AMP-activated Protein Kinase (AMPK) activators;
17. agonists of the G-protein-coupled receptors: (i) GPR-109, (ii) GPR-119 (e.g., MBX2082, and PSN821), and (iii) GPR-40 (e.g., TAK875, 5-[4-[[1(R)-4-[6-(3-hydroxy-3-methylbutoxy)-2-methylpyridine-3-yl]-2,3-dihydro-1H-indene-1-yl]oxy]phenyl]isothiazole-3-ol 1-oxide, 5-[(3,2-dimethyl-4-(3-(methylsulfonyl)propoxy)phenyl]methoxy]phenyl)iso, 5-4(3-(2-methyl-6-(3-hydroxypropoxy)pyridine-3-yl)-2-methylphenyl]methoxy)phenyl)isothiazole-3-ol 1-oxide, and 5-[(4-4-3-aminopropoxy)-2,6-dimethylphenyl]methoxy]phenyl)isothiazole-3-ol 1-oxide);
18. SSTR3 antagonists (e.g., such as those disclosed in WO 2009/01836);
19. neuremedin U receptor agonists (e.g., such as those disclosed in WO2009/042053, including, but not limited to, neuremedin S (NMS));
20. inhibitors of stearyl-coenzyme A delta-9 desaturase (SCD);
21. GPR-105 antagonists (e.g., such as those disclosed in WO 200/000087);
22. inhibitors of glucose uptake, such as sodium-glucose transport (SGLT) inhibitors and its various isofoms, such as SGLT-1; SGLT-2 (e.g., ASP1941, TS071, B110773, tofogliflozin, L4X211, canagliflozin, dapagliflozin and remogliflozin; and SGLT-3);
23. inhibitors of acyl coenzyme A: acyltransferase 1 and 2 (DGAT1 and DGAT2);
24. inhibitors of fatty acid synthase;
25. inhibitors of acyl coenzyme A: monooacylglycerol acyltransferase 1 and 2 (MGAT1 and MGAT2);
26. agonists of the TGR5 receptor (also known as GPBAR1, BG37, GPCR19, GPR131, and M-BAR);
27. bronocromesine mesylate and rapid-release formulations thereof, and
28. IL-1β antibodies (e.g., XOMA052, and canaknabumab); and
29. a pharmaceutically acceptable carrier.
30. When a co-agonist peptide of the present invention is used concomitaneously with one or more other drugs, peptides, or proteins, a pharmaceutical composition containing such other drugs, peptides, or proteins in addition to the co-agonist peptide of the present invention may be provided. Accordingly, the pharmaceutical compositions of the present invention include those that also contain one or more other active ingredients, in addition to a co-agonist peptide of the present invention.
31. Methods of administering the pharmacological compositions comprising the one or more co-agonist peptides disclosed herein to an individual include, but are not limited
to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compositions can be administered by any convenient route, for example by injection or bolus injection, by absorption through epithelial or mucocutaneous linings (for example, oral mucosa, rectal and intestinal mucosa, and the like), ocular, and the like and can be administered together with other biologically-active agents. Administration can be systemic or local. In addition, it may be advantageous to administer the composition into the central nervous system by any suitable route, including intraventricular and intrathecal injection. Intraventricular injection may be facilitated by an intraventricular catheter attached to a reservoir (for example, an Omniflow reservoir). Pulmonary administration may also be performed by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. It may also be desirable to administer the one or more co-agonist peptides disclosed herein locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, by injection, by means of a catheter, by means of a suppository, or by means of an implant.

[0233] Various delivery systems are known and can be used to administer the co-agonist peptides disclosed herein including, but not limited to, encapsulation in liposomes, microparticles, microcapsules; micelles; polymers; capsules; tablets; and the like. In one embodiment, the co-agonist peptides disclosed herein may be delivered in a vesicle, in particular a liposome. In a liposome, the co-agonist peptides disclosed herein are combined, in addition to other pharmaceutically acceptable carriers, with amphiphilic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfonates, lysollecithin, phospholipids, soya, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Pat. No. 4,837,028 and U.S. Pat. No. 4,737,323. In yet another embodiment, the co-agonist peptides disclosed herein can be delivered in a controlled release system including, but not limited to: a delivery pump (See, for example, Saudek, et al., New Engl. J. Med. 321: 574 (1989) and a semi-permeable polymeric material (See, for example, Howard, et al., J. Neurosurg. 71: 105 (1989)). Additionally, the controlled release system can be placed in proximity of the therapeutic target (for example, the brain), thus requiring only a fraction of the systemic dose. See, for example, Goodson, In: Medical Applications of Controlled Release, 1984. (CRC Press, Boca Raton, Fla.).

[0234] The amount of the compositions comprising one or more of the co-agonist peptides disclosed herein which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder and condition, and may be determined by standard clinical techniques by those of average skill in the art. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the overall seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Ultimately, the attending physician will decide the amount of the composition with which to treat each individual patient. Initially, the attending physician will administer low doses of the composition and observe the patient's response. Larger doses of the composition may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. In general, the daily dose range lies within the range of from about 0.001 mg to about 100 mg per kg body weight of a mammal, preferably 0.01 mg to about 50 mg per kg, and most preferably 0.1 to 10 mg per kg, in single or divided doses. On the other hand, it may be necessary to use dosages outside these limits in some cases. However, suitable dosage ranges for intravenous administration of the compositions comprising the one or more co-agonist peptides disclosed herein are generally about 5-500 micrograms (μg) of active compound per kilogram (Kg) body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems. Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient. Ultimately the attending physician will decide on the appropriate duration of therapy using compositions comprising the one or more co-agonist peptides disclosed herein of the present invention. Dosage will also vary according to the age, weight and response of the individual patient.

[0235] Further provided is a pharmaceutical pack or kit, comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions and co-agonist peptides disclosed herein. Optionally associated with such container(s) may be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[0236] Thus, the present invention further provides a pharmaceutical composition comprising (i) a peptide consisting of the amino acid sequence of native human glucagon having the amino acid sequence shown in SEQ ID NO:1 but in which the L-serine at position 2 is replaced with a D-serine; the tyrosine at position 10 is replaced with (i) L-lysine conjugated to a palmitoyl group by either a gamma-glutamyl acid (γE) spacer or a gamma-glutamyl acid-gamma-glutamyl acid dipeptide (γEγE) spacer or (ii) para-aminomethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamyl acid (γE) spacer or a gamma-glutamyl acid-gamma-glutamyl acid dipeptide (γEγE) spacer; and up to six additional amino acid substitutions; wherein the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ of the glucagon receptor to EC₅₀ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12, and wherein the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide, and (ii) a pharmaceutically acceptable carrier, with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

[0237] In particular aspects, the serine at position 16 is replaced with L-alanine, the arginine at position 18 is replaced with L-alanine, the methionine at position 27 is replaced with L-lysine, the asparagine at position 28 is replaced with L-aspartic acid, or combinations thereof.

[0238] The present invention further provides a method for treating a patient for obesity metabolic disease comprising
administering the patient an effective amount of the peptide of any one of the aforementioned peptides to treat the metabolic disease in the patient.

[0239] The present invention further provides method for treating a patient for obesity metabolic disease comprising administering the patient an effective amount of the composition of the aforementioned compositions to treat the metabolic disease in the patient.

[0240] In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

[0241] In particular aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

[0242] In particular aspects, the patient has more than one metabolic disease, for example, diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.

[0243] The present invention further provides for the use of any one of the aforementioned peptides for manufacture of a medicament for the treatment of obesity metabolic disease.

[0244] The present invention further provides for the use of any one of the aforementioned compositions for manufacture of a medicament for the treatment of obesity metabolic disease.

[0245] In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

[0246] In particular aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

[0247] In particular aspects, the medicament is for treatment of more than one metabolic disease, for example, diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.

[0248] In particular aspects of the compounds disclosed herein the C-terminal protecting group may be an amide or ester. For example, the carboxylic acid of the C-terminal amino acid is replaced with a charge-neutral group, such as an amide or ester.

[0249] Further provided is method for treating a metabolic disease in a patient or individual comprising: administering to the patient or individual an effective amount of any one of the aforementioned compositions comprising a co-agonist peptide and administering to the patient or individual an effective amount of a composition comprising an insulin or insulin analog to treat the metabolic disease in the patient or individual.

[0250] In particular aspects, the composition comprising the co-agonist peptide is administered at a time prior to the time the composition comprising the insulin or insulin analog is administered. In another aspect, the composition comprising the insulin or insulin analog is administered at a time prior to the time the composition comprising the co-agonist peptide is administered. In a further still aspect, the composition comprising the co-agonist peptide is administered at the same time as the composition comprising the insulin or insulin analog is administered.

[0251] In particular aspects, the insulin analog is insulin detemir, insulin glargine, insulin leuenir, insulin glulisine, or insulin lispro.

[0252] In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

[0253] In particular aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

[0254] In particular aspects, the patient has more than one metabolic disease, for example, diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.

[0255] The present invention further provides a composition comprising any one of the aforementioned peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier.

[0256] The present invention further provides for the use of a composition comprising any one of the aforementioned peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier for the treatment of a metabolic disease.

[0257] In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity. In further aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

[0258] The present invention further provides for the use of a composition comprising any one of the aforementioned peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier for the manufacture for a medicament for the treatment of a metabolic disease.

[0259] In particular aspects, the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity. In further aspects, the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

[0260] The following examples are intended to promote a further understanding of the present invention.

**Example 1**

[0261] Procedure for the synthesis of Co-agonist Peptide 1: His1-D-Ser2-Gly3-Thr4-Phe5-Thr6-Ser7-Asp8-Lys9-Glu10-Glu11-Glu12-Val13-Gly14-Xaa15-Leu16-Leu17-Asp18-Asp19-Thr20 CONH2 (SEQ ID NO:6) was as follows.

[0262] The peptide was synthesized on a Rink-amide PEPPS resin, Champion (Biosearch Technologies (150 μmol, loading 0.28 mmol/g) on a Symphony Protein Technologies Inc apparatus.

[0263] All the amino acids were dissolved at 0.3 M concentration in a solution of 0.3M HOBr (Hydroxybenzotriazole) in DMF. The acylation reactions were performed for 1 hour with 5-fold excess of activated amino acid over the resin free amino groups. The amino acids were activated with equimolar amounts of HATU (O-(7-azabenzotriazol-1-yl)-N, N,N',N'-tetramethyluronium hexafluorophosphate) and a 2-fold molar excess of DIEA (diisopropylethylamine) solution 2M in NMP.

[0264] Double acylation reactions of 45 minutes were performed from His1 to Trp15. The crude peptide (130 mg in 3 ml of DMSO) was purified by reverse-phase HPLC using preparative Waters Delta-Pak™ C4 cartridges (40x200 mm, 15 μm, 300 Å) and using as eluents (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile. The following gradient of eluent B was used: 30% B to 50% B over 5 min, 60% B to 60% B over 20 min-80% B, flow rate 80 mL/min, wavelength 214 nm. Yield 21%, 95% pure.

[0265] The final peptide was characterized on an Acquity UPLC Waters Chromatograph, with BEH300 C4 Acquity Waters 2.1x100 mm, 1.7 μm, at 45° C, using H2O, 0.1% TFA
Example 2

[0266] Procedure for the synthesis of Co-agonist Peptide 2: His1-Asp-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Lys10(γGlu-C14)-Ser-Lys-Tyr-Leu-Asp-Val-Arg-Ala-Ala-Gln-Asp-Phe2-Val15-Gln-Trp-Leu-Leu-Asp-Thr-CONH2 (SEQ ID NO:7) was as follows.

[0267] The peptide was synthesized as previously reported for SEQ ID NO:6. Example 1.
The crude peptide (130 mg in 3 ml of DMSO) was purified by reverse-phase HPLC using preparative Waters Delta-PakC4 cartridges (40x200 mm, 15 μm, 300 A) and using as eluents (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile. The following gradient of eluent B was used: 30% B to 30% B over 5 min, 50% B to 50% B over 20 min-80% B, flow rate 80 ml/min, wavelength 214 nm. Yield 16%, 95% pure.

[0268] The final peptide was characterized on an Acquity UPLC Waters Chromatograph, with BEH1300 C4 Acquity Waters 2.1x100 mm, 1.7 μm, at 45°C, using H2O, 0.1% TFA (A) and CH3CN, 0.1% TFA (B) as solvents and also characterized by electrospray mass spectrometry on a Acquity SQ Detector (Mw found: 3723.70 Da; Mw expected: 3724.18 Da).

Example 3

[0269] Procedure for the synthesis of Co-agonist Peptide 3: His1-Asp-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Lys10(γGlu-γGlu-C14)-Ser-Lys-Tyr-Leu-Asp-Val-Arg-Ala-Ala-Gln-Asp-Phe2-Val15-Gln-Trp-Leu-Leu-Asp-Thr-CONH2 (SEQ ID NO:8) was as follows.

[0270] The peptide was synthesized as previously reported for Co-agonist Peptide 1. The crude peptide (130 mg in 3 ml of DMSO) was purified by reverse-phase HPLC using preparative Waters Delta-PakC4 cartridges (40x200 mm, 15 μm, 300 A) and using as eluents (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile. The following gradient of eluent B was used: 30% B to 30% B over 5 min, 50% B to 60% B over 20 min-80% B, flow rate 80 ml/min, wavelength 214 nm. Yield 20%, >90% pure.

[0271] The final peptide was characterized on an Acquity UPLC Waters Chromatograph, with BEH1300 C4 Acquity Waters 2.1x100 mm, 1.7 μm, at 45°C, using H2O, 0.1% TFA (A) and CH3CN, 0.1% TFA (B) as solvents and the following gradient: 35% to 65% B in 4 min, flow 0.4 ml/min. The peptide was characterized by electrospray mass spectrometry on an Acquity SQ Detector (Mw found: 3852.80 Da; Mw expected: 3853.29 Da).

Example 4

[0272] Procedure for the synthesis of Co-agonist Peptide 4: His1-Asp2-Val-Gly-Thr-Phe-Thr-Ser-Asp-Lys10(γGlu-γGlu-C14)-Ser-Lys-Tyr-Leu-Asp15-Asp-Ala-Ala-Gln-Asp-Phe2-Val15-Gln-Trp2-Leu-Nle-Asp-Thr-CONH2 (SEQ ID NO:9) was as follows.

[0273] The peptide was synthesized by standard Solid-phase Peptide Synthesis (SPPS) using Fmoc-t-Bu chemistry. The assembly was performed on a Rink-amide PS resin, Champion (Biosense Technologies, 0.28 mmol/g) on a Symphony (Protein Technologies) peptide synthesizer.

[0274] All the amino acids were dissolved at a 0.3 M concentration in a solution of 0.5M HOBT (Hydroxybenzotriazole) in DMF. The acylation reactions were performed for 45 min with 5-fold excess of activated amino acid over the resin free amino groups. The amino acids were activated with equimolar amounts of HATU (O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate) and a 2-fold molar excess of DIEA (diisopropylethylamine) solution 2M in NMP. Double acylation reactions were performed from His1 to Trp25. During peptide assembly on solid phase, the side chain protecting groups were: tert-butyloxycarbonyl (Boc) for Lys, Trp, and Tyr; trityl for Gin and His; tert-butoxy-tyranosyl for Arg. The lysine position at lysine was introduced as Dde-Lys(Tf)COOH so to accomplish side chain derivatization just after assembly of Lys10 after Fmoc deprotection of the ε-amino group. The Dde-Lys10 side chain was derivatized with the linker and fatty acid by manual coupling of two Fmoc-Glu-Obu residues and palmitic acid activated with DIPC and HOAt. The Dde protecting group was removed by treatment with a solution of 2% hydrazine in DMF and the sequence assembly was continued to incorporate residues 1-9. The dry peptide-resin was treated for 2 h at room temperature with 88% TFA, 5% phenol, 2% triisopropylsilane and 5% water to afford protecting groups deprotection and cleavage from resin. The solution was filtered to remove the resin and the crude peptide solution was precipitated in cold methyl tert-buty ether (approximately 4 ml). peptide solution for 50 ml methyl tert-buty ether. The peptide pellet was suspended/washed/centrifuged in cold methanol tert-butyl ether for 2 times. The peptide pellet was dried under vacuum and then dissolved in water/acetonitrile and then lyophilized.

[0275] All the amino acids were dissolved at a 0.3 M concentration in a solution of 0.5M HOBT (Hydroxybenzotriazole) in DMF. The acylation reactions were performed for 45 min with 5-fold excess of activated amino acid over the resin free amino groups. The amino acids were activated with equimolar amounts of HATU (O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate) and a 2-fold molar excess of DIEA (diisopropylethylamine) solution 2M in NMP. Double acylation reactions were performed from His1 to Trp25. During peptide assembly on solid phase, the side chain protecting groups were: tert-butyloxycarbonyl (Boc) for Lys, Trp, and Tyr; trityl for Gin and His; tert-butoxy-tyranosyl for Arg. The lysine position at lysine was introduced as Dde-Lys(Tf)COOH so to accomplish side chain derivatization just after assembly of Lys10 after Fmoc deprotection of the ε-amino group. The Dde-Lys10 side chain was derivatized with the linker and fatty acid by manual coupling of two Fmoc-Glu-Obu residues and palmitic acid activated with DIPC and HOAt. The Dde protecting group was removed by treatment with a solution of 2% hydrazine in DMF and the sequence assembly was continued to incorporate residues 1-9. The dry peptide-resin was treated for 2 h at room temperature with 88% TFA, 5% phenol, 2% triisopropylsilane and 5% water to afford protecting groups deprotection and cleavage from resin. The solution was filtered to remove the resin and the crude peptide solution was precipitated in cold methyl tert-buty ether (approximately 4 ml). peptide solution for 50 ml methyl tert-buty ether. The peptide pellet was suspended/washed/centrifuged in cold methanol tert-butyl ether for 2 times. The peptide pellet was dried under vacuum and then dissolved in water/acetonitrile and then lyophilized.

Example 5

[0277] Procedure for the synthesis of Co-agonist Peptide 5: His1-Asp-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Lys10(γGlu-γGlu-C14)-Ser-Lys-Tyr-Leu-Asp-Val-Arg-Ala-Ala-Gln-Asp-Phe2-Val15-Gln-Trp2-Leu-Nle-Asp-Thr-CONH2 (SEQ ID NO:10) was as follows.

[0278] The peptide was synthesized by standard Solid-phase Peptide Synthesis (SPPS) using Fmoc-t-Bu chemistry. The assembly was performed on a Rink-amide PS resin (150 μmol, loading 0.47 mmol/g) on an APEX 396 peptide synthesizer.

[0279] All the amino acids were dissolved at a 0.5 M concentration in a solution of 0.5M HOBT (Hydroxybenzotriazole) in DMF. The acylation reactions were performed for 45
min with 5-fold excess of activated amino acid over the resin free amino groups. The amino acids were activated with equimolar amounts of HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate) and a 2-fold molar excess of DIPEA (diisopropylethylamine) solution 2M in NMP.

[0280] Double acylation reactions were performed on His\textsuperscript{1}, D-Ser\textsuperscript{2}, Phe\textsuperscript{3}, Val\textsuperscript{4}. During peptide assembly on solid phase, the side chain protecting groups were: tert-butyll for Asp, Ser, D-Ser, Thr and Tyr; trityl for Gin and His; tert-butylx-carbonyl for Lys, Trp; and 2,2,4,6,7-pentamethyldihydrobenzo[2,3]-5-sulfonyl for Arg. The Lys at position 10 was protected by the orthogonal Alloc\textdagger(allyloxy carbonyl) protecting group.

[0281] The N-terminal residue was acylated as Boc-His\textdagger(Trt)-OH. At the end of the peptide assembly on solid phase, the Alloc protecting group was removed from Lys\textsuperscript{10} and side chain derivatization was performed by manual coupling of Fmoc-Glu-OrBu residues and palmitic acid activated with DIPEA and HOAt. The dry peptide-resin was treated for 2 hr at room temperature with 88% TFA, 5% phenol, 2% trisopropanol and 5% water to afford protecting groups deprotection and cleavage from resin. The solution was filtered to remove the resin and the crude peptide solution was precipitated in cold methyl tert-butyl ether (approximately 4 mL peptide solution for 50 mL methyl tert-butyl ether). The peptide pellet was resuspended/washed/centrifuged in cold methyl tert-butyl ether for 2 times. The peptide pellet was dried under vacuum and then dissolved in water/acetonitrile and then lyophilized.

[0282] The crude peptide (130 mg in 3 ml of DMF) was purified by reverse-phase HPLC using preparative Waters Delta-Pak™ C4 cartridges (40x200 mm, 15 μm, 300 A) and using as eluents (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile. The following gradient of eluent B was used: 25% B to 25% B over 5 min, 25% B to 55% B over 20 min-80% B, flow rate 80 mL/min, wavelength 214 nm. Yield 27%, >90% pure.

[0288] The final peptide was characterized on an Acquity UPLC Waters Chromatograph, with BEH300 C4 Acquity Waters 2.1x100 mm, 1.7 μm, at 45 °C, using H2O, 0.1% TFA (A) and CH3CN, 0.1% TFA (B) as solvents and the following gradient: 30% to 30% over 1 min, 30% over 70% B in 4 min, flow 0.4 mL/min. The peptide was characterized by electrospray mass spectrometry on a Acquity SQ Detector (Mw found: 3909.31 Da; Mw expected: 3907.8 Da).

Example 8

[0289] Procedure for the synthesis of Co-agonist Peptide 8: His\textsuperscript{1}-D-Ser\textsuperscript{2}-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Lys\textsuperscript{10}(γGlu-γGlu\textsubscript{C16})-Ser-Lys-Tyr-Leu-Glu\textsuperscript{15}-Ala\textsuperscript{16}-Arg-Ala-Ala-Gln-Asp-Phe\textsuperscript{22}-Val\textsuperscript{23}-Gln-Trp\textsuperscript{25}-Leu-Leu-Asp-Thr-CONH (SEQ ID NO:12) was as follows.

[0290] The peptide was synthesized by standard Solid-phase Peptide Synthesis (SPPS) using Fmoc\textsuperscript{+}-Bu chemistry. The assembly was performed on a Rink-amide PEG-AM resin, (Chemipex, 0.47 mmol/g) or on Rink-amide PEG-PS resin, Champion (Biosearch Technologies, 0.28 mmol/g) on an Overture peptide synthesizer. The acylations were performed using HATU/DEPA as activators and 45 minutes reaction time. Double acylation reactions were performed on all the sequence. The Lys at position 10 was protected by the orthogonal Alloc\textdagger(allyloxy carbonyl) protecting group. The N-terminal residue was protected as Boc. At the end of the peptide assembly on solid phase, the Alloc protecting group was removed from Lys\textsuperscript{10} and side chain derivatization was performed by manual coupling of Fmoc-Glu-OrBu residues and palmitic acid activated with DIPEA and HOAt. The dry peptide-resin was treated for 2 hr at room temperature with 88% TFA, 5% phenol, 2% trisopropanol and 5% water to afford protecting groups deprotection and cleavage from resin. The solution was filtered to remove the resin and the crude peptide solution was precipitated in cold methyl tert-butyl ether (approximately 4 mL peptide solution for 50 mL methyl tert-butyl ether). The peptide pellet was resuspended/washed/centrifuged in cold methyl tert-butyl ether for 2 times. The peptide pellet was dried under vacuum and then dissolved in water/acetonitrile and then lyophilized.

[0291] The crude peptides (130 mg in 3 ml of DMF) were purified by reverse-phase HPLC using preparative Waters Delta-Pak™ C4 cartridges (40x200 mm, 15 μm, 300 A) and using as eluents (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile. The following gradient of eluent B was used: 35% B to 35% B over 5 min, 35% B to 55% B over 20 min-80% B, flow rate 80 mL/min, wavelength 214 nm. Yield 20%, >90% pure. The final peptide was characterized on an Acquity UPLC Waters Chromatograph, with BEH300 C4
Acquity Waters 2.1 x 100 mm, 1.7 µm, at 45°C, using H2O, 0.1% TFA (A) and CH3CN, 0.1% TFA (B) as solvent. The peptide was characterized by electrospray mass spectrometry on an Acquity SQ Detector (Mw found: 3839.10 Da; Mw expected: 3839.26 Da).

Example 9

[0293] The peptide was synthesized as previously reported for Co-agonist Peptide 4. Double acylation reactions were performed on His1, D-Ser2, Val20, Gly3, Thr3, Thr3, Phe22, Val22, Fmoc-p-fluoro-phenylalanine-COOH (Fmoc-pF-FH) was assembled by manual coupling with DIPC and HOAt.

[0294] The crude peptide (130 mg in 3 ml of DMSO) was purified by reverse-phase HPLC using preparative Waters Delta-Pak™ C4 cartridges (40 x 200 mm, 15 µm, 300 A) and using as eluents (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile. The following gradient of eluent B was used: 30% B to 30% B over 5 min, 30% B to 50% B over 20 min-80% B, flow rate 80 ml/min, wavelength 214 nm. The yield was 10%, and the purity above 90%. The final peptide was characterized on an Acquity UPLC Waters Chromatograph, with BEH300 C4 Acquity Waters 2.1 x 100 mm, 1.7 µm, at 45°C, using H2O, 0.1% TFA (A) and CH3CN, 0.1% TFA (B) as solvent. The peptide was characterized by electrospray mass spectrometry on an Acquity SQ Detector (Mw found: 3901.80 Da; Mw expected: 3901.26 Da).

Example 10
[0295] Procedure for the synthesis of Co-agonist Peptide 10: His1-D-Ser2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Lys10(γGlu-γGlu-C16)-Ser-Lys-Tyr-Leu-Asp16-Glu17-Ala-Ala-Gln-Asp-Phe22-Val23-Gln-Trp22-Leu-Met2(4)-Asp-Thr-CONH2 (SEQ ID NO:15) was as follows.

[0296] The peptide was synthesized as previously reported for Co-agonist Peptide 4. Double acylation reactions were performed on His1, D-Ser2, Gln3, Gly3, Thr3, Phe3, Thr3, Phe22, Val23, Fmoc-Methylsulphone-COOH was assembled by manual coupling with DIPC and HOAt. The crude peptide (130 mg in 3 ml of DMSO) was purified by reverse-phase HPLC using preparative Waters Delta-Pak™ C4 cartridges (40 x 200 mm, 15 µm, 300 A) and using as eluents (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile. The following gradient of eluent B was used: 30% B to 30% B over 5 min, 30% B to 50% B over 20 min-80% B, flow rate 80 ml/min, wavelength 214 nm. The yield was 15%, and the purity was >90% pure. The final peptide was characterized on an Acquity UPLC Waters Chromatograph, with BEH300 C4 Acquity Waters 2.1 x 100 mm, 1.7 µm, at 45°C, using H2O, 0.1% TFA (A) and CH3CN, 0.1% TFA (B) as solvent. The peptide was characterized by electrospray mass spectrometry on an Acquity SQ Detector (Mw found: 3932.70 Da; Mw expected: 3933.31 Da).

Example 11
[0297] Procedure for the synthesis of Co-agonist Peptide 11: His1-D-Ser2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Lys10(γGlu-γGlu-C16)-Ser-Leu-Tyr-Leu-Asp-Glu16(Ala5-Ala5-Ala5-A) with Acquity Waters BEH130 C18 Acquity 1.0 x 50 mm, 1.7 µm; Gradient: 10-99% B linear gradient over 1.6 min, back to 1% B in 0.4 min. A/B buffer system (A = 0.1% TFA in water; B = 0.1% TFA in acetonitrile); Flow rate: 0.5 ml/min; Temperature: 50°C; UV wavelength: 215 nm; Inject-
tion volume: 0.5 ul; Mass scan: 500-1500 m/z, 0.2 sec; Voltages: Capillary (KV)-3.25; Con (V)-27; Extract (V)-3; RF Lens (V)-0.3; Gas flow (L/hr): Desolvation-597; Cone-30; Temperature (C): Source temp-100; Desolvation temp-350.

Example 12

[0302] Procedure for the synthesis of Co-agonist Peptide 12: His<sup>1</sup>-D-Ser<sup>2</sup>-Gln-Gly-Thr-Phe-Thr-Ser-Asp-pAF<sup>10</sup> (γGlu-γGlu-C<sub>4</sub>)<sub>α</sub>-Ser-Lys-Tyr-Leu-Asp<sup>15</sup>-Arg-Ala-Ala-Gln-Asp-Phe<sup>22</sup>-Val<sup>23</sup>-Gln-Trp<sup>25</sup>-Leu-Leu-Asp-Thr-CONH<sub>2</sub> (SEQ ID NO:17), wherein pAF is para-aminomethyl phenylalanine was as follows.

[0303] The peptide was synthesized as previously reported for Co-agonist Peptide 4. Double acylation reactions were performed on His<sup>1</sup>, D-Ser<sup>2</sup>, Val<sup>3</sup>, Gly<sup>4</sup>, Thr<sup>5</sup>, Phe<sup>6</sup>, Thr<sup>7</sup>, Phe<sup>22</sup>, Val<sup>23</sup>. In position 10 Fmoc-para-aminomethyl phenylalanine-COOH orthogonally protected with Dde (see Example 15) was assembled by manual coupling activated with DIPC and HOAt. At the end of the assembly the Dde protecting group was removed by treatment with a solution of 2% hydrazine in DMF and side chain derivatization was accomplished with the linker and fatty acid by manual coupling of two Fmoc-Glu-OtBu residues and palmitic acid activated with DIPC and HOAt.

[0304] The crude peptide (130 mg in 3 ml of DMSO) was purified by reverse-phase HPLC using preparative Waters Delta-Pak<sup>TM</sup> C-4 cartridges (40x200 mm, 15 μm, 300 A) and as eluents (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile. The following gradient of eluent B was used: 30% B to 30% B over 5 min, 30% B to 55% B over 20 min-80% B, flow rate 80 ml/min, wavelength 214 nm. Yield 10%, >90% pure.

[0305] The final peptide was characterized on an Acquity UPLC Waters Chromatograph, with BEH300 C4 Acquity Waters 2.1x100 mm, 1.7 μm, at 45° C., using H2O, 0.1% TFA (A) and CH3CN, 0.1% TFA (B) as solvents and the following gradient: 35 over 55% B in 4 min, flow 0.4 ml/min. The peptide was characterized by electrospray mass spectrometry on a Acquity SQ Detector (Mw found: 3873.10 Da; Mw expected: 3873.28 Da)

Example 13

[0306] Procedure for the synthesis of Co-agonist Peptide 13: His<sup>1</sup>-a-methylcyclobutane<sup>2</sup>-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Lys<sup>10</sup>(γGlu-γGlu-C<sub>4</sub>)<sub>α</sub>-Ser-Lys-Tyr-Leu-Asp<sup>15</sup>-Glu<sup>16</sup>-Arg-Ala-Ala-Gln-Asp-Phe<sup>22</sup>-Val<sup>23</sup>-Gln-Trp<sup>25</sup>-Leu-Leu-Asp-Thr-CONH<sub>2</sub> (SEQ ID NO:18) as follows.

[0307] The peptide was synthesized as previously reported for SEQ ID NO:9/Example 4. Double acylation reactions were performed on His<sup>1</sup>, D-Ser<sup>2</sup>, Gln<sup>3</sup>, Gly<sup>4</sup>, Thr<sup>5</sup>, Phe<sup>6</sup>, Thr<sup>7</sup>, Phe<sup>22</sup>, Val<sup>23</sup>. Fmoc-a-methylcyclobutane carboxylic acid was assembled by manual coupling activated with DIPC and HOAt.

[0308] The crude peptide (130 mg in 3 ml of DMSO) was purified by reverse-phase HPLC using preparative Waters Delta-Pak<sup>TM</sup> C-4 cartridges (40x200 mm, 15 μm, 300 A) and as eluents (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile. The following gradient of eluent B was used: 35% B to 35% B over 5 min, 35% B to 50% B over 20 min-80% B, flow rate 80 ml/min, wavelength 214 nm. The final peptide was characterized on an Acquity UPLC Waters Chromatograph, with BEH300 C4 Acquity Waters 2.1x100 mm, 1.7 μm, at 45° C., using H2O, 0.1% TFA (A) and CH3CN, 0.1% TFA (B) as solvents and the following gradient: 20 over 60% B in 20 min, flow 0.4 ml/min. The product was also characterized by electrospray mass spectrometry on a Acquity SQ Detector Yield 12%, >90% pure (Mw found: 3899.3 Da; Mw expected: 3899.3 Da).

Example 14

[0309] Procedure for the synthesis of Co-agonist Peptide 14: His<sup>1</sup>-D-Ser-Gln-Gly-Thr-tBp-Thr-Ser-Asp-Lys<sup>10</sup>(γGlu-γGlu-C<sub>4</sub>)<sub>α</sub>-Ser-Lys-Tyr-Leu-Asp<sup>15</sup>-Glu<sup>16</sup>-Arg-Ala-Ala-Gln-Asp-Phe<sup>22</sup>-Val<sup>23</sup>-Gln-Trp<sup>25</sup>-Leu-Leu-Asp-Thr-CONH<sub>2</sub> (SEQ ID NO:19), wherein tBp=threo-β-Phenylserine, was as follows.

Example 15

[0310] The peptide was synthesized as previously reported for Co-agonist Peptide 4.

[0311] Double acylation reactions were performed on His<sup>1</sup>, D-Ser<sup>2</sup>, Gln<sup>3</sup>, Gly<sup>4</sup>, Thr<sup>5</sup>, Phe<sup>6</sup>, Thr<sup>7</sup>, Phe<sup>22</sup>, Val<sup>23</sup>. tBp was assembled by manual coupling activated with DIPC and HOAt.

[0312] The crude peptide (130 mg in 3 ml of DMSO) in which two isomers were present, due to the racemic tBp used in the synthesis, was purified by reverse-phase HPLC using preparative Waters Delta-Pak<sup>TM</sup> C-4 cartridges (40x200 mm, 15 μm, 300 A) and as eluents (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile. The following gradient of eluent B was used: 35% B to 35% B over 5 min, 35% B to 45% B over 20 min-80% B, flow rate 80 ml/min, wavelength 214 nm. The two isomers were separated in the preparative run with different elution times. The most hydrophilic isomer having the shorter retention time t=12.45 was identified with no. L-005472682 on an Acquity UPLC Waters Chromatograph, with BEH300 C18 Acquity Waters 2.1x100 mm, 1.7 μm, at 45° C., using H2O, 0.1% TFA (A) and CH3CN, 0.1% TFA (B) as solvents and the following gradient: 20 over 60% B in 20 min, flow 0.4 ml/min. The product was also characterized by electrospray mass spectrometry on a Acquity SQ Detector Yield 12%, >90% pure (Mw found: 3899.3 Da; Mw expected: 3899.3 Da).

[0313] Synthesis of Fmoc-4-(Dde-aminomethyl)-phenylalanine was as follows.

![Image](https://via.placeholder.com/150)

**tBp = threo-β-phenylserine was prepared from (2R,3S)/(2S,3R)- racemic Fmoc-β-hydroxy-phenylalanine:**
Fmoc-4-(Boc-aminomethyl)-phenylalanine was stirred in DCM/TFA 2/1 for 1 hour. The solvents were removed under reduced pressure and the residue was treated with diethyl ether to obtain a white solid. The crude material obtained was dissolved in EtOH (19 mM), DIPEA (5 eq) and Dimedone (1.1 eq) were added to the reaction mixture. After 3 hours at 60°C, the solution was acidified with TFA to pH 1.4. The solvents were removed under reduced pressure and the residue was treated with AcOEt and washed with HCl 1N.

The organic phase was washed with brine and dried over Na2SO4. The solvents were removed under reduced pressure and the final product was obtained as yellow solid.

The final compound was characterized on an Acquity UPLC Waters Chromatograph, with BEH1300 C18 Acquity Waters 2.1x100 mm, 1.7 µm, at 45°C, using H2O, 0.1% TFA (A) and CH3CN, 0.1% TFA (B) as solvents and the following gradient: 10% to 10% B in 1 min, 10% B over 90% B in 4 min, flow 0.4 mL/min. The protected amino acid was characterized by electrospray mass spectrometry on an Acquity SQ Detector (Mw found: 581.5 Da; Mw expected: 580.67 Da).

Example 16

The cAMP protocol was as follows.

Peptides were dissolved in 100% DMSO and serially diluted to generate 11 point titrations. The peptide solutions were then transferred into 384-well assay plates (150 nL/well). Assay ready frozen cells expressing human GLP-1R or human GCGR were suspended in growth media consisting of DMEM/F12 medium (GIBCO), 10% FBS (GIBCO), lx P/S (GIBCO), 800 µg/mL Genetin (GIBCO), and 300 µg/mL Hygromycin (Invitrogen). Cells were then diluted in assay buffer consisting of PBS (GIBCO), 7.5% BSA (Perkin Elmer), 100 µM RO 20-1724 (Sigma), and 20% human serum (MP Biomedical). The cell suspensions (15 µL) were then added to the assay plates containing the peptide solutions (30,000 cells/well for human GCGR; 20,000 cells/well for GLP1R). The cells were incubated for 1 hour at room temperature in the dark. Production of cAMP was determined using HitHunter™ cAMPXS kits (DiscoverX). ED buffer (20 µL) was then added to each well and the plates were incubated for 1 hour at room temperature in the dark. Subsequently, EA buffer (20 µL) was added to each well, and the plates were incubated for 1-18 hours at room temperature in the dark. Luminescence was measured using an EnVision Multilabel plate reader (Perkin Elmer). EC50 values were calculated using ADA data analyzer software. The results are shown in Table 2.

<table>
<thead>
<tr>
<th>SEQ ID NO.</th>
<th>Peptide</th>
<th>GCGR EC50 (nM) human</th>
<th>GLP1R EC50 (nM) human</th>
<th>hGCGR/hGLP1R (nTone)</th>
</tr>
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<tbody>
<tr>
<td>6</td>
<td>HsGQTTSKsl[YEYEC] SKYLDARAQDFVQNLDT-NH2</td>
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<td>0.048</td>
<td>0.88</td>
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<tr>
<td>7</td>
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<td>0.160</td>
<td>0.30</td>
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<tr>
<td>8</td>
<td>HsGQTTSKsl[YEYEC] SKYLDVRAAQDFVQNLDT-NH2</td>
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<td>0.130</td>
<td>0.60</td>
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<tr>
<td>9</td>
<td>HsGQTTSKsl[YEYEC] SKYLDARAQDFVQNLDT-NH2</td>
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<td>0.033</td>
<td>0.90</td>
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<td>10</td>
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<td>0.007</td>
<td>1.30</td>
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<td>HsGQTTSKsl[YEYEC] SKYLDARAQDFVQNLDT-NH2</td>
<td>0.027</td>
<td>0.070</td>
<td>1.00</td>
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<td>12</td>
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<td>0.020</td>
<td>0.90</td>
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<td>13</td>
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TABLE 2 - continued

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<th>SEQ ID NO:</th>
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<th>GLP1R EC50 (nM)</th>
<th>hGCGR/ hNLGBP EC50 (nM)</th>
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</thead>
<tbody>
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<td>15</td>
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<td>0.060</td>
<td>0.91</td>
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<td>0.050</td>
<td>0.98</td>
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<td>1.040</td>
<td>1.020</td>
<td>1.02</td>
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</table>

* = D-serine; D = D-aspartate; E = N-acetyl; H = norleucine (Nle); P = para-aminomethyl phenylalanine; 2 = methionine sulfoxide; 1 = 1-amino-1-cyclobutane carboxylic acid; 3 = 3-amino-3-fluorophenylalanine; 4 = acetyl; 5 = p-fluorophenylalanine

Example 17

[0318] Diet induced obesity (DIO) mice have long been used as surrogates for humans in the study of the efficacy of anti-obesity compounds. The results obtained from such mice in the study of obesity compounds are translatable to humans (See for example, Nilsson et al. Acta Pharmacologica Sinica 33: 173-181 (2012), which is incorporated herein by reference in its entirety). Thus, DIO mice are useful surrogates for humans for the testing the efficacy of compounds intended to treat obesity.

[0319] For the studies in Examples 18, 19, 20, and 21, diet induced obesity (DIO) mice (strain C57Bl6/NTac; 20 weeks on a high fat diet, about 47 g) were divided into groups of eight mice per group and the initial average body weight, food intake and basal glucose of each group were matched before peptide administration. Each group of mice was subcutaneously injected daily with a dose of Co-agonist Peptide or vehicle control for 7 to 10 days. The peptides tested in this study were Co-agonist Peptides 1, 2, 3, 4, 5, and 6 (SEQ ID NOs: 6, 7, 8, 9, 10, and 11, respectively) at the doses that varied from 3 nmol/kg to 25 nmol/kg. Body weight, food intake and basal glucose were measured periodically during treatment. All peptides have comparable pharmacokinetic properties and similar plasma protein binding.

Example 18

[0320] The in vivo effects of certain peptides of the invention were tested in diet-induced obese (DIO) mice (strain C57Bl6/NTac) that were maintained on a high fat diet for 20 weeks and had an initial body weight of about 47 grams. Mice were administered a vehicle control or a dose of a peptide daily for nine days. The peptides in this study included peptides of SEQ ID NOs: 6, 7, 8, 9, 10 and 11 administered at a dose of 9 nmol/kg. The peptide of SEQ ID NO: 9 was administered at doses of 9 and 25 nmol/kg. Cumulative body weight changes (grams) was measured each day of the study except on day 3. Results are shown in FIG. 1 and are expressed as mean±SEM. Each of the peptides tested in this study demonstrated a significant (p<0.05 vs vehicle, 2way ANOVA) weight loss over the course of the study compared to the vehicle treated groups of mice. On day 9 of the study, mice that were administered 9 nmol/kg of the peptide of SEQ ID NOs: 7 and 9 exhibited an approximate weight loss of 3 grams, while a dose of 25 nmol/kg of the peptide of SEQ ID NO: 9 led to a body weight loss of 10.5 grams. Mice that were administered 9 nmol/kg of the peptide of SEQ ID NOs: 8 and 6 exhibited weight loss of 8 and 10.2 grams, respectively. Mice that were administered 9 nmol/kg of the peptide of SEQ ID NOs: 10 and 11 exhibited approximately 13 grams of weight loss, respectively.

Example 19

[0321] In a second study in DIO mice (strain C57Bl6/NTac) the doses of each peptide were varied—each of the peptides of SEQ ID NOs: 6 to 11 was administered at a dose 9 nmol/kg. In addition, the peptide of SEQ ID NO: 8 was administered at 15 nmol/kg, while the peptide of SEQ ID NOs: 10 and 11 were administered at 3 nmol/kg. Body weight was measured daily for eight days and body weight change is expressed as percent relative to vehicle (FIG. 2). Mice administered a dose of 9 nmol/kg the peptide of SEQ ID NOs: 7 or 9 exhibited approximately 5% weight loss compared to mice administered with a dose of 9 nmol/kg the peptide of SEQ ID NOs: 6 or 8, who exhibited weight loss of approximately 20% or 17% respectively. Mice administered with a dose of 15 nmol/kg the peptide of SEQ ID NO: 8 exhibited weight loss of approximately 25%. Mice administered with a dose 3 nmol/kg the peptide of SEQ ID NO: 10 exhibited weight loss of approximately 17%, while mice administered with a dose of 3 nmol/kg of the peptide of SEQ ID NO: 11, exhibited no weight loss efficacy.

Example 20

[0322] As shown in FIG. 3, DIO mice (strain C57Bl6/NTac) that were administered with 9 nmol/kg of the peptides of SEQ ID NOs: 6 to 11, with exception of peptides of SEQ ID NOs: 7, exhibited a significant reduction in cumulative food intake after 8 days of dosing (*p<0.05, **p<0.01, ***p<0.001 vs Vehicle, 1 way ANOVA, Dunns multiple comparisons). Mice that were administered with 3 nmol/kg of the peptide of SEQ ID NO: 10 exhibited also a significant suppression of food intake, while mice that were administered with 3 nmol/kg of the peptide of SEQ ID NO: 11 exhibited no change in food intake.

Example 21

[0323] In addition to cumulative body weight change, ambient glucose levels of the DIO mice (strain C57Bl6/NTac)
were measured periodically throughout the study. As shown in FIG. 4, mice that were administered with the peptide of SEQ ID NO: 9 at a dose 25 nmol/kg demonstrated a significant decrease in ambient glucose by the second day of dosing compared to vehicle treated control mice (p<0.05 vs Vehicle). Mice that were dosed with 9 nmol/kg of the peptide of SEQ ID NOs: 10 and 11 demonstrated a similar decrease in ambient glucose. Mice that were dosed with 9 nmol/kg of the peptide of SEQ ID NO: 6 demonstrated a significant decrease in ambient glucose by day 7 of dosing (p<0.05 vs Vehicle). Mice that were dosed with 9 nmol/kg of the peptides of SEQ ID NOs: 8 demonstrated a significant decrease in ambient glucose by day 9 (p<0.01 vs Vehicle, 2-way ANOVA). Mice that were dosed with 9 nmol/kg of the peptides of SEQ ID NOs: 7 or 9 did not exhibit significant changes in ambient glucose after 9 days of dosing.

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<th>Name</th>
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<td>Glucagon (Homo sapiens)</td>
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<td>Oxyntomodulin (Homo sapiens)</td>
<td>HGQTPTS8SYKLD8RAQDFVQMLMTKRNR</td>
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<td>Exendin-4 (Heloderma suspectum)</td>
<td>HAGTSPLGQM726ZAVLRPI26W42UGPG</td>
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<tr>
<td>6</td>
<td>Co-agonist Peptide 1</td>
<td>X2-D-Ser</td>
</tr>
<tr>
<td>7</td>
<td>Co-agonist Peptide 2</td>
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</tr>
<tr>
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<td>Co-agonist Peptide 3</td>
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<td>Co-agonist Peptide 9</td>
<td>X6-p-fluoro phenylalanine (pFF)</td>
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<td>E27-methionine sulfoxide</td>
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<td>Co-Terminal amidation</td>
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<td>X2-D-Ser, L-Ala, aib, or 1-amino-cyclobutane carboxylic acid</td>
<td>E6-L-Phe, p-fluoro phenylalanine (pFP) or threeo-β-phenylserine</td>
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<td>X10-K conjugated to EC16, K conjugated to γEYEC16, or pAF conjugated to γEYEC16</td>
<td>E12-L-Lys or L-Leu</td>
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<tr>
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<td>X15-L-Glu or L-Leu or L-Amp</td>
<td>X16-aib, L-Ala, L-Glu, or L-Val</td>
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<tr>
<td></td>
<td>X18-L-Ala or L-Arg</td>
<td>X27-L-Leu, methionine sulfoxide, or L-Nle</td>
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<tr>
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<td>Co-Terminal amidation</td>
<td></td>
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<tr>
<td>21</td>
<td>X is D-Ser</td>
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<tr>
<td></td>
<td>K conjugated to γEYEC16 or γEYEC16</td>
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While the present invention is described herein with reference to illustrated embodiments, it should be understood that the invention is not limited hereto. Those having ordinary skill in the art and access to the teachings herein will recognize additional modifications and embodiments within the scope thereof. Therefore, the present invention is limited only by the claims attached herein.

SEQUENCE LISTING

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ORGANISM: Heloderma suspectum
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His Gly Glu Gly Thr Ser Ser Asp Ser Leu Ser Lys Glu Met Glu Gly
1 5 10 15
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
20 25 30
Ser Gly Ala Pro Pro Pro Ser
35

<210> SEQ ID NO 5
His Ala Asp Gly Ser Phe Ser Asp Glu Met Asn Thr Ile Leu Asp Arg
1  5  10  15
Leu Ala Ala Arg Asp Phe Ile Asn Trp Leu Ile Gln Thr Lys Ile Thr
20 25 30
Asp

Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20 25

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Ala
1  5  10  15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20 25

Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20 25
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<220> FEATURE:
<223> OTHER INFORMATION: Co-agonist peptide 3

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)...(2)
<223> OTHER INFORMATION: Xaa = D-serine

<220> FEATURE:
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<222> LOCATION: (10)...(10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via a gamma-Glu gamma-Glu spacer

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29)...(29)
<223> OTHER INFORMATION: C-terminal amide

<400> SEQUENCE: 8
His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lye Tyr Leu Asp Val
1 5 10 15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20 25

<210> SEQ ID NO 9
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: Co-agonist peptide 4

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)...(2)
<223> OTHER INFORMATION: Xaa = alpha-aminoisobutyric acid (Aib)

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)...(10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer

<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (16)...(16)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (27)...(27)
<223> OTHER INFORMATION: Xaa = norleucine

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29)...(29)
<223> OTHER INFORMATION: C-terminal amide

<400> SEQUENCE: 9
His Xaa Val Gly Thr Phe Thr Ser Asp Lys Ser Lye Tyr Leu Asp Xaa
1 5 10 15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Xaa Asp Thr
20 25

<210> SEQ ID NO 10
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence

<220> FEATURE:
<223> OTHER INFORMATION: Co-agonist peptide 5

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)...(2)
<223> OTHER INFORMATION: Xaa = D-Serine

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)...(10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu
gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) .. (29)
<223> OTHER INFORMATION: C-terminal amide

<400> SEQUENCE: 10

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Aln
  1  5  10  15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
  20  25

<210> SEQ ID NO 11
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2) .. (2)
<223> OTHER INFORMATION: Xaa = D-Serine

<400> SEQUENCE: 11

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
  1  5  10  15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
  20  25

<210> SEQ ID NO 12
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1) .. (1)
<223> OTHER INFORMATION: N-terminal acetyl group

<400> SEQUENCE: 12

His Ala Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
  1  5  10  15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
  20  25

<210> SEQ ID NO 13
<211> LENGTH: 29
<212> TYPE: PRT
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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Co-agonist peptide 8
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2) (2)
<223> OTHER INFORMATION: Xaa = D-Serine
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) (10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6) (6)
<223> OTHER INFORMATION: Xaa = p-fluoro phenylalanine (pFF)
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) (10)
<223> OTHER INFORMATION: C-terminal amide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) (29)
<223> OTHER INFORMATION: C-terminal amide

<400> SEQUENCE: 13
His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Gln Ala
1   5
10   15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20
25

<210> SEQ ID NO 14
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Co-agonist peptide 9
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2) (2)
<223> OTHER INFORMATION: Xaa = D-Serine
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6) (6)
<223> OTHER INFORMATION: Xaa = p-fluoro phenylalanine (pFF)
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) (10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) (29)
<223> OTHER INFORMATION: C-terminal amide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) (29)
<223> OTHER INFORMATION: C-terminal amide

<400> SEQUENCE: 14
His Xaa Gln Gly Thr Xaa Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1   5
10   15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20
25

<210> SEQ ID NO 15
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Co-agonist peptide 10
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2) (2)
<223> OTHER INFORMATION: Xaa = D-Serine
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) (10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) (29)
<223> OTHER INFORMATION: C-terminal amide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Xaa Asp Thr
20 25

<210> SEQ ID NO 16
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (27) .. (27)
<223> OTHER INFORMATION: C-terminal amide

<400> SEQUENCE: 15

His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1 5 10 15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Xaa Asp Thr
20 25

<210> SEQ ID NO 17
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) .. (29)
<223> OTHER INFORMATION: C-terminal amide

<400> SEQUENCE: 17

His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Ala
1 5 10 15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20 25

<210> SEQ ID NO 18
<211> LENGTH: 29
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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<222> LOCATION: (2) ... (2)
<223> OTHER INFORMATION: Co-agonist peptide 13
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) ... (10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2) ... (2)
<223> OTHER INFORMATION: C-terminal amide

<400> SEQUENCE: 19
His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Tyr Leu Asp Glu
1  5  10  15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20  25

<210> SEQ ID NO 19
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<222> LOCATION: (6) ... (6)
<223> OTHER INFORMATION: Xaa = D-Serine
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) ... (10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) ... (29)
<223> OTHER INFORMATION: C-terminal amide

<400> SEQUENCE: 19
His Xaa Gln Gly Thr Xaa Thr Ser Asp Lys Ser Tyr Leu Asp Glu
1  5  10  15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20  25

<210> SEQ ID NO 20
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<222> LOCATION: (1) ... (1)
<223> OTHER INFORMATION: Optional N-terminal acetyl group
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2) ... (2)
<223> OTHER INFORMATION: Xaa = D-Serine, L-Alanine aib, or L-amino-cyclobutane carboxylate acid
<220> FEATURE:
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<210> SEQ ID NO 22
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<222> OTHER INFORMATION: CO-agonist peptide 16
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2) .. (2)
<223> OTHER INFORMATION: Xaa = D-Serine
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) .. (10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) .. (29)
<223> OTHER INFORMATION: Optional C-terminal amide or ester

<400> SEQUENCE: 22
His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Ala Arg Ala Ala Gln Phe Val Gln Trp Leu Leu Asp Thr
1 5 10 15

<210> SEQ ID NO 23
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<222> OTHER INFORMATION: CO-agonist peptide 17
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2) .. (2)
<223> OTHER INFORMATION: Xaa = D-Serine
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) .. (10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) .. (29)
<223> OTHER INFORMATION: Optional C-terminal amide or ester

<400> SEQUENCE: 23
His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Val Arg Arg Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
1 5 10 15

<210> SEQ ID NO 24
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<222> OTHER INFORMATION: CO-agonist peptide 18
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2) .. (2)
<223> OTHER INFORMATION: Xaa = D-Serine
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) .. (10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) .. (29)
<223> OTHER INFORMATION: Optional C-terminal amide or ester
<400> SEQUENCE: 24
His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Val
1 5 10 15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20 25

<210> SEQ ID NO 25
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Co-agonist peptide 19
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)...(2)
<223> OTHER INFORMATION: Xaa = alpha-aminoisobutyric acid (aib)
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (16)...(16)
<223> OTHER INFORMATION: Xaa = alpha-aminoisobutyric acid (aib)
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (27)...(27)
<223> OTHER INFORMATION: Xaa = L-norleucine (Nle)
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29)...(29)
<223> OTHER INFORMATION: Optional C-terminal amide or ester

<400> SEQUENCE: 25
His Xaa Val Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Xaa
1 5 10 15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Xaa Asp Thr
20 25

<210> SEQ ID NO 26
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Co-agonist peptide 20
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)...(2)
<223> OTHER INFORMATION: Xaa = D-serine
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)...(10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29)...(29)
<223> OTHER INFORMATION: Optional C-terminal amide or ester

<400> SEQUENCE: 26
His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Ala
1 5 10 15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20 25
-continued

<210> SEQ ID NO 27
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Co-agonist peptide 21
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2) (2)
<223> OTHER INFORMATION: Xaa = D-Serine
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) (10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) (29)
<223> OTHER INFORMATION: Optional C-terminal amide or ester

<400> SEQUENCE: 27
His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1  5  10  15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20  25

<210> SEQ ID NO 28
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Co-agonist peptide 22
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1) (1)
<223> OTHER INFORMATION: N-terminal acetyl group
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) (10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) (29)
<223> OTHER INFORMATION: Optional C-terminal amide or ester

<400> SEQUENCE: 28
His Ala Gln Gly Thr Phe Thr Ser Asp Lys Ser Lys Tyr Leu Asp Glu
1  5  10  15
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20  25

<210> SEQ ID NO 29
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Co-agonist peptide 23
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2) (2)
<223> OTHER INFORMATION: Xaa = D-Serine
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10) (10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29) (29)
<210> SEQ ID NO: 32
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (10)...(10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (29)...(29)
<223> OTHER INFORMATION: Optional C-terminal amide or ester

<400> SEQUENCE: 32
His Xaa Gln Gly Thr Phe Thr Ser Asp Lys Ser Tyr Leu Asp Glu
1  5  10
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20  25

<210> SEQ ID NO: 33
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (2)...(2)
<223> OTHER INFORMATION: Xaa = D-Serine
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (10)...(10)
<223> OTHER INFORMATION: Xaa = para-aminomethyl phenylalanine 9pAF) conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (29)...(29)
<223> OTHER INFORMATION: Optional C-terminal amide or ester

<400> SEQUENCE: 33
His Xaa Gln Gly Thr Phe Thr Ser Asp Xaa Ser Lys Tyr Leu Asp Ala
1  5  10
Arg Ala Ala Gln Asp Phe Val Gln Trp Leu Leu Asp Thr
20  25

<210> SEQ ID NO: 34
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (2)...(2)
<223> OTHER INFORMATION: Xaa = 1-amino-1-cyclobutane carboxylic acid
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (10)...(10)
<223> OTHER INFORMATION: Lysine conjugated to C16 via gamma-Glu gamma-Glu spacer
<220> FEATURE:
<221> NAME/KEY: Misc_feature
1. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, or 19.

2. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, or 19.

3. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of native human glucagon having the amino acid sequence shown in SEQ ID NO: 1 but in which the L-serine at position 10 is replaced with a D-serine.

4. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, or 19.

5. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of native human glucagon having the amino acid sequence shown in SEQ ID NO: 1 but in which the L-serine at position 10 is replaced with a D-serine.

6. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of native human glucagon having the amino acid sequence shown in SEQ ID NO: 1 but in which the L-serine at position 10 is replaced with a D-serine.

7. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of native human glucagon having the amino acid sequence shown in SEQ ID NO: 1 but in which the L-serine at position 10 is replaced with a D-serine.

8. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of native human glucagon having the amino acid sequence shown in SEQ ID NO: 1 but in which the L-serine at position 10 is replaced with a D-serine.

9. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of native human glucagon having the amino acid sequence shown in SEQ ID NO: 1 but in which the L-serine at position 10 is replaced with a D-serine.

10. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of native human glucagon having the amino acid sequence shown in SEQ ID NO: 1 but in which the L-serine at position 10 is replaced with a D-serine.

11. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of native human glucagon having the amino acid sequence shown in SEQ ID NO: 1 but in which the L-serine at position 10 is replaced with a D-serine.

12. The peptide of claim 103, wherein the peptide comprises the amino acid sequence of native human glucagon having the amino acid sequence shown in SEQ ID NO: 1 but in which the L-serine at position 10 is replaced with a D-serine.
position 2 is replaced with a D-serine; the tyrosine at position 10 is replaced with (i) L-lysine conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer or (ii) para-aminomethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamic acid spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide spacer; and up to six additional amino acid substitutions; wherein the peptide is an agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC50 at the glucagon receptor to EC50 at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12, and wherein the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide, and (ii) a pharmaceutically acceptable carrier.

111: The composition of claim 110, wherein the peptide further comprises one or more of the following substitutions: a serine at position 16 of the peptide is replaced with L-alanine; a histidine at position 18 of the peptide is replaced with L-lysine; a histidine at position 27 of the peptide is replaced with L-leucine; and an asparagine at position 28 of the peptide is replaced with L-aspartic acid.

112: The composition of claim 110, wherein the peptide comprises a serine at position 16 of the peptide replaced with L-alanine, a histidine at position 18 of the peptide replaced with L-lysine, a histidine at position 27 of the peptide replaced with L-leucine, and an asparagine at position 28 of the peptide replaced with L-aspartic acid.

113: A method for treating a patient or individual for having a metabolic disease, comprising administering the patient or individual an effective amount of the composition of claim 1 to treat the metabolic disease in the patient or individual.

114: The method of claim 113, wherein the metabolic disease comprises diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

115: The method of claim 114, wherein the diabetes comprises Type I diabetes, Type II diabetes, or gestational diabetes.

116: A method for treating a patient or individual for a metabolic disease comprising administering the patient or individual an effective amount of the composition of claim 10 to treat the metabolic disease in the patient or individual.

117: The method of claim 116, wherein the metabolic disease comprises diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

118: The method of claim 117, wherein the diabetes comprises Type I diabetes, Type II diabetes, or gestational diabetes.

119: A method for treating a metabolic disease in a patient or individual comprising:

administering to the patient or individual (i) an effective amount of a composition comprising a peptide of claim 103 and administering to the patient or individual an effective amount of a composition comprising an insulin or insulin analog or (ii) the composition of claim 108 to treat the metabolic disease in the patient or individual.

120: The method of claim 119, wherein the method in (i) comprises administering the composition comprising the peptide at a time prior to the time the composition comprising the insulin or insulin analog is administered; or administering the composition comprising the insulin or insulin analog at a time prior to the time the composition comprising the peptide is administered; or administering the composition comprising the peptide at the same time as the composition comprising the insulin or insulin analog is administered.

121: The method of claim 119, wherein the insulin analog comprises insulin detemir, insulin glargine, insulin levoemir, insulin glulisine, or insulin lispro.

122: The method of claim 119, wherein the metabolic disease comprises diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

123: The method of claim 122, wherein the diabetes comprises Type I diabetes, Type II diabetes, or gestational diabetes.

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