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[Continued on next page]

(54) **Title:** CO-AGONISTS OF THE GLUCAGON AND GLP-1 RECEPTORS

	1	10	20	29
Glucagon	HSQGTFTSDYSKYLSRRAQDFVQWLMNT			
Libr 1	XXXXXXXXFTSDYSKYLSRRAQDFVQWLXXT			
Libr 2	XXXXXXXXSDYSKYLSRRAQDFVQWLXXT			
Libr 3	HSQGTFTXXXXXXXXLSRRAQDFVQWLXXT			
Libr 4	HSQGTFTSDYSKYXXXXXXXXDFVQWLXXT			
Libr 5	HSQGTFTSDYSKYLSRRAQXXXXXXXXXX			

FIG. 1(57) **Abstract:** Co-agonists of the glucagon and GLP-1 receptors are described.



— with sequence listing part of description (Rule 5.2(a))

TITLE OF THE INVENTION

CO-AGONISTS OF THE GLUCAGON AND GLP-1 RECEPTORS

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Application No. 62/264,928 filed December 9, 2015, which is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

(1) Field of the Invention

10 The present invention is related to co-agonist peptides of the glucagon and GLP-1 receptors.

(2) Description of Related Art

15 Pre-proglucagon 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-
20 proglucagon, while GLP-1 is produced as a 37-amino acid peptide that corresponds to amino acids 72 through 108 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.

25 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.

30 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 diabetics.

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.

5 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.

10 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
15 body weight (Salter, *Am. J. Clin. Nutr.* 8: 535–539 (1960)). Nonetheless, the inherent risk of hyperglycemia, especially in insulin resistant states such T2DM, has complicated the translation of these observations to human study.

20 The hormone oxyntomodulin (OXM, glucagon-37) is a posttranslational product of proglucagon processing in the intestine and central nervous system (CNS) and is secreted from L-cells in the gut in response to food intake. Discovered in 1983, OXM has been implicated in the regulation of food intake and energy expenditure (Jarrouse et al., *Endocrinol.* 115: 102-105 (1984); Schjoldager et al., *Eur. J. Clin. Invest.*, 18: 499-503 (1988)). Central or peripheral administration of OXM in rats causes a decrease in short term food intake with minimal effects on gastric emptying (Dakin et al. *Endocrinology*, 142: 4244-4250 (2001), Dakin
25 et al. *Endocrinology*, 145: 2687-2695 (2004)). Repeated intracerebroventricular administration of OXM in rats results in elevated core temperatures and reduced weight gain compared to pair-fed animals, suggesting effects on both caloric intake and energy expenditure (Dakin et al. *Am. J. Physiol. Endocrinol. Metab.*, 283: E1173-E1177 (2002)).

30 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 c-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)).

Studies of OXM action in mice have demonstrated that although OXM can
5 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
10 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 (Sowden et al., *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292: R962-R970 (2007)). OXM has also been shown to differentially affect GLP-1 receptor beta-
15 arrestin recruitment and signaling through Galpha (Jorgensen et al., *J. Pharma. Exp. Therapeut.* 322: 148-154 (2007)) and to differentially affect hypothalamic neuronal activation following peripheral injection of OXM (Choudhri et al., *Biochem. Biophys. Res. Commun.* 350: 298-306 (2006)).

In humans, a single 90 minute intravenous infusion of OXM in normal weight
20 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 volunteers (BMI about 33)
25 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
30 incidence of mild and transient nausea (about 3%) despite the relatively high doses necessitated

by the poor in vivo stability of OXM (plasma $t_{1/2}$ < 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 inactivated by the cell surface dipeptidyl peptidase IV (DPP-IV) (Zhu et al., J. Biol. Chem. 278: 22418-22423 (2002)).

5 However, DPP-IV inhibitors are weight-neutral in the clinic, suggesting that supraphysiological 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. WO03/022304, WO2004/062685, WO2006/134340, and WO2010/096052.

10 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 jejuno-
15 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, WO2012/177444, and
20 WO2012/177443. 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 activity or potency at the GLP-1 receptor and GCG receptor.

BRIEF SUMMARY OF THE INVENTION

25 The present invention provides co-agonist peptides of the human glucagon (GCG) receptor and the human glucagon-like protein 1 (GLP-1) receptor that include substitution of one or more amino acids of the native glucagon peptide with an L-amino acid not native to glucagon to produce a non-native glucagon peptide that is a co-agonist at the glucagon and GLP-1 receptors. Native human glucagon has about 10-fold greater activity at the glucagon receptor
30 than at the GLP-1 receptor. Phage display technology was used to construct libraries of randomly mutagenized human glucagon peptides, which were screened for activity at both the human

glucagon receptor and the human GLP-1 receptor. The technology has enabled the inventors to discover peptides that have co-agonist activity at the glucagon and GLP-1 receptors. The co-agonist peptides identified by the phage display screens may be stabilized against inactivation *in vivo* by dipeptidyl peptidase-4 (DPP-IV) cleavage at the *N*-terminus by replacing the serine residue at position 2 of the co-agonist peptide with an amino acid that inhibits DPP-IV cleavage. For example an amino acid selected from the group consisting of Val, Ile, Asp, Glu, Met, Trp, Asn, D-Ala, D-Ser, and alpha-aminoisobutyric (aib). The co-agonist peptides further include amidation of the C-terminus amino acid to stabilize the co-agonist peptide from potential degradation *in vivo* by carboxypeptidases.

The co-agonist peptides may have a blood serum half-life of at least one day, two days, three days, four days, five days, six days, or seven days. The co-agonist peptides further comprise modifications that control the relative activity at the GLP-1 receptor versus the glucagon receptor. Thus, yet another aspect of the invention provides co-agonist peptides that have higher activity at the glucagon receptor versus the GLP-1 receptor, co-agonist peptides that have approximately equivalent activity at both receptors, and co-agonist peptides that have higher activity at the GLP-1 receptor versus the glucagon receptor. The latter category of co-agonist peptides may be engineered to exhibit little or no activity at the glucagon receptor, and yet retain ability to activate the GLP-1 receptor with the same or better potency than native GLP-1. Any of these analogs may also include modifications that confer enhanced biophysical stability and/or aqueous solubility. The co-agonist peptides herein are useful for the treatment of metabolic diseases or 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.

The present invention provides a co-agonist peptide comprising a peptide selected from following peptides

SEQ ID NO:	Name	Sequence
1	PD17	HSVGNFWSDYSKYLDSSRAQDFVQWLMLT-CONH ₂
2	PD18	HSQGTFTSDYSKYVEDRRAHDFVQWLMNT-CONH ₂
3	PD19	HSQGTFTSDYRKYLDERAAWDFVQWLMNT-CONH ₂
4	PD20	HSQGTFTSDYSKYLNSWMTQDFVQWLMNT-CONH ₂

5	PD21	HSQGTFTSDYSKYLD IG RAQDFVQWLLNT-CONH ₂
6	PD22	HSQGTFTSDYSKYLD SLMA QDFVQWLMST-CONH ₂
7	PD23	HSQGTFTSDYSKYLD W RRRAQDFVQWLLNT-CONH ₂
8	PD24	HSQGTFTSDY IKLL DSRRRAQDFVQWLMNT-CONH ₂
9	PD25	HSQGTFTSDYSKYLD ARRA QDFVQWL IRT -CONH ₂
10	PD26	HSQGTFTSDYSKYLD V RRRAQDFVQWLMNT-CONH ₂
11	PD27	HSQGTFTSDYSKYLD ELRAY DFVQWLMNT-CONH ₂
12	PD28	HSQGTFTSDYSKYLD YM RAYDFVQWLMNT-CONH ₂
13	PD29	HSQGTFTSDYSKYLDSRRRA H DFVQWLLNT-CONH ₂
14	PD30	HSQGTFTSDYSKYLDSRRRAQDFVQWLMN P -CONH ₂
15	PD31	HSQGTFTSDYSKYLDSRRRAQDFVQWL IN Y-CONH ₂
16	PD32	INHEQW AFTSDYSKYLDSRRRAQDFVQWLMNT-CONH ₂
17	PD33	ASMTFF SDYSKYLDSRRRAQDFVQWLM LT -CONH ₂
18	PD34	HSQGT FL SDYSK LL DSRRRAQDFVQWLM QT -CONH ₂
19	PD35	HSQGT FLHDY YYYLDSRRRAQDFVQWLM DT -CONH ₂
20	PD36	HSQGTFTSDYSKYLDS IRA QDFVQWLM DT -CONH ₂
21	PD37	HSQGTFTSDYSKYLD NK RAQDFVQWLM QT -CONH ₂
22	PD38	HSQGTFTSDYSKYLDSRRRAQDFV D WLMN E -CONH ₂
23	PD39	HSQGTFTSDYSKYLDSRRRAQ EFV EWM DE -CONH ₂
24	PD40	HSQGTFTSDYSKYLDSRRRAQDFVQWL INT -CONH ₂
25	PD41	KALGQ FTFTSDYSKYLDSRRRAQDFVQWLMNT-CONH ₂
26	PD42	HSQGT FFH DYSKYLDSRRRAQDFVQWLLNT-CONH ₂
27	PD43	HSQGT FFS DY HW LDSRRRAQDFVQWLMNT-CONH ₂
28	PD44	HSQGTFTSDYSKYLD W RRRAQDFVQWL QNT -CONH ₂
29	PD45	HSQGTFTSDYSKYLDS KRAH DFVQWLLNT-CONH ₂
30	PD46	HSQGTFTSDYSKYLDSRRRAQDF WID LMNT-CONH ₂
31	PD47	HSQGTFTSDYSKYLDSRRRAQDFV MTS MNT-CONH ₂
32	PD48	HSQGTFTSDYSKYLDSRRRAQDFV DW LL NA -CONH ₂
33	PD49	HSQGTFTSDYSKYLDSRRRAQDFV E WLMN N -CONH ₂
34	PD50	HSQGTFTSDYSKYLDSRRRAQDFV DW L INS -CONH ₂
35	PD51	HS H GTFTSDYSKYLDSRRRAQDFVQWLM TT -CONH ₂
36	PD52	HSQ GIF SDYSKYLDSRRRAQDFVQWLMNT-CONH ₂
37	PD53	HSQGT FL SDYSKYLDSRRRAQDFVQWLMNT-CONH ₂
38	PD54	HSQGTFTSDY WY LDSRRRAQDFVQWLMNT-CONH ₂
39	PD55	HSQGTFTSDYSKYLD MQRAH DFVQWLMNT-CONH ₂

40	PD56	HSQGTFTSDYSKYLDSRM AY DFVQWLMNT-CONH ₂
41	PD57	HSQGTFTSDYSKYLDSRRAQDFVQWLLN Q -CONH ₂
42	PD58	HSQGTFFSDYSKYLDSRRAQDFVQWLL ET -CONH ₂
43	PD59	HSQGTFTSDYSKYLDSRRAQDFVQWLL DS -CONH ₂

wherein the L-Serine at position 2 is replaced with Val, Ile, Asp, Glu, Met, Trp, Asn, D-Ala, D-Ser, or α -aminoisobutyric acid (aib), the Tyrosine at position 10 is replaced with a Lysine residue conjugated to a fatty acid or fatty diacid via a linking moiety, wherein CONH₂ indicates the C-terminal amino acid carboxy group is amidated, and the peptide may comprise 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 additional amino acid substitutions.

In particular embodiments, the co-agonist peptide of claim 1, wherein the fatty acid or fatty diacid comprises 14 to 20 methylene groups. In a further embodiment, the fatty acid or fatty diacid comprises 16 carbon atoms.

In particular embodiments, the linking moiety comprises a PEG₂ (8-amino-3,6-dioxaoctanoic acid) Gamma-Glutamic acid (γ Glu), a γ Glu, a γ Glu γ Glu, or a PEG₂PEG₂ γ Glu.

The present invention further provides a co-agonist peptide comprising a peptide selected from the following peptides

SEQ ID NO:	Name	Sequence
44	TP534	HsQGTFTSDK(γ E γ EC16)SKYLD NK RAQDFVQWLM QT -CONH ₂
45	TP535	HsQGTFTSDK(γ E γ EC16)SKYLDSRRA H DFVQWLLNT-CONH ₂
46	TP536	HsQGTFLSDK(γ E γ EC16)SKLLDSRRAQDFVQWLM QT -CONH ₂
47	TP552	HsQGTFLSDYSKLLDSRAAQDFVQWLL QT -CONH ₂
48	TP559	HsQGTFTSDK(γ E γ EC16)SKYLD ARAA HDFVQWLLNT-CONH ₂
49	TP572	HUQGTFTSDK(γEγEC16)SKYLDSRRA H DFVQWLLNT KγE -CONH ₂
50	TP573	HUQGTFTSDK(γEγEC16)SKYLD ARAA HDFVQWLLNT KγE -CONH ₂
51	TP574	HUQGTFTSDK(γEγEC16)SKYLD NK RAQDFVQWLM QT KγE -CONH ₂

wherein U is aminoisobutyric acid, s is D-Ser, γ E = γ -glutamic acid, and C16 = -CO-(CH₂)₁₄-CH₃ and wherein CONH₂ indicates the C-terminal amino acid carboxy group is amidated.

In particular embodiments, the present invention provides a composition comprising one or more co-agonist peptides of claims 1-6 and a pharmaceutically acceptable carrier and/or pharmaceutically acceptable salt.

The present invention further provides a method for treating a patient for a metabolic disease or disorder comprising administering the patient an effective amount of any

one or more of the co-agonist peptides of claims 1-6 to treat the metabolic disease or disorder in the patient.

In particular aspects, the metabolic disease or disorder comprises diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity. In particular aspects, the diabetes comprises Type I diabetes, Type II diabetes, or gestational diabetes. In a further aspect, the patient has more than one metabolic disease or disorder, which may be for example, a metabolic disease or disorder which comprises, 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 method for treating a patient for a metabolic disease or disorder comprising administering the patient an effective amount of the composition of claim 6 to treat the metabolic disease or disorder in the patient.

In particular aspects, the metabolic disease or disorder comprises diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity. In particular aspects, the diabetes comprises Type I diabetes, Type II diabetes, or gestational diabetes. In a further aspect, the patient has more than one metabolic disease or disorder, which may be for example, a metabolic disease or disorder which comprises, 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 for the use of one or more co-agonist peptides of claims 1-6 for manufacture of a medicament for the treatment of a metabolic disease or disorder.

In particular aspects, the metabolic disease or disorder comprises diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity. In particular aspects, the diabetes comprises Type I diabetes, Type II diabetes, or gestational diabetes. In a further aspect, the use may be for treatment of more than one metabolic disease or disorder, which may be for example, a metabolic disease or disorder which comprises, 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 method for treating a metabolic disease or disorder 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 agonist and administering to the patient or individual an effective amount of a composition comprising an insulin or insulin analog to treat the metabolic disease or disorder in the patient or individual.

5 In particular aspects, the insulin analog comprises insulin detemir, insulin glargine, insulin levemir, insulin glulisine, insulin degludec, or insulin lispro.

 In particular aspects, the metabolic disease or disorder comprises diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity. In particular aspects, the diabetes comprises Type I diabetes, Type II diabetes, or gestational
10 diabetes. In a further aspect, the patient has more than one metabolic disease or disorder, which may be for example, a metabolic disease or disorder which comprises, 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 comprising one or more of
15 any one of the aforementioned co-agonist peptides and a pharmaceutically acceptable carrier and/or pharmaceutically acceptable salt.

 The present invention further provides a method for treating a patient for a metabolic disease or disorder comprising administering the patient an effective amount of any one or more of the aforementioned co-agonist peptides to treat the metabolic disease or disorder
20 in the patient.

 The present invention further provides method for treating a patient for a metabolic disease or disorder comprising administering the patient an effective amount of a composition comprising any one or more of the co-agonist peptides to treat the metabolic disease or disorder in the patient.

25 In particular aspects, the metabolic disease or disorder 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 or disorder, for example, diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity;
30 diabetes, NAFLD, and obesity; or diabetes and obesity.

The present invention further provides for the use of any one of the aforementioned co-agonist peptides for manufacture of a medicament for the treatment of a metabolic disease or disorder.

5 The present invention further provides for the use of any one of the aforementioned co-agonist peptides or compositions for manufacture of a medicament for the treatment of a metabolic disease or disorder.

In particular aspects, the metabolic disease or disorder 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
10 aspects, the medicament is for treatment of more than one metabolic disease or disorder, 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.

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

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
20 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.

25 In particular aspects, the insulin analog is insulin detemir, insulin glargine (U100 or U300), insulin levemir, insulin glulisine, insulin degludec, or insulin lispro.

In particular aspects, the metabolic disease or disorder 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
30 aspects, the patient has more than one metabolic disease or disorder, 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 comprising any one of the aforementioned co-agonist peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier. In particular aspects, the insulin analog is insulin detemir, insulin glargine (U100 or U300), insulin levemir, insulin glulisine, insulin degludec, or insulin lispro.

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

In particular aspects, the metabolic disease or disorder 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 comprising any one of the aforementioned co-agonist peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier for the manufacture of a medicament for the treatment of a metabolic disease or disorder. In particular aspects, the metabolic disease or disorder 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.

In particular aspects, the insulin analog is insulin detemir, insulin glargine (U100 or U300), insulin levemir, insulin glulisine, insulin degludec, or insulin lispro.

Definitions

The term "about" as used herein means greater or lesser than the value or range of values stated by 10 percent, but is not intended to designate any value or range of values to only this broader definition. Each value or range of values preceded by the term "about" is also intended to encompass the embodiment of the stated absolute value or range of values.

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

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

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

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" of a glucagon peptide refers to a nontoxic but sufficient amount of the peptide 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 inducing weight loss/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.

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, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr. "Coded" as used 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, Ile, 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. The term further includes peptides wherein one or more amino acids is conjugated to a second molecule via a linker.

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

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:

I. Small aliphatic, nonpolar or slightly polar residues:

Ala, Ser, Thr, Pro, Gly;

II. Polar, negative- charged residues and their amides and esters:

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

III. Polar, positive- charged residues:

His, Arg, Lys; Ornithine (Orn)

IV. Large, aliphatic, nonpolar residues:

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

V. Large, aromatic residues:

Phe, Tyr, Trp, acetyl phenylalanine

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

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.

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.

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 incorporating the amino acid into a peptide, or acylation after incorporation into a peptide).

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 acylated amino acids and alkylated amino acids of the present disclosures are non-coded amino acids.

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.

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.

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

Figure 1: Phage display library used to select GCGR/GLP1R co-agonists is composed of five sub-libraries, each one with nine randomized positions. For selection on GCGR⁺ and GLP1R⁺ cells, the five libraries were pulled and selected together. X = any amino acid with the proviso that for each library X is not Cys and at least one X in each series of Xs in the library is not a wild-type amino acid. Glucagon = SEQ ID NO:52; Libr 1 = SEQ ID NO:53; Libr 2 = SEQ ID NO:54; Libr 3 = SEQ ID NO:55; Libr 4 = SEQ ID NO:56; and Libr 5 = SEQ ID NO:57.

Figure 2: The single-strand oligonucleotides were converted into double-strand nucleotide fragments by primer extension, combining the relative oligonucleotide forward and reverse for each library.

Figure 3 is a table showing the composition for each of the codon mixes.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides peptide co-agonists of the glucagon (GCG) receptor and the glucagon-like protein 1 (GLP-1) receptor. The co-agonists comprise the amino acid sequence of glucagon peptide wherein specific mutations have been introduced which result in peptides that have co-agonist activity. The co-agonist peptides further comprise modifications that control the relative activity at the GLP-1 receptor verses the glucagon receptor. Thus, yet another aspect of the invention provides co-agonist peptides that have higher activity at the glucagon receptor versus the GLP-1 receptor, co-agonist peptides that have approximately equivalent activity at both receptors, and co-agonist peptides that have higher activity at the GLP-1 receptor versus the glucagon receptor. The latter category of co-agonist peptides may be engineered to exhibit little or no activity at the glucagon receptor, and yet retain ability to activate the GLP-1 receptor with the same or better potency than native GLP-1. Any of these analogs may also include modifications that confer enhanced biophysical stability and/or aqueous solubility. The co-agonist 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.

One of the most promising emerging areas for the treatment of obesity and Diabetes is combinatorial hormone therapies [1-3]. In particular, single molecule peptides have been discovered integrating the complementary actions of multiple endogenous metabolically-related hormones [4-14].

Collectively, these studies had proven that the high evolutionary relatedness of peptides like glucagon (GCG), GLP-1, and GIP and their cognate receptors enables using the sequence of one these hormones as a starting point, onto which co-agonism for one or more additional hormone receptors can be installed, with relatively few sequence changes [7-8, 10-11]. Sometimes, as little as one amino acid change is sufficient to switch between single- and dual-hormone agonism [9, 15].

These studies have been carried out by “rational design”, through an iterative cycle combining hybridization of the parent peptides with single-point mutations guided by the developing SAR: while successful and a testimony to the ingenuity of peptide medicinal chemists, this has necessarily limited the exploration of the chemical space to the immediate vicinity of the parent sequences. For example, the effect of multiple mutations in different parts of the molecule has rarely been explored, precluding a thorough investigation of conformational cross-talk.

One alternative way to develop unimolecular polypharmacy agents is the use of selection methods, for example those based on phage-displayed peptide libraries [16-19]. The defining feature of the libraries is the existence of a physical linkage between the peptide displayed on the surface (which determines the phenotype) and its encoding DNA (the genotype). Very large, diverse, random libraries can be constructed and selected, and the results of the screening can be rapidly decoded by DNA sequencing.

Peptide agonists and antagonists of cell membrane receptors have been successfully identified using this process [19-22], including peptide agonists of G-protein-coupled receptors (GPCRs) [23-28].

In 1997-98 Szardenings *et al.* [23] and Rousch *et al.* [24] first showed that phages bearing a GPCR agonist, the peptide hormones α -MSH or somatostatin, fused to the pIII or the pVIII protein could bind to cells expressing the respective receptor and most importantly, could activate it, acting as an agonist with lower [23] or even comparable [24] potency to the free peptide.

For the class B GPCRs, Yin *et al.* screened a library of Exendin-4 mutants (2-3 average mutations/molecule) on the N-terminal region including the ectodomain (aa 21-145) of rat GLP1R [26]. Sequencing of 60 clones from the third biopanning led to the identification of a phage which bound the receptor fragment better than the WT peptide; the mutant phage featured the Glu¹⁶Val, Pro³¹Gln, and Ser³³Asn mutations.

Koth *et al.* selected a library of glucagon mutants on the ectodomain of the GCG receptor (GCGR), immobilized on a plate through a biotin tag [28]. In the library, residues 16-29 were “soft randomized” to be a mixture of 50% WT and 50% random amino acid. In the terminology first proposed by Schwyzzer [29-31], this corresponds to the “address” segment of GCG, mostly responsible for receptor selectivity, while the N-terminal region represents the “message” segment, mostly responsible for receptor activation. Structural and modeling studies indicate that the “address” includes the key molecular determinants for binding to the receptor ectodomain, while the “message” is in contact with the 7TM membrane-embedded domain [28, 32-33].

Chen *et al.* selected a 12-amino acid random library on stable recombinant cells overexpressing the rat GLP1 receptor (GLP1R) [27]. Four rounds of selection enriched for GLP1R cell-binding phages. About 1% of the specifically bound phages (10 phages) were capable of activating the GLP1R, with the best peptide, KS-12, featuring high sequence homology to the N-terminal “message” region of GPL-1 [27]. KS-12 showed EC₅₀ of 0.8 μM, vs 0.8 nM of GLP-1 in the same assay.

Collectively, these studies established that (i) a peptide hormone – including GLP-1 – on the surface of a phage particle is capable of activating its receptor expressed on the cell surface; (ii) it is possible to identify active mutants both in the “message” and the “address” segments of the peptide ligands of class B GPCRs.

The above studies were aimed at evolving a single receptor-specific peptide, however, inventors have developed a selection strategy for identifying peptides able to activate more than one receptor. As shown herein, the selection of GCG/GLP-1 receptor co-agonists from a phage display library based on randomization of the glucagon sequence, sequentially selected on GCGR- and GLP1R-overexpressing cells. Two or three rounds of selection enabled the discovery of novel unimolecular co-agonists, with comparable *in vitro* potency to the reported designed peptides [7, 10].

Library design. In order to independently interrogate the “message” and the “address” segments of GCG the library design was split into four sub-libraries, each focusing on one of four seven-amino acid long consecutive segments (**Figure 1**).

The two C-terminal residues 27 and 28, together with the N-terminal residues, exhibit more diversity among GCG, GLP-1 and GIP and are critical for the interaction of each hormone with the respective receptor [11]. These residues were mutagenized in all four sub-libraries.

Additionally, motivated by the results of Chen et al. showing that an additional N-terminal Lys residue was well tolerated for GLP1R agonism and offered increased resistance to DPP-IV [27, 35], the inventors designed a fifth library exploring variants harboring a two amino acid extension at the GCG N-terminus.

Both GCG and GLP-1 are low picomolar ligands of the respective receptors, suggesting that their sequence is highly optimized; moreover, the work of Tschöp and coworkers had shown that relatively few mutations suffice to install dual- or even triple-agonism in the GCG sequence [7-8, 10-11]. Therefore in all the sub-libraries, instead of a standard full-randomization scheme (equal ratios of all amino acids except Cys) which would have predominantly provided variants with ≥ 4 mutations, the inventors chose a randomization scheme where each mutagenized position retained a constant fraction of 45% wt residue: for a library with a targeted complexity 6×10^7 , this scheme produced variants with a small number (1–3) of mutations (**Table 1**).

Table 1 Distribution of the variants in a library of complexity 6×10^7 with 45% wild-type amino acid at each of the nine randomized positions.							
No. of Mutations In GCG	Fraction	Cumulative Fraction	No. of physical clones	Theoretical Diversity	Poisson estimate Coverage	Number of unique clones *	Average # of duplicates
0 (wt)	0.08%	0.08%	4.54×10^4	1.00	100.00%	1.00	45400.84
1	0.83%	0.91%	4.99×10^5	1.62×10^2	100.00%	1.62×10^2	3082.77
2	4.07%	4.98%	2.44×10^6	1.17×10^4	100.00%	1.17×10^4	209.32

3	11.60%	16.58%	6.96x10 ⁶	4.90x10 ⁵	100.00%	4.90x10 ⁵	14.21
4	21.28%	37.86%	1.28x10 ⁷	1.32x10 ⁷	61.91%	8.19x10 ⁶	1.56
5	26.00%	63.86%	1.56x10 ⁷	2.38x10 ⁸	6.34%	1.51x10 ⁷	1.03
6	21.19%	85.05%	1.27x10 ⁷	2.86x10 ⁹	0.44%	1.27x10 ⁷	<<1
7	11.10%	96.15%	6.66x10 ⁶	2.20x10 ¹⁰	0.03%	6.66x10 ⁶	<<1
8	3.39%	99.54%	2.03x10 ⁶	9.92x10 ¹⁰	0.00%	2.03x10 ⁶	<<1
9	0.46%	100.00%	2.76x10 ⁵	1.98x10 ¹¹	0.00%	2.76x10 ⁵	<<1
Sum:	100%		6.00x10 ⁷			4.55x10 ⁷	
* Based on Poisson estimate for coverage of theoretical diversity (fractional completeness)							

Exemplary co-agonist peptides identified using phage display are shown in **Table**

2. The peptides are shown in alignment with GCG and GLP1. The EC₅₀ in nm of the peptides
 5 at the human GCG and GLP1 receptors are shown.

Table 2				
SEQ ID No.	PD-#	Sequence	EC ₅₀ hGCGR (nm)	EC ₅₀ hGLP1R (nm)
52	GLP-1	HAEGTFT SDVSSY LEGQAAK EFIAWLVKGRG-CONH ₂	NA	0.008
53	GCG	HSQGTFT SDYSKY LDSRRAQ DFVQWLMNT	0.005	0.06
6	PD22	HSQGTFT SDYSKY LDS L MAQ DFVQWLMST-CONH ₂	0.02	0.03
7	PD23	HSQGTFT SDYSKY LD W RRAQ DFVQWLLNT-CONH ₂	0.03	0.05
9	PD25	HSQGTFT SDYSKY LD A RAQ DFVQWL I RT-CONH ₂	0.04	0.02
10	PD26	HSQGTFT SDYSKY LD V RRAQ DFVQWLMNT-CONH ₂	0.02	0.06
11	PD27	HSQGTFT SDYSKY LD E LRAY DFVQWLMNT-CONH ₂	0.05	0.03
13	PD29	HSQGTFT SDYSKY LDSRRA H DFVQWLLNT-CONH ₂	0.02	0.02
14	PD30	HSQGTFT SDYSKY LDSRRAQ DFVQWLM N P-CONH ₂	0.04	0.12
15	PD31	HSQGTFT SDYSKY LDSRRAQ DFVQWL I NY-CONH ₂	0.06	0.04
20	PD36	HSQGTFT SDYSKY LDS I RAQ DFVQWLMD T -CONH ₂	0.04	0.06
22	PD38	HSQGTFT SDYSKY LDSRRAQ DFV D WLMNE-CONH ₂	0.01	0.14

24	PD40	HSQGTFT SDYSKY LDSRRAQ DFVQWLINT-CONH ₂	0.03	0.02
27	PD43	HSQGTFF SDYSHWLDLSRRAQ DFVQWLMNT-CONH ₂	0.03	0.04
28	PD44	HSQGTFT SDYSKY LDWRRAQDFVQWLQNT-CONH ₂	0.02	0.01
29	PD45	HSQGTFT SDYSKY LDSKRAH DFVQWLLNT-CONH ₂	0.03	0.02
32	PD48	HSQGTFT SDYSKY LDSRRAQ DFVDWLLNA-CONH ₂	0.07	0.02
33	PD49	HSQGTFT SDYSKY LDSRRAQ DFVEWLMNN-CONH ₂	0.01	0.01
34	PD50	HSQGTFT SDYSKY LDSRRAQ DFVDWLINS-CONH ₂	0.02	0.01
35	PD51	HSQGTFT SDYSKY LDSRRAQ DFVQWLMNT-CONH ₂	0.03	0.02
36	PD52	HSQGIFF SDYSKY LDSRRAQ DFVQWLMNT-CONH ₂	0.03	0.02
37	PD53	HSQGTFL SDYSKY LDSRRAQ DFVQWLMNT-CONH ₂	0.02	0.01
38	PD54	HSQGTFT SDYSWY LDSRRAQ DFVQWLMNT-CONH ₂	0.03	0.02
39	PD55	HSQGTFT SDYSKY LDMQRAH DFVQWLMNT-CONH ₂	0.01	0.01
40	PD56	HSQGTFT SDYSKY LDSRMAY DFVQWLMNT-CONH ₂	0.08	0.05
41	PD57	HSQGTFT SDYSKY LDSRRAQ DFVQWLLNQ-CONH ₂	0.02	0.01
42	PD58	HSQGTFF SDYSKY LDSRRAQ DFVQWLLET-CONH ₂	0.03	0.02
43	PD59	HSQGTFT SDYSKY LDSRRAQ DFVQWLIDS-CONH ₂	0.02	0.01
CONH ₂ indicates that the carboxy terminus of the C-terminal peptide is amidated.				

Co-agonist-based therapy has the potential to favorably impact both obesity and diabetes. Weight loss efficacy and reduction in food intake upon peripheral administration of the co-agonist oxyntomodulin (OXM) has been well validated in humans (Wynne *et al.*, Diabetes 54: 2390-2395 (2005)). OXM has also been shown to reduce body weight in humans (*See*, for example, Published International Application Nos. WO03/022304, WO2004/062685, and WO2006/134340); however, the effects of the OXM peptide and similar co-agonist GLP-1 receptor/glucagon receptor (GLP-1R/GCGR) agonists on the glycemic control independent of weight loss have not been systematically studied. Furthermore, while OXM is a co-agonist of the GLP-1 and GCG receptors, its potency at the two receptors is relatively unbalanced: OXM is about 10-fold less potent at the GLP-1 receptor than the GCG receptor.

Native OXM, glucagon, and GLP-1 have a very short half-life and are rapidly inactivated by the cell surface dipeptidyl peptidase-4 (DPP-IV). Mutations can be incorporated into these peptides to render them resistant to DPP-IV cleavage; however, many of these mutations have also been found to inactivate the native peptide or adversely affect the ability of

the peptide to interact with the glucagon receptor (GCGR) or GLP-1 receptor (GLP-1R) (*See* Published International Application No. WO2007/100535, which is incorporated herein in its entirety). OXM, glucagon, and GLP-1 are also rapidly cleared by the kidneys. Conjugation of these peptides with bulky substituents such as polyethylene glycol may reduce renal clearance of the peptides; however, when these bulky substituents have been incorporated into the OXM peptide, many of resulting peptides have been found to have a reduced ability or no ability to effectively interact with the glucagon receptor.

Prior to the instant application, identification of peptides with appropriately balanced co-agonist activity and stability were identified empirically by trial and error. The phage display approach taken by the inventors has simplified the process of identifying co-agonist peptides. Using phage display technology, the inventors were able to identify from randomly mutated human glucagon a number of peptides that are co-agonists of the GCG and GLP-1 receptors. These peptides have previously unknown amino acid substitutions that have the effect of rendering the peptides co-agonists of the GCG and GLP-1 receptors. For example, substituting the threonine at position 7 of human glucagon with leucine results in a peptide with relatively balanced potent activity at the GCG and GLP-1 receptors (see peptide PD53) as did substituting the tyrosine at position 16 of human glucagon with tryptophan (see peptide PD44) or the methionine at position 27 of human glucagon with isoleucine (see peptide PD40) or substituting the threonine at position 29 of human glucagon with proline (see peptide PD30). As shown in **Table 2**, other combinations of previously unknown amino acid substitutions also resulted in peptides with relatively balanced activity and relatively potent activity at the GCG and GLP-1 receptors.

The limited *in vivo* stability of native GCG (and the peptides shown in **Table 2** due to cleavage of the penultimate residue at the *N*-terminus by DPP-IV and rapid renal clearance, which necessitates frequent dosing (t.i.d. s.c. injections or continuous infusion) at high doses in humans, may be solved by mutating the site of DPP-IV cleavage and lipidating the peptide to increase its *in vivo* half-life ($t_{1/2}$), which is expected to render the co-agonist peptides suitable for at least once daily administration.

The tolerability profile of co-agonist peptides disclosed herein is anticipated to be similar to that of native OXM or GCG or GLP-1 in humans, which may be superior to that of conventional GLP-1 mimetics like exenatide and liraglutide. The chronic efficacy of the co-

agonist peptides disclosed herein may therefore be better than conventional GLP-1 mimetics due to reduced nausea and vomiting, which have typically been dose limiting for the GLP-1 mimetics.

The co-agonist peptides herein are expected to the potential for improving glucose control. It has been shown that co-agonist peptides that have a balanced GLP-1 receptor and GCGR co-agonism give enhanced reductions in food intake and body weight with chronic administration and result in improved glucose tolerance.

To produce long-acting co-agonist peptides has not been straight forward. For example, as disclosed in Published International Application No. WO2010096142, site-specific conjugation of a bulky polyethylene glycol (PEG) substituent at various locations throughout the peptide resulted in a significant reduction in GCG receptor and/or GLP-1 receptor potency. Other methods to improve pharmacokinetic properties while maintaining GCG receptor activity such as adding a cholesterol conjugate to various positions in the peptide resulted in a significant serum shift of potency and decreased *in vivo* efficacy. However, as disclosed in Published International Application No. WO2010096142 and WO2009/155258, inclusion of a hydrophilic linker between the peptide and cholesterol group, or direct addition of an acyl chain to an amino acid at position 10, 20, 24, or the C-terminus provided co-agonist peptides with improved pharmacokinetic properties.

Based on published human studies with OXM, the co-agonist peptides disclosed herein are anticipated to exhibit at least comparable if not superior efficacy and a better safety profile than current anti-obesity agents such as orlistat (Xenical® (Roche), a lipase inhibitor) and sibutramine (Meridia® (Abbott Laboratories), a serotonin/norepinephrine re-uptake inhibitor), for which GI intolerance (diarrhea, flatulence) and hypertension are common side effects, respectively.

In particular aspects, the co-agonist peptides disclosed herein optionally include a protecting group covalently joined to the N-terminal amino group. A protecting group covalently joined to the N-terminal amino group of the peptide reduces the reactivity of the amino terminus under *in vivo* conditions. Amino protecting groups include -C1-10 alkyl, -C1-10 substituted alkyl, -C2-10 alkenyl, -C2-10 substituted alkenyl, aryl, -C1-6 alkyl aryl, -C(O)-(CH₂)₁₋₆-COOH, -C(O)-C1-6 alkyl, -C(O)-aryl, -C(O)-O-C1-6 alkyl, or -C(O)-O-aryl. In particular embodiments, the amino terminus protecting group is selected from the group

consisting of acetyl, propyl, succinyl, benzyl, benzyloxycarbonyl, and t-butyloxycarbonyl. Deamination of the N-terminal amino acid is another modification that is contemplated for reducing the reactivity of the amino terminus under *in vivo* conditions.

In particular aspects, the co-agonist peptides may be modified to have a protecting
5 group covalently joined to the C-terminal carboxy group, which reduces the reactivity of the carboxy terminus under *in vivo* conditions. For example, carboxylic acid groups of the peptide, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmacologically-acceptable cation or esterified to form a C1-6 ester, or converted to an amide of formula NRR_2 wherein R and R_2 are each independently H or C1-6 alkyl, or combined to
10 form a heterocyclic ring, such as a 5- or 6-membered ring. The carboxy terminus protecting group is preferably attached to the α -carbonyl group of the last amino acid. Carboxy terminus protecting groups include, but are not limited to, amide, methanamide, and ethanamide. Amino groups of the peptide, whether N-terminal or side chain, may be in the form of a pharmacologically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene
15 sulfonic, maleic, tartaric, and other organic salts, or may be modified to C1-6 alkyl or dialkyl amino or further converted to an amide.

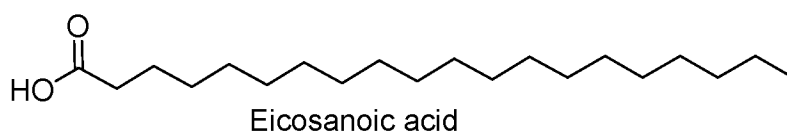
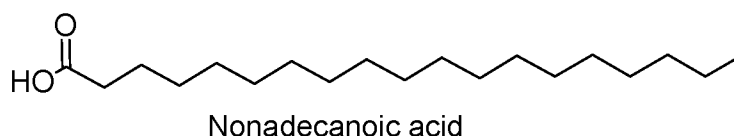
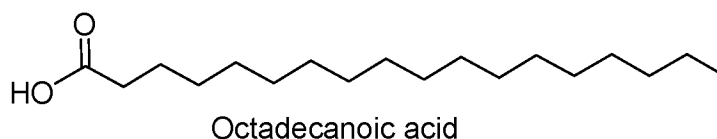
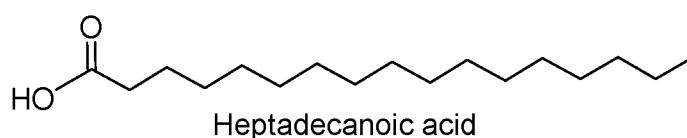
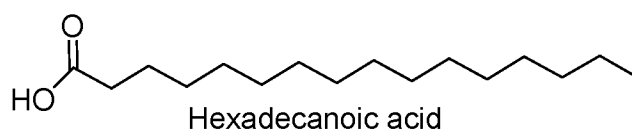
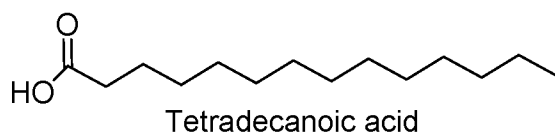
The co-agonist peptides that are capable of acting as dual GLP-1 and glucagon agonists comprise the amino acid sequence of a peptide selected from **Table 2** wherein the second amino acid from the N-terminus is substituted with an amino acid that renders the peptide
20 resistant to cleavage and inactivation by dipeptidyl peptidase IV; a lipid moiety covalently linked to the co-agonist peptide at a lysine residue substituted for the tyrosine residue at position 10; optionally 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 additional amino acid substitutions in addition to the substitution at position 2; and a protecting group that is joined to the C-terminal carboxy group. Optionally, the peptide may further comprises a protecting group joined to the N-terminal amino
25 group.

In general, the co-agonist peptides comprises a substitution of the Ser at position 2 with Val, Ile, Asp, Glu, Met, Trp, Asn, D-Ala, D-Ser, or α -aminoisobutyric acid (aib). In particular embodiments, the Ser is substituted with D-Ser or aib.

In particular embodiments, the co-agonist peptide may further include
30 substitutions of the amino acid at positions 16 and 18 with aib or Ala.

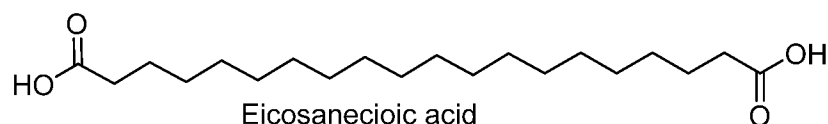
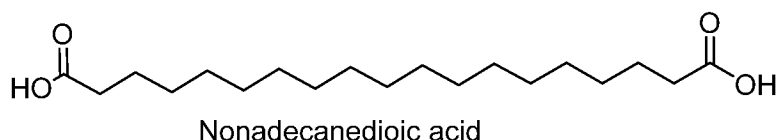
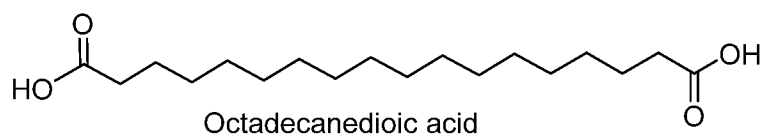
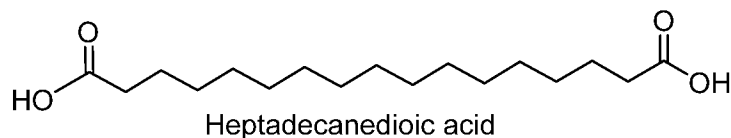
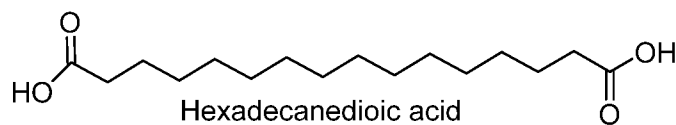
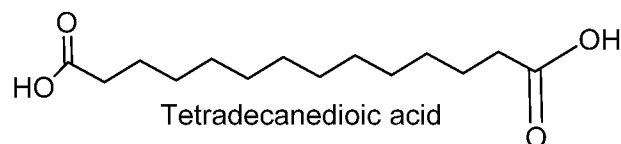
The lipid moiety may be a monocarboxylic acid comprising an aliphatic chain of 13 to 20 methylene groups (fatty acid) wherein one end of the molecule is the proximal end and the other end is the distal end and only one of the proximal end and the distal end has a carboxyl (COOH) group. The fatty acid may be represented by the structure $\text{HO}_2\text{C}(\text{CH}_2)_n\text{CH}_3$, wherein

5 n is 11, 12, 13, 14, 15, 16, 17, or 18. The fatty acid may have one of the following structures



10 The lipid moiety may be an α,ω -dicarboxylic acid comprising an aliphatic chain of 13 to 20 methylene groups (fatty diacid) wherein one end of the molecule is the proximal end and the other end is the distal end and wherein the proximal end and the distal end both have a carboxyl (COOH) group. The fatty diacid may be represented by the structure

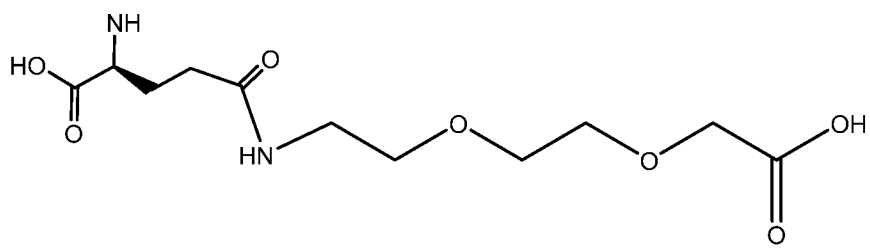
$\text{HO}_2\text{C}(\text{CH}_2)_n\text{CO}_2\text{H}$, wherein n is 11, 12, 13, 14, 15, 16, 17, or 18. The fatty diacid may have one of the following structures



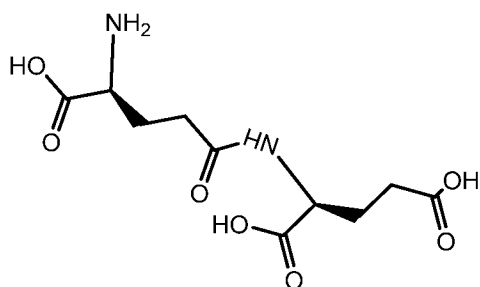
- 5 As a component of the co-agonist peptide, the acid functionality at the proximal end of the fatty diacid is conjugated to the amino group of a linker in a C(O)-NH linkage and the acid functionality at the distal end of the fatty diacid is a free carboxyl group (COOH). The COOH group at the distal end helps confer a longer half-life to the co-agonist peptide by its ability to non-covalently bind to serum albumin, a known carrier for fatty acids in serum. The
- 10 COOH group enhances duration of action as it provides a better non-covalent interaction with serum albumin than peptides that have been acylated using a fatty acid, which bind serum albumin less efficiently and form a less stable non-covalent interaction with the serum albumin.

When the fatty acid or diacid is conjugated to a linking moiety or linker, it is subsequently referred to as a fatty acid component.

The linker may be PEG₂ (8-amino-3,6-dioxaoctanoic acid) linked to Gamma-Glutamic acid (gamma-Glu, γ Glu, or γ E), which has the structure

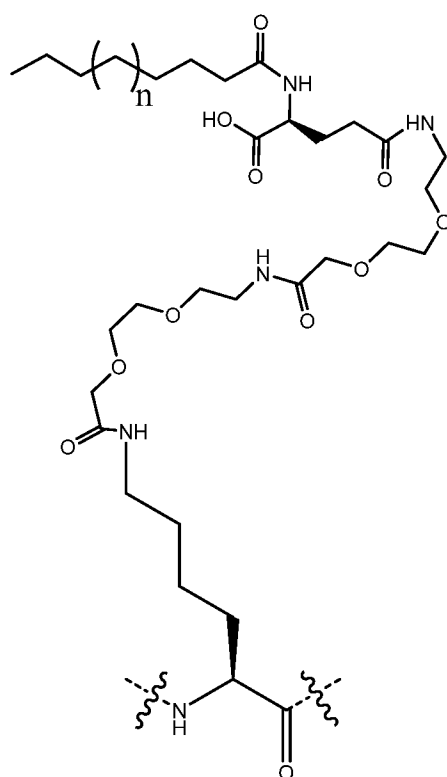


- 5 or the linker may be Gamma-Glutamic acid- gamma glutamic acid (gamma-Glu-gamma-Glu, or γ Glu- γ Glu, or γ E γ E), which has the structure



The structure of K(PEG₂PEG₂ γ E-fatty acid) wherein the linker is PEG₂PEG₂ γ E and the fatty acid component comprises C14, C16, C17, C18, C19, or C20 fatty acid is

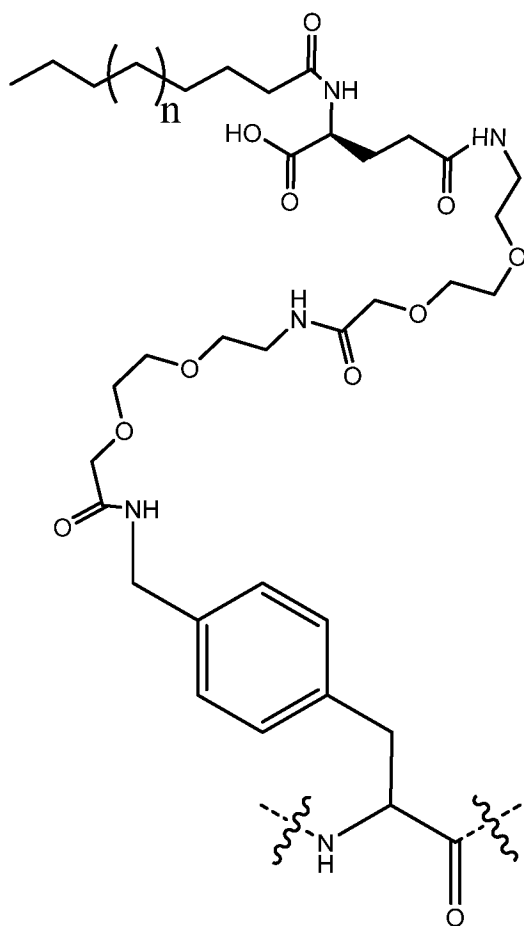
- 10 represented by



wherein n is 7, 9, 10, 11, 12, 13, or 14 respectively, and the wavy lines represent the bonds between adjacent amino acids in the co-agonist peptide sequence.

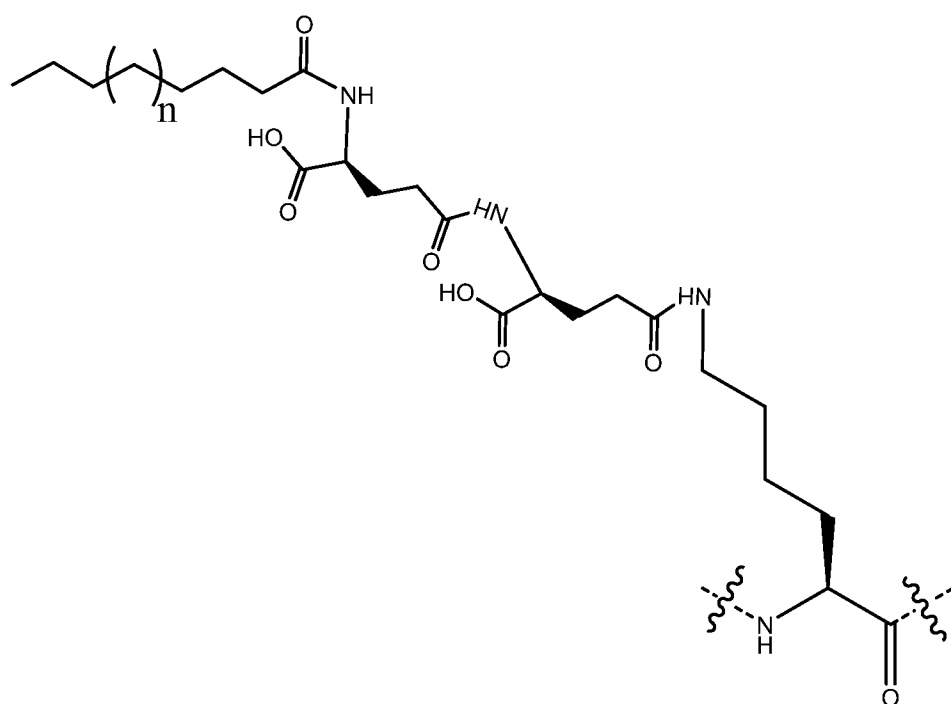
The structure of pAF(PEG₂PEG₂γE-fatty acid) wherein the linker is

- 5 PEG₂PEG₂γE and the fatty acid component comprises C14, C16, C17, C18, C19, or C20 is represented by



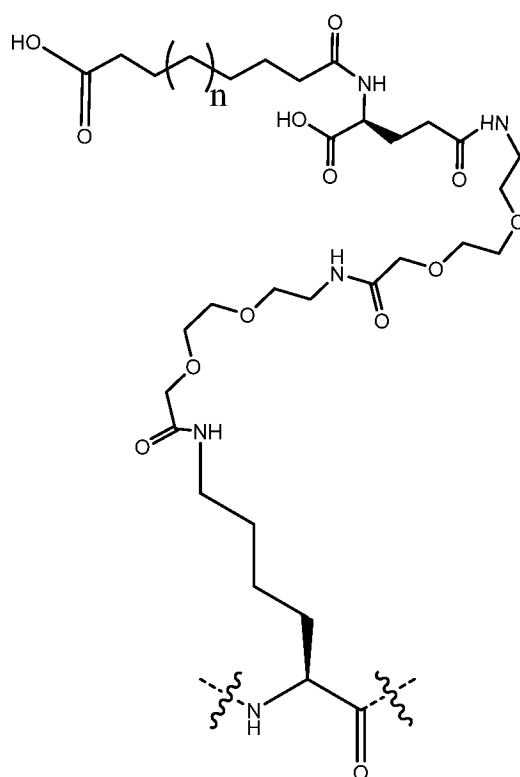
wherein n is 7, 9, 10, 11, 12, 13, or 14, respectively, and the wavy lines represent the bonds between adjacent amino acids in the co-agonist peptide sequence.

The structure of K(γ E γ E-fatty acid) wherein the linker is γ E γ E and the fatty acid component comprises C14, C16, C17, C18, C19, or C20 fatty acid is represented by



wherein n is 7, 9, 10, 11, 12, 13, or 14, respectively, and the wavy lines represent the bonds between adjacent amino acids in the co-agonist peptide sequence.

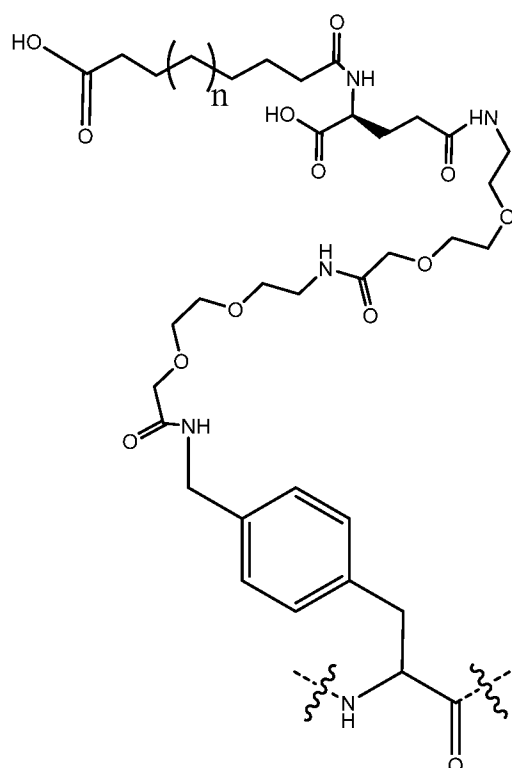
The structure of K(PEG₂PEG₂γE-fatty acid) wherein the linker is PEG₂PEG₂γE
 5 and the fatty acid component comprises C14, C16, C17, C18, C19, or C20 fatty diacid is represented by



wherein n is 7, 9, 10, 11, 12, 13, or 14 respectively, and the wavy lines represent the bonds between adjacent amino acids in the co-agonist peptide sequence.

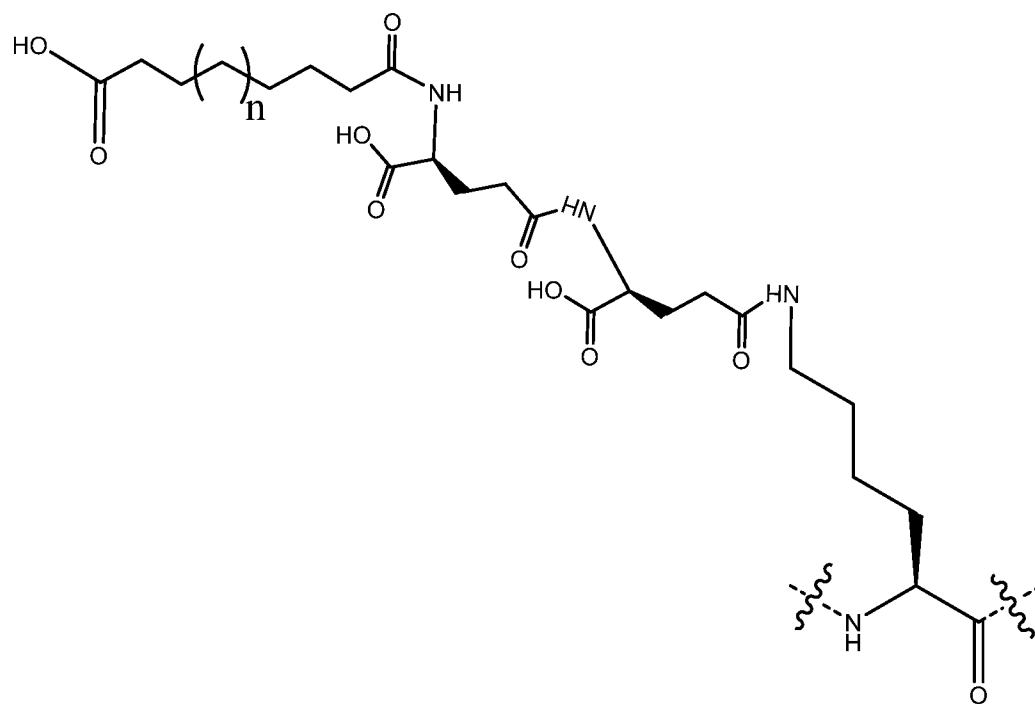
The structure of pAF(PEG₂PEG₂γE-fatty acid) wherein the linker is

- 5 PEG₂PEG₂γE and the fatty acid component comprises C14, C16, C17, C18, C19, or C20 fatty diacid is represented by



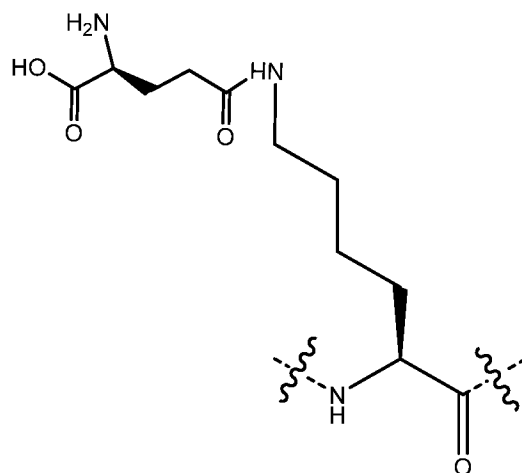
wherein n is 7, 9, 10, 11, 12, 13, or 14, respectively, and the wavy lines represent the bonds between adjacent amino acids in the co-agonist peptide sequence.

The structure of K(γ E γ E-fatty acid) wherein the linker is γ E γ E and the fatty acid component comprises C14, C16, C17, C18, C19, or C20 fatty diacid is represented by



wherein n is 7, 9, 10, 11, 12, 13, or 14, respectively, and the wavy lines represent the bonds between adjacent amino acids in the co-agonist peptide sequence.

In particular aspects, the co-agonist peptide may comprise a lysine residue at the C-terminus that is conjugated to a γ E residue to provide a K γ E at position 30 in the co-agonist peptide, which is represented by



wherein the wavy lines represent the bonds between adjacent amino acids in the co-agonist peptide sequence.

The co-agonist peptides disclosed herein may have anywhere from at least about 1% (including at least about 1.5%, 2%, 5%, 7%, 10%, 20%, 30%, 40%, 50%, 60%, 75%, 100%, 125%, 150%, 175%) to about 200% or higher activity at the GLP-1 receptor relative to native GLP-1 and anywhere from 5 at least about 1% (including about 1.5%, 2%, 5%, 7%, 10%, 20%, 30%, 40%, 50%, 60%, 75%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%) to about 500% or higher activity at the glucagon receptor relative to native glucagon. In some embodiments, the co-agonist peptides described herein exhibit no more than about 100%, 1000%, 10,000%, 100,000%, or 1,000,000% of the activity of native glucagon at the glucagon receptor. In some embodiments, the co-agonist peptides described herein exhibit no more than about 100%, 1000%, 10,000%, 100,000%, or 1,000,000% of the activity of native GLP-1 at the GLP-1 receptor. In exemplary embodiments, a co-agonist peptide may exhibit at least 10% of the activity of native glucagon at the glucagon receptor and at least 50% of the activity of native GLP-1 at the GLP-1 receptor, or at least 40% of the activity of native glucagon at the glucagon receptor and at least 40% of the activity of native GLP-1 at the GLP-1 receptor, or at least 60%

of the activity of native glucagon at the glucagon receptor and at least 60% of the activity of native GLP-1 at the GLP-1 receptor.

Selectivity of a glucagon peptide for the glucagon receptor versus the GLP-1 receptor can be described as the relative ratio of glucagon/GLP-1 activity (the peptide analog's activity at the glucagon receptor relative to native glucagon, divided by the peptide's activity at the GLP-1 receptor relative to native GLP-1). For example, a co-agonist peptide that exhibits 60% of the activity of native glucagon at the glucagon receptor and 60% of the activity of native GLP-1 at the GLP-1 receptor has a 1:1 ratio of glucagon/GLP-1 activity. Exemplary ratios of glucagon/GLP-1 activity include about 1:1, 1.5:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 or 10:1, or about 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, or 1:1.5. As an example, a glucagon/GLP-1 activity ratio of 10:1 indicates a 10-fold selectivity for the glucagon receptor versus the GLP-1 receptor. Similarly, a GLP-1/glucagon activity ratio of 10:1 indicates a 10-fold selectivity for the GLP-1 receptor versus the glucagon receptor.

Pharmaceutical Compositions

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.

"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/m^2). "Obesity" refers to a condition whereby an otherwise healthy subject has a Body Mass Index (BMI) greater than or equal to 30 kg/m^2 , or a condition whereby a subject with at least one co-morbidity has a BMI greater than or equal to 27 kg/m^2 . An "obese subject" is an otherwise healthy subject with a Body Mass Index (BMI) greater than or equal to 30 kg/m^2 or a subject with at least one co-morbidity with a BMI greater than or equal to 27 kg/m^2 . A "subject at risk for obesity" is an otherwise healthy subject with a BMI of 25 kg/m^2 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.

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
5 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
10 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.

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

Obesity-induced or obesity-related co-morbidities include, but are not limited to,
15 diabetes, non-insulin dependent diabetes mellitus - type 2, impaired glucose tolerance, impaired fasting glucose, insulin resistance syndrome, dyslipidemia, hypertension, hyperuricacidemia, 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,
20 orthopedic disorders, arthritis deformans, lumbodynia, emmeniopathy, and infertility. In particular, co-morbidities include: hypertension, hyperlipidemia, dyslipidemia, glucose intolerance, cardiovascular disease, sleep apnea, diabetes mellitus, and other obesity-related conditions.

“Treatment” (of obesity and obesity-related disorders) refers to the administration
25 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
30 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.

“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.

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, craniopharyngioma, the Prader-Willi Syndrome, Frohlich’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-
5 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.

10 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),
15 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
20 diabetes mellitus.

U.S. Patent No. 6,852,690, which is incorporated herein 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 insulintropic peptides. The co-agonist peptides disclosed
25 herein are insulintropic 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,
30 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 Cushing's 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 hypo- 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 co-agonist peptides disclosed herein.

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 term "individual" is meant to include humans and companion or domesticated animals such as dogs, cats, horses, and the like. Therefore, the compositions comprising a compound as disclosed herein are also useful for treating or preventing obesity and obesity-related disorders in cats and dogs. As such, the term "mammal" includes companion animals such as cats and dogs.

The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and

inorganic or organic acids. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic
5 bases 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'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine,
10 isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like. The term "pharmaceutically acceptable salt" further includes all acceptable salts such as acetate, lactobionate, benzenesulfonate, laurate, benzoate, malate, bicarbonate, maleate, bisulfate, mandelate, bitartrate, mesylate, borate, methylbromide, bromide, methylnitrate,
15 calcium edetate, methylsulfate, camsylate, mucate, carbonate, napsylate, chloride, nitrate, clavulanate, N-methylglucamine, citrate, ammonium salt, dihydrochloride, oleate, edetate, oxalate, edisylate, pamoate (embonate), estolate, palmitate, esylate, pantothenate, fumarate, phosphate/diphosphate, gluceptate, polygalacturonate, gluconate, salicylate, glutamate, stearate, glycolylarsanilate, sulfate, hexylresorcinate, subacetate, hydrabamine, succinate, hydrobromide,
20 tannate, hydrochloride, tartrate, hydroxynaphthoate, teoclate, iodide, tosylate, isothionate, triethiodide, lactate, pantoate, 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 OXM analogs disclosed herein are meant to also include the pharmaceutically acceptable salts.

25 As utilized herein, the term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s), approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals and, more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or
30 vehicle with which the therapeutic is administered and includes, but is not limited to such sterile liquids as water and oils. The characteristics of the carrier will depend on the route of

administration. 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.

5 As used herein, the term "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. When applied to an individual active ingredient, administered
10 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 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
15 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
20 certain forms of cancers.

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
25 receptor antagonists, lipase inhibitors, leptin, tetrahydrolipstatin, 2-4-dinitrophenol, acarbose, sibutramine, phentamine, fat absorption blockers, simvastatin, mevastatin, ezetimibe, atorvastatin, sitagliptin, metformin, orlistat, Qnexa, topiramate, naltrexone, bupriopion, phentermine, losartan, losartan with hydrochlorothiazide, and the like.

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

(1) other dipeptidyl peptidase-IV (DPP-4) inhibitors (e.g., sitagliptin, alogliptin, linagliptin, vildagliptin, saxagliptin and omarigliptin);

(2) insulin sensitizers, including (i) PPAR γ agonists, such as the glitazones (e.g. pioglitazone, AMG 131, MBX2044, mitoglitazone, lobeglitazone, IDR-105, rosiglitazone, and

5 balaglitazone), and other PPAR ligands, including (1) PPAR α/γ dual agonists (e.g., ZYH2, ZYH1, GFT505, chiglitazar, muraglitazar, aleglitazar, sodelglitazar, and naveglitazar); (2)

PPAR α agonists such as fenofibric acid derivatives (e.g., gemfibrozil, clofibrate, ciprofibrate, fenofibrate, bezafibrate), (3) selective PPAR γ modulators (SPPAR γ M's), (e.g., such as those disclosed in WO 02/060388, WO 02/08188, WO 2004/019869, WO 2004/020409, WO

10 2004/020408, and WO 2004/066963); and (4) PPAR γ partial agonists; (ii) biguanides, such as metformin and its pharmaceutically acceptable salts, in particular, metformin hydrochloride, and extended-release formulations thereof, such as GlumetzaTM, FortametTM, and GlucophageXRTM; 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 15 degludec, insulin glargine (U100 or U300), insulin lispro and inhalable formulations of each);

(4) leptin and leptin derivatives and agonists;

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

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

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

(8) glucagon receptor antagonists (e.g., such as those disclosed in WO 98/04528, WO 99/01423, WO 00/39088, and WO 00/69810);

(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, 25 lixisenatide, taspoglutide, CJC-1131, and BIM-51077, including intranasal, transdermal, and once-weekly formulations thereof);

(10) LDL cholesterol lowering agents such as (i) HMG-CoA reductase inhibitors (e.g., simvastatin, lovastatin, pravastatin, crivastatin, fluvastatin, atorvastatin, pitavastatin and rosuvastatin), (ii) bile acid sequestering agents (e.g., colestilan, colestimide, colesevalam 30 hydrochloride, colestipol, cholestyramine, and dialkylaminoalkyl derivatives of a cross-linked

dextran), (iii) inhibitors of cholesterol absorption, (e.g., ezetimibe), and (iv) acyl CoA:cholesterol acyltransferase inhibitors, (e.g., avasimibe);

(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);

(12) antiobesity compounds;

(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;

(14) antihypertensive 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;

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

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

(17) CETP inhibitors (e.g., anacetrapib, and torcetrapib);

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

(19) inhibitors of acetyl CoA carboxylase-1 or 2 (ACC1 or ACC2);

(20) AMP-activated Protein Kinase (AMPK) activators;

(21) other agonists of the G-protein-coupled receptors: (i) GPR-109, (ii) GPR-119 (e.g., MBX2982 and PSN821), and (iii) GPR-40 (e.g., TAK875, 5-[4-[[[(1R)-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-(4-((3-(2,6-dimethyl-4-(3-(methylsulfonyl)propoxy)phenyl)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-[[3-[4-(3-aminopropoxy)-2,6-dimethylphenyl]phenyl]methoxy]phenyl]isothiazole-3-ol 1-oxide);

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

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

(24) SCD inhibitors;

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

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

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

10 (28) inhibitors of fatty acid synthase;

(29) inhibitors of acyl coenzyme A:monoacylglycerol acyltransferase 1 and 2 (MGAT-1 and MGAT-2);

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

15 (31) ileal bile acid transporter inhibitors;

(32) PACAP, PACAP mimetics, and PACAP receptor 3 agonists;

(33) PPAR agonists;

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

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

20 (36) bromocriptine mesylate and rapid-release formulations thereof;

(37) FGF-21 and analogs and derivatives thereof; and

(38) FGF21 mimetics such as agonist antibodies that binds the β -Klotho and FCFR1c complex.

25 Of particular interest are metformin hydrochloride, pioglitazone, rosiglitazone, simvastatin, atorvastatin, or a sulfonylurea.

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;

30

neuropeptide Y₁ or Y₅ antagonists (such as MK-0557); CB1 receptor inverse agonists and antagonists (such as rimonabant and taranabant); β ₃ adrenergic receptor agonists; ghrelin antagonists; bombesin receptor agonists (such as bombesin receptor subtype-3 agonists); and 5-hydroxytryptamine-2c (5-HT_{2c}) 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;

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

(1) other dipeptidyl peptidase-IV (DPP-4) inhibitors;

(2) insulin sensitizers, including (i) PPAR γ agonists, such as the glitazones (e.g. AMG 131, MBX2044, mitoglitazone, lobeglitazone, IDR-105, pioglitazone, rosiglitazone, and balaglitazone) and other PPAR ligands, including (1) PPAR α/γ dual agonists, such as ZYH1, YYH2, chiglitazar, GFT505, muraglitazar, aleglitazar, sodelglitazar, and naveglitazar, (2) PPAR α agonists, such as fenofibric acid derivatives (e.g., gemfibrozil, clofibrate, ciprofibrate, fenofibrate and bezafibrate), (3) selective PPAR γ modulators (SPPAR γ M's), and (4) PPAR γ 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 ISI-113715, and TTP814;

(3) sulfonylurea and non-sulfonylurea insulin secretagogues, (e.g., tolbutamide, glyburide, glipizide, glimepiride, mitiglinide, 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 rosuvastatin), (ii) bile acid sequestering agents (e.g., colestilan, cholestyramine, colestimide, colesevelam hydrochloride, colestipol, and dialkylaminoalkyl 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 11 β -hydroxysteroid dehydrogenase type 1 (e.g., such as those disclosed in U.S. Patent 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. Patent Nos. 6,054,587; 6,110,903; 6,284,748; 6,399,782; and 6,489,476);

(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., MBX2982, and PSN821), and (iii) GPR-40 (e.g., TAK875, 5-[4-[(1R)-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-(4-((3-(2,6-dimethyl-4-(3-(methylsulfonyl)propoxy)phenyl)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-[[3-[4-(3-aminopropoxy)-2,6-dimethylphenyl]phenyl]methoxy]phenyl]isothiazole-3-ol 1-oxide);

(18) SSSTR3 antagonists (e.g., such as those disclosed in WO

5 2009/011836);

(19) neuromedin U receptor agonists (e.g., such as those disclosed in WO2009/042053, including, but not limited to, neuromedin S (NMS));

(20) inhibitors of stearoyl-coenzyme A delta-9 desaturase (SCD);

(21) GPR-105 antagonists (e.g., such as those disclosed in WO

10 2009/000087);

(22) inhibitors of glucose uptake, such as sodium-glucose transporter (SGLT) inhibitors and its various isoforms, such as SGLT-1; SGLT-2 (e.g., ASP1941, TS071, BI10773, tofogliflozin, LX4211, canagliflozin, dapagliflozin and remogliflozin; and SGLT-3);

(23) inhibitors of acyl coenzyme A:diacylglycerol acyltransferase 1 and 2

15 (DGAT-1 and DGAT-2);

(24) inhibitors of fatty acid synthase;

(25) inhibitors of acyl coenzyme A:monoacylglycerol acyltransferase 1 and 2 (MGAT-1 and MGAT-2);

(26) agonists of the TGR5 receptor (also known as GPBAR1, BG37,

20 GPCR19, GPR131, and M-BAR);

(28) bromocriptine mesylate and rapid-release formulations thereof, and

(29) IL-1b antibodies (e.g., XOMA052, and canakinumab);

(30) FGF-21 or analog or derivative;

(31) FGF21 mimetics such as agonist antibodies that binds the β -Klotho

25 and FCFR1c complex; and

(c) a pharmaceutically acceptable carrier.

When a co-agonist peptide of the present invention is used contemporaneously 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
30 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.

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 infusion 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 Ommaya reservoir). Pulmonary administration may also be employed 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.

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; minicells; 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 amphipathic 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, sulfatides, lysolecithin, phospholipids, saponin, 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. Patent No. 4,837,028 and U.S. Patent 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, 5 Bocca Raton, Fla.).

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 or condition, and may be determined by standard clinical techniques by those of average skill within the art. In addition, *in vitro* assays 10 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 15 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 lie 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 20 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 μg /kg body weight to 1 25 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 30 present invention. Dosage will also vary according to the age, weight and response of the individual patient.

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.

The present invention further provides a method for treating a patient for a metabolic disease or disorder comprising administering the patient an effective amount of a composition comprising any one or more of the aforementioned co-agonist peptides to treat the metabolic disease or disorder in the patient.

In particular aspects, the metabolic disease or disorder 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 or disorder, 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 for the use of any one of the aforementioned co-agonist peptides for manufacture of a medicament for the treatment of a metabolic disease or disorder.

The present invention further provides for the use of any one of the aforementioned co-agonist peptides for manufacture of a medicament for the treatment of a metabolic disease or disorder.

In particular aspects, the metabolic disease or disorder 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 or disorder, 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.

Further provided is method for treating a metabolic disease or disorder 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 or disorder in the patient or individual.

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.

In particular aspects, the insulin analog is insulin detemir, insulin glargine (U100 or U300), insulin levemir, insulin glulisine, insulin degludec, or insulin lispro.

In particular aspects, the metabolic disease or disorder 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 or disorder, 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 comprising any one of the aforementioned co-agonist peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier.

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

In particular aspects, the metabolic disease or disorder 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 comprising any one of the aforementioned co-agonist peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier for the manufacture of a medicament for the treatment of a metabolic disease or disorder.

In particular aspects, the insulin analog comprises insulin detemir, insulin glargine (U100 or U300), insulin levemir, insulin glulisine, insulin degludec, or insulin lispro.

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

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

EXAMPLE 1

10 Co-agonists were identified using a phage display protocol as set forth below.

Cell lines

Hek 293 Tet GCGr and Hek 293 Tet GLP1r stable inducible cell lines were generated to obtain high level of expressed receptors. DNA encoding the human GCG receptor and human GLP1 receptor, both including DNA encoding Myc-DDK tag for FLAG detection
15 were each subcloned as a *NheI*/*ClaI* (*NheI* #R0131S and *ClaI* #R0197S, BioLabs) fragments into the pTRE2hyg vector (Clontech), using Rapid Ligation kit (N. 04898125001, Roche), and transformed into DH5 α CaCl₂ competent cells. The following oligos were used to introduce *NheI* and *ClaI* restriction site in the amplified fragment for the cloning step:

***GCGR* *NheI* For:** 5'-GAC TGC TAG CAC CAT GCC CCC CTG CCA GCC AC-3' (SEQ ID
20 NO:58);

***GLP1R* *NheI* For:** 5'-GAC TGC TAG CAC CAT GGC CGG CGC CCC CGG C-3' (SEQ ID NO:59); and

***GCGR/GLP1R* *ClaI* Rev:** 5'-ATC GAT CGA TTT AAA CCT TAT CGT CGT CAT CCT TGT AAT C-3' (SEQ ID NO:60). Once obtained, recombinant plasmids were purified using Wizard
25 SV Clean-Up System (Promega) and checked by sequencing.

Cells were cultured at 37°C with 5% CO₂ in DMEM medium (Gibco, Life technologies) supplemented with 10% Fetal Bovine Serum (Gibco, Life technologies), 100 μ g/mL Penicillin/Streptomycin (Gibco Life technologies REF 1514-122), 1% L-Glutamine (Gibco Life Technologies REF 25030-024), 250 μ g/mL Geneticin (G418 Sulfate 11811-031,
30 Life Technologies), 0.5 μ g/mL Puromycin (Sigma Aldrich P8833-100MG) and 100 μ g/mL HygromycinB (Sigma Aldrich H3274-1G).

Transfections of Hek293 Tet cells either with pTRE2hyg GCGr or with pTRE2hyg GLP1r plasmid were made using Lipofectamine reagent (Invitrogen 18324-020), according to manufacturer's instructions. Stable clones were selected in culture medium containing 100 µg/mL of HygromycinB in about 2/3 weeks. Individual clones were then
5 characterized by Western Blot Cell based Elisa assay for their level of expression upon induction with 1 µg/mL Doxycycline (Sigma Aldrich N.D9891) for 20 hours. The highest responding clones were selected, frozen and stored in liquid nitrogen for further experiments.

PROTEIC EXTRACTS AND WESTERN BLOT ANALYSIS

GCG receptor and GLP1 receptor gene expression in *Hek293 Tet GCGr* and
10 *Hek293 Tet GLP1r* cells were induced with 1 µg/mL Doxycycline (Sigma Aldrich N.D9891) for 20 hours. Cells were washed with cold PBS1X, harvested in falcon and centrifuged at 4°C 1200 rpm for 5 minutes. To each pellet was added 1 mL of Complete Lysis-M (Roche Cat. No. 04719956001) for efficient protein extraction. Protein extracts were analyzed by electrophoresis on polyacrylamide gel (SDS-PAGE), and samples were loaded on a 4-12% Bis-Tris gel (NuPage
15 Invitrogen). After blotting on a nitrocellulose filter (Nitrocellulose Blotting Membrane 0.2 µm #10600001, GE Healthcare Life Science), the membrane was incubated with α-Flag 1:1000 (Monoclonal ANTI-FLAG M2-Peroxidase, Sigma Aldrich Cat. A8592) followed by incubation with α-mouse secondary antibody (Goat α-mouse IRDYe680 92632210 Lot#B70907-01 ODYSSEY Infrared Imaging System LI-COR)

CELL BASED ELISA ASSAY

Hek293 Tet GCGr or Hek293 Tet GLP1r cells were seeded at 8×10^6 cells/10 cm plate and induced, if the case, with 1 µg/mL Doxycycline. After 20 hours cells were detached and plated on 96-well poly-lysine coated plate (Poly-L-lysine, Sigma Aldrich Cat. P4707) at about 50,000 cells/well over night at 37°C. The day after, cells were fixed with 4% of
25 paraformaldehyde (Electron microscopy sciences Cat N.157-8) for 20 minutes at room temperature, and α-FlagHRP conjugated mAb (Monoclonal ANTI-FLAG M2-Peroxidase, Sigma Aldrich Cat. A8592) diluted 1:1000 in 3%Milk-PBS1X-Tween 0.05% was added. Development was made with Tetramethylbenzidine (TMB N.8665 SIGMA Aldrich) liquid substrate for 30 minutes reading Absorbance at 450 and 620 nm with Elisa Reader Thermoscientific Multiskan
30 Ascent.

LIBRARY CONSTRUCTION

pIII-GCG pIII-GLP1 positive control phages and peptide phage libraries

In order to obtain positive control phages for libraries screening, DNA sequences coding for Glucagon (GCG) and for GLP-1 peptides were sub-cloned as a NcoI-NotI (NcoI
5 #R0193S and NotI #R0189L BioLabs) fragment into pCANTAB6 phagemid, fused with the N-terminus sequence of M13 bacteriophage coat protein pIII. Once obtained, the GCG and GLP-1 phagemids were converted to phage particles by superinfecting TG1 transformed cells with helper phage K07, purified on a CsCl gradient and titrated as CFU (colony forming units).

To introduce diversity and design the peptide phage library, the GCG sequence
10 was divided in five domains to generate five sub-libraries. In each sub-library, a cassette of seven amino acids was mutagenized and combined with the two amino acids in the position 27 and 28 of the sequence. In one sub-library two extra amino acids at N-terminus were also added. In each position 45% of amino acids were maintained as wild type, while 55% were the other possible amino acids (except cysteine to avoid disulfide bridges formation) in equal percentages.

15 (See **Figure 1**).

The sequences shown in Fig. 1 are also shown in **Table 3**.

Table 3 Library Sequences		
SEQ ID NO:	Name	Sequence
53	Lib1 (1)-(7) X is any amino acid with the proviso that for each position in 45% of the peptides the amino acid is maintained as the wild type amino acid while in 55% of the peptides the amino acid is any other amino acid except cysteine (29)-(30) X is any amino acid with the proviso that for each position in 45% of the peptides the amino acid is maintained as the wild type amino acid while in 55% of the peptides the amino acid is any other amino acid except cysteine	XXXXXXXXFTSDYSKYLDSRRAQDFVQWLXXT
54	Lib2 (1)-(7) X is any amino acid with the proviso that for each position in 45% of the peptides the amino acid is maintained as the wild type amino acid while in 55% of the peptides the amino acid is any other amino acid	XXXXXXXXSDYSKYLDSRRAQDFVQWLXXT

	except cysteine (27)-(28) X is any amino acid with the proviso that for each position in 45% of the peptides the amino acid is maintained as the wild type amino acid while in 55% of the peptides the amino acid is any other amino acid except cysteine	
55	Lib3 (7)-(13) X is any amino acid with the proviso that for each position in 45% of the peptides the amino acid is maintained as the wild type amino acid while in 55% of the peptides the amino acid is any other amino acid except cysteine (27)-(28) X is any amino acid with the proviso that for each position in 45% of the peptides the amino acid is maintained as the wild type amino acid while in 55% of the peptides the amino acid is any other amino acid except cysteine	HSQGTFTXXXXXXLDSRRAQDFVQWLXXT
56	Lib4 (14)-(20) X is any amino acid with the proviso that for each position in 45% of the peptides the amino acid is maintained as the wild type amino acid while in 55% of the peptides the amino acid is any other amino acid except cysteine (27)-(28) X is any amino acid with the proviso that for each position in 45% of the peptides the amino acid is maintained as the wild type amino acid while in 55% of the peptides the amino acid is any other amino acid except cysteine	HSQGTFTSDYSKYXXXXXXDFVQWLXXT
57	Lib5 (21)-(29) X is any amino acid with the proviso that for each position in 45% of the peptides the amino acid is maintained as the wild type amino acid while in 55% of the peptides the amino acid is any other amino acid except cysteine	HSQGTFTSDYSKYLDSRRAQXXXXXXXXXX

The randomized single strand oligonucleotides for the library constructions were generated by trimer-based synthesis (ELLA Biotech). The oligo sequences are shown in the **Table of Primers** wherein the presence of diversity is represented by X_n wherein X represents a mixture of 19 codons, each codon encoding one of 19 amino acids except for Cys and wherein n is one of 21 different codon mixtures as shown in **Figure 3** wherein each mixture has a particular frequency of the 18 codons.

Each X_n represents a codon of three nucleotides $X_1X_2X_3$ wherein each X is independently A,G,C, or T with the proviso that X_1 is not T if X_2 is G or C and X_3 is T or if X_1

is T, then X₃ is not T and wherein the combinations of X₁X₂X₃ have the triplet nucleotide sequences as shown in **Figure 3** for each particular X_n. For example, **Figure 3** shows that for X₁₀ the codons encode 19 different amino acids (excluding Cys) and the codon for each amino acid is 5.26% of the mixture; for X₁ the codons encode 19 different amino acids (excluding Cys) and the codon encoding Asp comprises 45% of the mixture of codons and the codons for each of the remainder of the amino acids comprises 3% of the mixture.

Table of Primers		
Name	Sequence	SEQ ID NO:
GCG_lib1_for	5'- GCCGGATGGCAX ₁₀ X ₁₀ X ₀₄ X ₀₇ X ₀₂ X ₀₃ X ₀₈ TTTACCTCTGATTACAGCAAA TACCT GGATTCTAGACGTGCTCAAGAC-3'	61
GCG_lib2_for	5'- GCCGGCCATGGCAX ₀₄ X ₀₇ X ₀₂ X ₀₃ X ₀₈ X ₀₆ X ₀₈ TCTGATTACAGCAAATACC TGGA TTCTAGACGTGCTCAAGAC-3'	62
GCG_lib3_for	5'- GCCGGCCATGGCACATTCTCAGGGTACCTTTX ₀₈ X ₀₇ X ₀₁ X ₀₉ X ₀₇ X ₀₅ X ₀₉ C TGGA TTCTAGACGTGCTCAAGAC-3'	63
GCG_lib4_for	5'- GCCGGCCATGGCACATTCTCAGGGTACCTTCACCAGCGACTACAGCAAGTA C-3'	64
GCG_lib5_for	5'- CCGGCCATGGCACATTCTCAGGGTACCTTTACCTCTGATTATAGCAAGTATC TGGA TTCTCGTCGTG-3'	65
GCG_lib123_rev	5'- CTGTGCGGCCGACCAGTX ₁₃ X ₁₇ CAGCCACTGAACAAAGTCTTGAGCACG TCT AGAATCC 3'	66
GCG_lib4_rev	5'- CTGTGCGGCCGACCAGTX ₁₃ X ₁₇ CAGCCACTGAACGAAGTC X ₁₅ X ₁₁ X ₁₂ X ₁₂ X ₁₉ X ₁₄ X ₁₆ GTACTTGCTGTAGTCGCTGGTG-3'	67
GCG_lib5_rev	5'- CTGTGCGGCCGCACTX ₂₀ X ₁₃ X ₁₇ X ₁₆ X ₂₁ X ₁₅ X ₂₂ X ₁₈ X ₁₄ CTGAGCACGAC GAGA GTCCAGATACTTG-3'	68

SEQ ID NOs:61, 62, 63, 66, 67, and g8 may also be represented as shown in **Table 4**.

[illegible]

[illegible]

[illegible]

[illegible]

	are each present 5.26% of the time $n_{28}n_{29}n_{30}$ have the triplet sequence CCA present 45% percent of the time and the triplet sequences TTT, GTT, CGT, AAT, CAT, CTG, ATG, CGG, TCT, CAG, TTC, ATC, TGC, ACC, AAC, ATA, ACT, or AAA are each present 5.26% of the time $n_{31}n_{32}n_{33}$ have the triplet sequence CTG present 45% percent of the time and the triplet sequences TTT, GTT, CGT, AAT, CAT, ATG, CGG, TCT, CAG, TTC, ATC, TGC, ACC, AAC, ATA, ACT, CCA, or AAA are each present 5.26% of the time $n_{34}n_{35}n_{36}$ have the triplet sequence AAC present 45% percent of the time and the triplet sequences TTT, GTT, CGT, AAT, CAT, CTG, ATG, CGG, TCT, CAG, TTC, ATC, TGC, ACC, ATA, ACT, CCA, or AAA are each present 5.26% of the time $n_{37}n_{38}n_{39}$ have the triplet sequence AAA present 45% percent of the time and the triplet sequences TTT, GTT, CGT, AAT, CAT, CTG, ATG, CGG, TCT, CAG, TTC, ATC, TGC, ACC, AAC, ATA, ACT, or CCA are each present 5.26% of the time $n_{40}n_{41}n_{42}$ have the triplet sequence ATC present 45% percent of the time and the triplet sequences TTT, GTT, CGT, AAT, CAT, CTG, ATG, CGG, TCT, CAG, TTC, TGC, ACC, AAC, ATA, ACT, CCA, or AAA are each present 5.26% of the time	
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The single strand oligonucleotides were converted into double strand fragments by primer extension, combining the relative oligo forward (for) and reverse (rev) for each library. 600 pmol of oligo for and rev were denatured at 95°C for 30 seconds, annealed at 65°C for 30 seconds and extended in the presence of 0.2 mM dNTS and 1 unit of Phusion High –Fidelity DNA Polymerase (#F-530 Thermo Scientific) for 30 seconds at 72°C as shown in **Figure 2**.

Once checked on agarose gel, the fragments were digested with NcoI-NotI (NcoI #R0193S and NotI #R0189L Bio Labs), purified through Qiaquick nucleotide Removal Kit (Qiagen), quantified by OD₂₆₀ and ligated into NcoI-NotI pCANTAB6 phagemid. Preparative ligations were assembled by mixing vector and inserts at 1:5 molar ratio and incubated overnight at 16°C with T4 DNA ligase (M02024 Bio Labs). The ligation product was desalted and transformed to the electrocompetent E.Coli XL1Blue cells (Agilent Technologies) using an electroporator (Bio-Rad, Hercules, CA) at 1.8kV, 25µF and 200Ω. Transformed bacteria were

plated on 23x23 2XTY/Amp (100µg/ml)/2%Glucose square plates and incubated o/n at 37°C.

The day after the plates were scraped to harvest bacteria in 10ml 2XTY Amp (100µg/ml)/2%Glucose containing 17% glycerol and stored at -80°C. For each library 6×10^7 individual clones were collected for a total complexity of 3×10^8 for the five sub-libraries. Phage libraries were prepared by super-infection of transformed bacteria with 10 multiplicity of Infection (MOI) of M13K07 helper phage, purified through CsCl gradient, according to standard protocols, and titrated. The library mix was prepared mixing equivalent amount of phages from each sub-library.

10 SELECTION OF GCG/GLP1 CO-AGONISTS FROM PHAGE LIBRARIES

About 10^{11} phages of the library were used for the first round of selection on HEK293 glucagon receptor (GCGr) and GLP1 receptor (GLP1r) cells, after depletion on HEK293 naïve cells. Four rounds of selection were carried out on cells, where two different selection scheme (M10 and M20) were followed, alternating rounds of selection on the two different cell lines: in the selection scheme M10 the first round was made on HEK293 GCGr cells, while in the selection scheme M20 the first round was made on HEK293 GLP1r.

Briefly phage particles were blocked in 0.5 ml PBS containing 3%non-fat dry milk (MPBS) for 30 min at room temperature (RT), then incubated with 10^7 HEK293 wild type cells previously resuspended in MPBS for 1 additional hour in a rotary mixer. HEK293 cells were pelleted, the depleted phage supernatant was recovered, then incubated with either HEK293 GLP1r or HEK293 GCGr resuspended into 0.5 ml of MPBS for 1hr in a rotary mixer.

After incubation, the mixture of cells and phages was centrifuged 1 minute at 2000 rpm, the supernatant was discarded, and pellet was washed 6 times with 1X PBS. For the elution the pellet was resuspended in 100 mM HCl (500µl) for 15 minutes at RT in agitation then neutralized with 100mM TrisHCl pH 7.5. Eluted phages were transferred in 50 mL Falcon tube, 10 mL of TG1 cells were added and the mixture was incubated for 1hr 37°C 150 rpm. TG1 infected cells were plated out onto 2XTY Amp/Glu bioassay plate and incubated overnight at 30°C. The day after the selected pool of phages was rescued by scraping bacteria from bioassay plate in 10 mL of 2X TY Amp/Glu. 50 µL of the scraping was inoculated in 50 mL 2X TY Amp/Glu and grown at 37°C to OD_{600nm}=0.5 in a shaker at 250 rpm. M13K07 helper phage was added to bacteria at 10 MOI, incubated 30 min 37°C in static and 30min 37°C in agitation

(120 rpm). The culture was centrifuged 10 minutes 3200rpm, supernatant was removed, pellet was resuspended in 50ml 2XTY Amp(100 µg/mL)/Kan(25 µg/mL) and grown overnight at 25°C 250 rpm. The day after culture was centrifuged 45 minutes 4°C 4200 rpm, the supernatant was recovered and used to perform the following selection round.

5

SCREENING by cAMP ACTIVITY ASSAY

Each round of selection was screened by activity assay both as pool and as single clone phage supernatant. From each round of selection about 200 single clones were isolated and prepared as 96 well supernatants. Phages were then precipitated from the supernatants by adding 1/3 volume of 20%PEG8000/2.5M NaCl overnight, centrifuged at 4200 rpm 4°C for 45minutes, and resuspended in 1/10 volume of PBS1X. Phage pool and single phages were tested by using cAMP activity assay (HitHunter cAMP XS+ Assay *DiscoverX* 90-0075), following manufacture instruction. HEK293 GCGr and HEK293 GLP1r stable cell lines Doxy-induced and not induced were harvested and counted and re-suspended in Assay Buffer (7.5%BSA, 10%FBS, PBS1X + R01724 phosphatase inhibitor). Eight µL of each phage supernatant was diluted 1:5 into Assay Buffer (7.5%BSA, 10%FBS, PBS1X + R01724 phosphatase inhibitor) and incubated 3 hours at room temperature with 30.000 cells/well resuspended in Assay Buffer. The specific activity was detected in luminescence with Safire2 TECAN instrument. Phages able to co-activate GCGr and GLP1r were isolated, phagemids were extracted and subjected to DNA sequencing. Peptides corresponding to the identified sequences were synthetized and further tested.

20

EXAMPLE 2

Peptides corresponding to the identified sequences were synthetized and are shown in **Table 5**.

25

Table 5		
SEQ ID NO:	Name	Sequence
1	PD17	HSVGNFWSDYSKYLDSRRAQDFVQWLMLT-CONH ₂
2	PD18	HSQGTFTSDYSKYVEDRRAHDFVQWLMNT-CONH ₂
3	PD19	HSQGTFTSDYRKYLDERAAWDFVQWLMNT-CONH ₂

4	PD20	HSQGTFTSDYSKYLS NSWMT QDFVQWLMNT-CONH ₂
5	PD21	HSQGTFTSDYSKYLD IGRA QDFVQWLLNT-CONH ₂
6	PD22	HSQGTFTSDYSKYLDS LMA QDFVQWLMST-CONH ₂
7	PD23	HSQGTFTSDYSKYLD WRR AQDFVQWLLNT-CONH ₂
8	PD24	HSQGTFTSDY IKLL DSRRAQDFVQWLMNT-CONH ₂
9	PD25	HSQGTFTSDYSKYLD ARR AQDFVQWL IRT -CONH ₂
10	PD26	HSQGTFTSDYSKYLD VRR AQDFVQWLMNT-CONH ₂
11	PD27	HSQGTFTSDYSKYLD ELRAY DFVQWLMNT-CONH ₂
12	PD28	HSQGTFTSDYSKYLD YMRAY DFVQWLMNT-CONH ₂
13	PD29	HSQGTFTSDYSKYLDSRRA H DFVQWLLNT-CONH ₂
14	PD30	HSQGTFTSDYSKYLDSRRAQDFVQWLMN P -CONH ₂
15	PD31	HSQGTFTSDYSKYLDSRRAQDFVQWL INY -CONH ₂
16	PD32	INHEQW AFTSDYSKYLDSRRAQDFVQWLMNT-CONH ₂
17	PD33	ASMFTF SDYSKYLDSRRAQDFVQWML T -CONH ₂
18	PD34	HSQGT FL SDYSK LL DSRRAQDFVQWLM QT -CONH ₂
19	PD35	HSQGT FLHDY YYYLDSRRAQDFVQWLM DT -CONH ₂
20	PD36	HSQGTFTSDYSKYLDS IRA QDFVQWLM DT -CONH ₂
21	PD37	HSQGTFTSDYSKYLD NKRA QDFVQWLM QT -CONH ₂
22	PD38	HSQGTFTSDYSKYLDSRRAQDFV D WLMN E -CONH ₂
23	PD39	HSQGTFTSDYSKYLDSRRAQ EFVE WLM DE -CONH ₂
24	PD40	HSQGTFTSDYSKYLDSRRAQDFVQWL INT -CONH ₂
25	PD41	KALGQF TFTSDYSKYLDSRRAQDFVQWLMNT-CONH ₂
26	PD42	HSQGT FHD YSKYLDSRRAQDFVQWLLNT-CONH ₂
27	PD43	HSQGT FFSDY HWL DSRRAQDFVQWLMNT-CONH ₂
28	PD44	HSQGTFTSDYSKYLD WRR AQDFVQWL QNT -CONH ₂
29	PD45	HSQGTFTSDYSKYLDS KRAH DFVQWLLNT-CONH ₂
30	PD46	HSQGTFTSDYSKYLDSRRAQDF WID LMNT-CONH ₂
31	PD47	HSQGTFTSDYSKYLDSRRAQDFV MTS MNT-CONH ₂
32	PD48	HSQGTFTSDYSKYLDSRRAQDFV DWLLNA -CONH ₂
33	PD49	HSQGTFTSDYSKYLDSRRAQDFV EWLMNN -CONH ₂
34	PD50	HSQGTFTSDYSKYLDSRRAQDFV DWLINS -CONH ₂
35	PD51	HS HGT FFTSDYSKYLDSRRAQDFVQWLM TT -CONH ₂
36	PD52	HSQ GIF SDYSKYLDSRRAQDFVQWLMNT-CONH ₂
37	PD53	HSQGT FL SDYSKYLDSRRAQDFVQWLMNT-CONH ₂
38	PD54	HSQGTFTSDYS WY LDSRRAQDFVQWLMNT-CONH ₂

39	PD55	HSQGTFTSDYSKYLD MQ RAHDFVQWLMNT-CONH ₂
40	PD56	HSQGTFTSDYSKYLDSR MAY DFVQWLMNT-CONH ₂
41	PD57	HSQGTFTSDYSKYLDSRRAQDFVQWLL NQ -CONH ₂
42	PD58	HSQGTFFSDYSKYLDSRRAQDFVQWLL ET -CONH ₂
43	PD59	HSQGTFTSDYSKYLDSRRAQDFVQWLL DS -CONH ₂
CONH ₂ indicates that the carboxy terminus of the C-terminal peptide is amidated.		

Selected peptides from **Table 5** were synthesized as modified peptides that included lipidation and insertion of non-natural amino acids. These peptides are shown in **Table 6**.

Table 6		
SEQ ID NO:	Name	Sequence
44	TP534	HsQGTFTSDK(γEγEC16)SKYLD NK RAQDFVQWLMQT-CONH ₂
45	TP535	HsQGTFTSDK(γEγEC16)SKYLDSRRAHDFVQWLLNT-CONH ₂
46	TP536	HsQGTFLSDK(γEγEC16)SKLLDSRRAQDFVQWLMQT-CONH ₂
47	TP552	HsQGTFLSDYSKLLDSRAAQDFVQWLLQT-CONH ₂
48	TP559	HsQGTFTSDK(γEγEC16)SKYLD ARAA HDFVQWLLNT-CONH ₂
49	TP572	HUQGTFTSDK(γEγEC16)SKYLDSRRAHDFVQWLLNT KγE -CONH ₂
50	TP573	HUQGTFTSDK(γEγEC16)SKYLD ARAA HDFVQWLLNT KγE -CONH ₂
51	TP574	HUQGTFTSDK(γEγEC16)SKYLD NK RAQDFVQWLMQT KγE -CONH ₂
U= aminoisobutyric acid; s = D-Ser; γE= γ-glutamic acid; C16 = -CO-(CH ₂) ₁₄ -CH ₃ CONH ₂ indicates that the carboxy terminus of the C-terminal peptide is amidated.		

5

General synthetic procedure for synthesizing the peptides shown in Table 5 and Table 6.

Peptides SEQ ID NOs:1-48 were synthesized on a Rink-amide PEG-PS resin, Champion (Biosearch Technologies (150 μmol scale, loading 0.28 mmol/g) on a Symphony Protein Technologies Inc synthesizer. The amino acids were dissolved at a 0.3 M concentration in DMF and 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 2 M in NMP. The acylation reactions were performed for 1 hour with 5-fold excess of activated amino acid over the resin free amino groups; double acylation reactions of 45 minutes were performed from amino acid position 1 to amino acid position 7 and from Phe₂₂ to Val₂₃.

15

Peptides SEQ ID NOs:49-51 were synthesized on a Liberty Blue microwave synthesizer (CEM). The amino acids were dissolved at a 0.2 M concentration in DMF and activated with equimolar amounts of diisopropylcarbodiimide (0.5 M solution) and Oxyma (1 M solution). Both acylation and deprotection cycles were done using microwave heating using
5 standard protocols provided by CEM. Namely the acylation reactions were performed for 2,5 minutes by microwave heating at 90°C with 5-fold excess of activated amino acid over the resin free amino groups; for Arg and His acylations double acylation reactions were performed for 10 minutes by microwave heating at 50°C. From amino acid at position 1 to amino acid at position 7 and for the Phe-Val residues double acylation reactions were performed for 5 minutes by
10 microwave heating at 90°C. The deprotection step was performed by microwave heating at 90°C for 1,5 minutes using 20% piperidine solution in DMF.

For all the synthesis, the amino acids side chain protecting groups were: *tert*-butyl for Asp, Glu, Ser, D-Ser, Thr and Tyr; trityl for Asn, Gln and His; *tert*-butoxy-carbonyl for Lys, Trp; 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl for Arg. For seq ID 49, 50, 51, the C-
15 terminal amino acid γ -glutamic acid was incorporated as Fmoc-Glu-OtBu. For peptide SEQ ID NOs:44, 45, 46, 47, 48, 49, 50, and 51 the N-terminal residue was incorporated as Boc-His(Trt)-OH.

For peptide SEQ ID NOs: 44, 45, 46, 49, 50, and 51, the lysine at position 10 was incorporated with a Dde [1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl] protecting group
20 on the side chain amino group. At the end of the assembly, the Dde protecting group of Lys(Dde) was removed by treatment of 2% hydrazine in DMF. The side chain of Lys was derivatized by incorporation of two Fmoc-Glu-OtBu (γ -glutamic acid) residues and palmitic acid using HOAt and DIC as activators.

The dry peptide-resins were treated for two hours at room temperature with 88%
25 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*-butyl ether. The peptide pellet was resuspended, washed and centrifuged in cold methyl *tert*-butyl ether for two times. The peptide pellet was dried under vacuum and then resuspended in H₂O, 20% acetonitrile, and lyophilized.

The crude peptides (140 mg in 3 mL of DMSO) were purified by reverse-phase HPLC using preparative Waters XBridge C18 (50 x 150 mm, 5 μ m, 100Å) and using as eluents (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile.

Analytical HPLC was performed on a Acquity UPLC Waters Chromatograph with a BEH130 C18 or BEH300 C4 Acquity Waters 2.1x100 mm, 1.7 μ m, at 45°C, using H₂O, 0.1% TFA (A) and CH₃CN, 0.1% TFA (B) as solvents. The peptides were characterized by electrospray mass spectrometry on an Acquity SQ Detector.

EXAMPLE 3

Activity of the peptides at the Glucagon receptor (GCGR) and GLP-1 receptor (GLP1R) was measured in a cAMP activity assay.

Peptides were dissolved in 100% DMSO and serially diluted to generate 10 point titrations. The peptide solutions were then transferred into 384-well assay plates (150 nL/well). Assay ready frozen cells expressing human GLP1R or human GCGR were suspended in growth media consisting of DMEM medium (GIBCO), 10% FBS (GIBCO), 1x NEAA(GIBCO), 1x P/S (GIBCO), 10ug/ml Blasticidin (GIBCO) and 200 μ g/mL Hygromycin (GIBCO). Cells were then diluted in assay buffer consisting of PBS (GIBCO), 7.5% BSA (Perkin Elmer), 100 μ M RO 20-1724 (Sigma), with or without 20% human serum (MP Biomedical). The cell suspensions (15 μ L) were then added to the assay plates containing the peptide solutions (30,000 cells/well for human GCGR; 10,000 cells/well for human GLP1R). The cells were incubated for 1 hour at room temperature in the dark. Production of cAMP was determined using HitHunterTM cAMPXS kits (DiscoverX) following manufacturer protocol. The plates were incubated for overnight at room temperature in the dark. Luminescence was measured using an EnVision Multilabel plate reader (Perkin Elmer). Native GLP-1 and Glucagon (Bachem) are used as control peptides. EC₅₀ values were calculated using a 4 parameter logistic fit based on the Levenberg-Marquardt algorithm. The EC₅₀ results are shown in **Table 2** and **Table 7**.

EXAMPLE 4

In this example, the EC₅₀ activity and balance of the five peptides from **Table 6** that included d-Ser at position 2 and Lys(γ E γ EC16 at position 10) were compared to their

respective non-derivatized parental peptides. As shown, the lipidated analogs retained their parental peptide potency at the GCG and/or GLP-1 receptor and retained the relative balance displayed by the parent peptide.

Table 7					
SEQ ID NO:	Name	Sequence	EC ₅₀ hGCGR	EC ₅₀ hGLP1	Ratio
13	PD29	HSQGTFTSDYSKYLDSRRAHDFVQWLLNT-CONH ₂	0.02	0.02	1
45	TP535	HsQGTFTSDK(γEγEC16)SKYLDSRRAHDFVQWLLNT-CONH ₂	0.02	0.05	0.4
48	TP559	HsQGTFTSDK(γEγEC16)SKYLDARAADHDFVQWLLNT-CONH ₂	0.02	0.05	0.4
18	PD34	HSQGTFLSDYSKLLDSRAAQDFVQWLLQT-CONH ₂	0.09	>5	<0.018
47	TP552	HsQGTFLSDYSKLLDSRAAQDFVQWLLQT-CONH ₂	0.02	5.000	0.004
46	TP536	HsQGTFLSDK(γEγEC16)SKLLDSRAAQDFVQWLLQT-CONH ₂	0.04	5.00	0.008
21	PD37	HSQGTFTSDYSKYLD NK RAQDFVQWLMQT-CONH ₂	0.16	0.05	3.2
44	TP534	HsQGTFTSDK(γEγEC16)SKYLD NK RAQDFVQWLMQT-CONH ₂	0.01	0.02	0.5
U= aminoisobutyric acid; s = D-Ser; γE= γ-glutamic acid; C16 = -CO-(CH ₂) ₁₅ CONH ₂ indicates that the carboxy terminus of the C-terminal peptide is amidated.					

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5

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.

10

WHAT IS CLAIMED:

1. A co-agonist peptide comprising a peptide selected from following peptides

SEQ ID NO:	Name	Sequence
1	PD17	HSVGNFWSDYSKYLDSRRAQDFVQWLMLT-CONH ₂
2	PD18	HSQGTFTSDYSKYVEDRRAHDFVQWLMNT-CONH ₂
3	PD19	HSQGTFTSDYRKYLDERAAWDFVQWLMNT-CONH ₂
4	PD20	HSQGTFTSDYSKYLSWMTQDFVQWLMNT-CONH ₂
5	PD21	HSQGTFTSDYSKYLDIGRAQDFVQWLLNT-CONH ₂
6	PD22	HSQGTFTSDYSKYLDSLMAQDFVQWLMST-CONH ₂
7	PD23	HSQGTFTSDYSKYLDWRRRAQDFVQWLLNT-CONH ₂
8	PD24	HSQGTFTSDYIKLLDSRRAQDFVQWLMNT-CONH ₂
9	PD25	HSQGTFTSDYSKYLDARRAQDFVQWLIRT-CONH ₂
10	PD26	HSQGTFTSDYSKYLDVRRRAQDFVQWLMNT-CONH ₂
11	PD27	HSQGTFTSDYSKYLDELRAYDFVQWLMNT-CONH ₂
12	PD28	HSQGTFTSDYSKYLDYMRAYDFVQWLMNT-CONH ₂
13	PD29	HSQGTFTSDYSKYLDSRRAHDFVQWLLNT-CONH ₂
14	PD30	HSQGTFTSDYSKYLDSRRAQDFVQWLMNP-CONH ₂
15	PD31	HSQGTFTSDYSKYLDSRRAQDFVQWLINY-CONH ₂
16	PD32	INHEQWAFSTSDYSKYLDSRRAQDFVQWLMNT-CONH ₂
17	PD33	ASMFTFFSDYSKYLDSRRAQDFVQWLMLT-CONH ₂
18	PD34	HSQGTFLSDYSKLLDSRRAQDFVQWLMQT-CONH ₂
19	PD35	HSQGTFLHDYYYLDSRRAQDFVQWLMDDT-CONH ₂
20	PD36	HSQGTFTSDYSKYLDSIRAQDFVQWLMDDT-CONH ₂
21	PD37	HSQGTFTSDYSKYLDNKRAQDFVQWLMQT-CONH ₂
22	PD38	HSQGTFTSDYSKYLDSRRAQDFVDWLMNE-CONH ₂
23	PD39	HSQGTFTSDYSKYLDSRRAQEFVEWLMDE-CONH ₂
24	PD40	HSQGTFTSDYSKYLDSRRAQDFVQWLINT-CONH ₂
25	PD41	KALGQFTFTSDYSKYLDSRRAQDFVQWLMNT-CONH ₂
26	PD42	HSQGTFFHDYSKYLDSRRAQDFVQWLLNT-CONH ₂
27	PD43	HSQGTFFSDYSHWLDISRRAQDFVQWLMNT-CONH ₂

28	PD44	HSQGTFTSDYSKYLD W RRAQDFVQWL Q NT-CONH ₂
29	PD45	HSQGTFTSDYSKYLDS K RAHDFVQWLLNT-CONH ₂
30	PD46	HSQGTFTSDYSKYLDSRRAQDF W IDLMNT-CONH ₂
31	PD47	HSQGTFTSDYSKYLDSRRAQDFV M TSMNT-CONH ₂
32	PD48	HSQGTFTSDYSKYLDSRRAQDFV D WLLNA-CONH ₂
33	PD49	HSQGTFTSDYSKYLDSRRAQDFV E WLMNN-CONH ₂
34	PD50	HSQGTFTSDYSKYLDSRRAQDFV D WLINS-CONH ₂
35	PD51	HS H GTFTSDYSKYLDSRRAQDFVQWLM T T-CONH ₂
36	PD52	HSQGI F SDYSKYLDSRRAQDFVQWLMNT-CONH ₂
37	PD53	HSQGT F LSDYSKYLDSRRAQDFVQWLMNT-CONH ₂
38	PD54	HSQGTFTSDYS W YLSRRAQDFVQWLMNT-CONH ₂
39	PD55	HSQGTFTSDYSKYLD M QRAHDFVQWLMNT-CONH ₂
40	PD56	HSQGTFTSDYSKYLDSR M AYDFVQWLMNT-CONH ₂
41	PD57	HSQGTFTSDYSKYLDSRRAQDFVQWLL N Q-CONH ₂
42	PD58	HSQGTFFSDYSKYLDSRRAQDFVQWL L ET-CONH ₂
43	PD59	HSQGTFTSDYSKYLDSRRAQDFVQWL L DS-CONH ₂

wherein the L-Serine at position 2 is replaced with Val, Ile, Asp, Glu, Met, Trp, Asn, D-Ala, D-Ser, or α -aminoisobutyric acid (aib), the Tyrosine at position 10 is replaced with a Lysine residue conjugated to a fatty acid or fatty diacid via a linking moiety, wherein CONH₂ indicates the C-terminal amino acid carboxy group is amidated, and the peptide may comprise 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 additional amino acid substitutions.

2. The co-agonist peptide of claim 1, wherein the fatty acid or fatty diacid comprises 14 to 20 methylene groups.

3. The co-agonist peptide of claim 1, wherein the fatty acid or fatty diacid comprises 16 carbon atoms.

4. The co-agonist peptide of claim 1, wherein the linking moiety comprises a PEG₂ (8-amino-3,6-dioxaoctanoic acid) Gamma-Glutamic acid (γ Glu), a γ Glu, a γ Glu γ Glu, or a PEG₂PEG₂ γ Glu.

5. A co-agonist peptide comprising a peptide selected from the following peptides

SEQ ID NO:	Name	Sequence
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44	TP534	HsQGTFTSDK(γ E γ EC16)SKYLD NK RAQDFVQWLM QT -CONH ₂
45	TP535	HsQGTFTSDK(γ E γ EC16)SKYLD SRRAH DFVQWLLNT-CONH ₂
46	TP536	HsQGTFLSDK(γ E γ EC16)SKLL DSRAA QDFVQWLM QT -CONH ₂
47	TP552	HsQGTFLSDYSKLL DSRAA QDFVQWLL QT -CONH ₂
48	TP559	HsQGTFTSDK(γ E γ EC16)SKYLD ARAAH DFVQWLLNT-CONH ₂
49	TP572	HUQGTFTSDK(γEγEC16)SKYLD SRRAH DFVQWLLNT KγE -CONH ₂
50	TP573	HUQGTFTSDK(γEγEC16)SKYLD ARAAH DFVQWLLNT KγE -CONH ₂
51	TP574	HUQGTFTSDK(γEγEC16)SKYLD NK RAQDFVQWLM QTγE -CONH ₂

wherein U is aminoisobutyric acid, s is D-Ser, γ E= γ -glutamic acid, and C16 = -CO-(CH₂)₁₄-CH₃, and wherein CONH₂ indicates the C-terminal amino acid carboxy group is amidated.

6. A composition comprising one or more co-agonist peptides of claims 1-6 and a pharmaceutically acceptable carrier and/or pharmaceutically acceptable salt.

5

7. A method for treating a patient for a metabolic disease or disorder comprising administering the patient an effective amount of any one or more of the co-agonist peptides of claims 1-6 to treat the metabolic disease or disorder in the patient.

10

8. The method of claim 7, wherein the metabolic disease or disorder comprises diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

9. The method of claim 8, wherein the diabetes comprises Type I diabetes, Type II diabetes, or gestational diabetes.

15

10. The method of claim 7, wherein the patient has more than one metabolic disease or disorder

20

11. The method of claim 10, wherein the metabolic disease or disorder comprises, diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.

12. A method for treating a patient for a metabolic disease or disorder comprising administering the patient an effective amount of the composition of claim 6 to treat the metabolic disease or disorder in the patient.

5 13. The method of claim 12, wherein the metabolic disease or disorder comprises diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

10 14. The method of claim 12, wherein the patient has more than one metabolic disease or disorder

15 15. The method of claim 14, wherein the metabolic disease or disorder comprises, diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.

16. Use of one or more co-agonist peptides of claims 1-6 for manufacture of a medicament for the treatment of a metabolic disease or disorder.

20 17. The use of claim 15, wherein the metabolic disease or disorder comprises diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

25 18. The use of claim 17, wherein the diabetes comprises Type I diabetes, Type II diabetes, or gestational diabetes.

30 19. A method for treating a metabolic disease or disorder 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 agonist and administering to the patient or individual an effective amount of a composition comprising an insulin or insulin analog to treat the metabolic disease or disorder in the patient or individual.

20. The method of claim 19, wherein the insulin analog comprises insulin detemir, insulin glargine, insulin levemir, insulin glulisine, insulin degludec, or insulin lispro.

21. The method of claim 20, wherein the metabolic disease or disorder
5 comprises diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

22. The method of claim 21, wherein the diabetes comprises Type I diabetes, Type II diabetes, or gestational diabetes.
10

23. The method of claim 19, wherein the patient has more than one metabolic disease or disorder selected from diabetes and NASH, NAFLD, or obesity; obesity and NASH or NAFLD; diabetes, NASH, and obesity; diabetes, NAFLD, and obesity; or diabetes and obesity.
15

1/3

	1	10	20	29
Glucagon	HSQGTFTSDYSKYLSRRAQDFVQWLMNT			
Libr 1	XXXXXXXXFTSDYSKYLSRRAQDFVQWLXT			
Libr 2	XXXXXXXXSDYSKYLSRRAQDFVQWLXT			
Libr 3	HSQGTFTXXXXXXXXLSRRAQDFVQWLXT			
Libr 4	HSQGTFTSDYSKYXXXXXXXXDFVQWLXT			
Libr 5	HSQGTFTSDYSKYLSRRAQXXXXXXXXXX			

FIG. 1

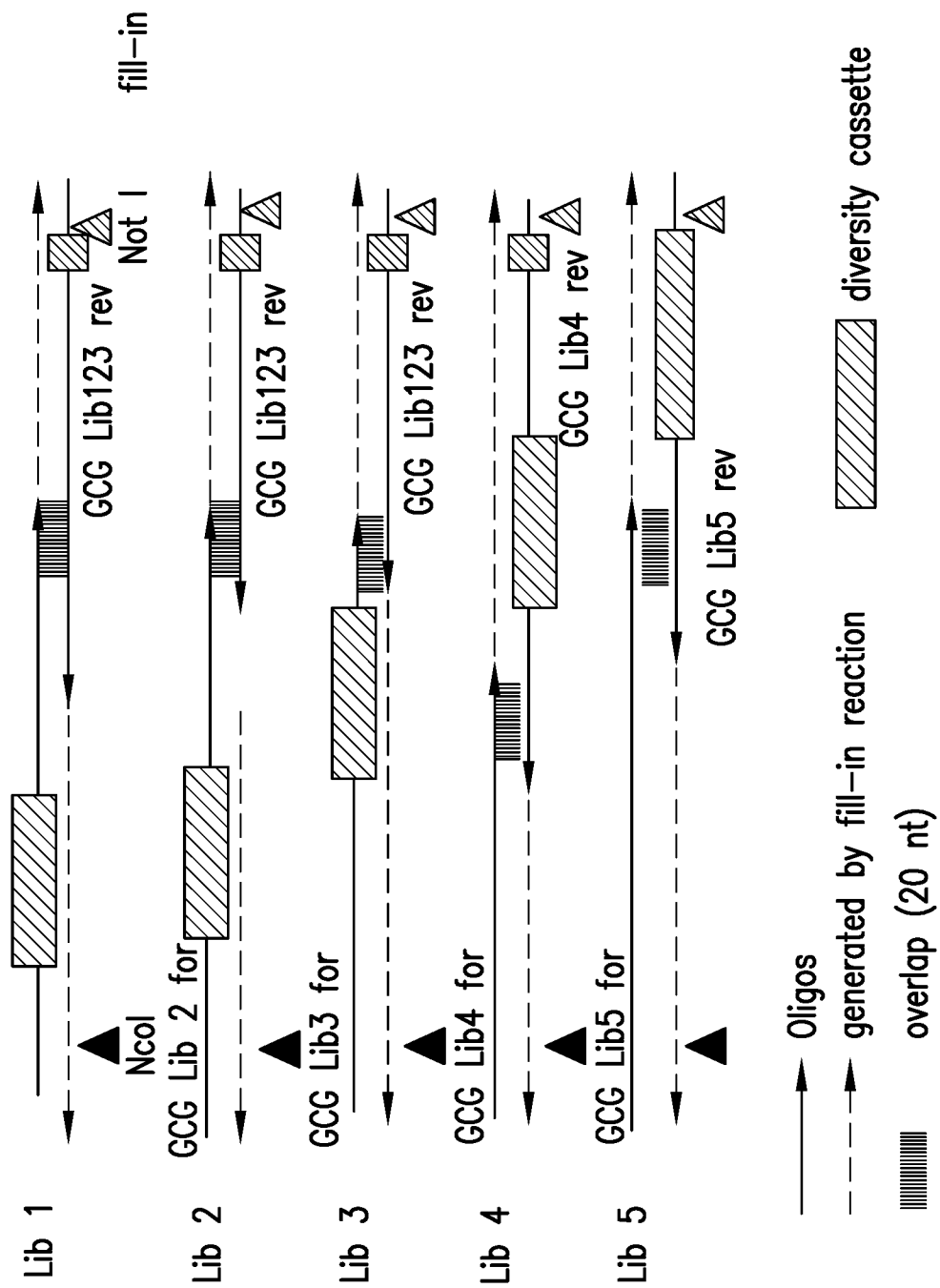


FIG. 2

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X _n	Description	Codons																		
		Lys	Asn	Thr	Ile	Met	Gln	His	Pro	Arg	Leu	Glu	Asp	Ala	Gly	Val	Tyr	Ser	Trp	Phe
X10	all 5.26 (no Cys)	AAA	AAT	ACT	ATA	ATG	CAG	CAT	CCG	CGT	CTG	GAA	GAT	GCT	GGT	GTT	TAC	TCT	TGG	TTT
X1	Asp 45% other 3% (no Cys)	AAA	AAT	ACT	ATA	ATG	CAG	CAT	CCG	CGT	CTG	GAA	GAT	GCT	GGT	GTT	TAC	TCT	TGG	TTT
X2	Gln 45% other 3% (no Cys)	AAA	AAT	ACT	ATA	ATG	CAG	CAT	CCG	CGT	CTG	GAA	GAT	GCT	GGT	GTT	TAC	TCT	TGG	TTT
X3	Gly 45% other 3% (no Cys)	AAA	AAT	ACT	ATA	ATG	CAG	CAT	CCG	CGT	CTG	GAA	GAT	GCT	GGT	GTT	TAC	TCT	TGG	TTT
X4	His 45% other 3% (no Cys)	AAA	AAT	ACT	ATA	ATG	CAG	CAT	CCG	CGT	CTG	GAA	GAT	GCT	GGT	GTT	TAC	TCT	TGG	TTT
X5	Lys 45% other 3% (no Cys)	AAA	AAT	ACT	ATA	ATG	CAG	CAT	CCG	CGT	CTG	GAA	GAT	GCT	GGT	GTT	TAC	TCT	TGG	TTT
X6	Phe 45% other 3% (no Cys)	AAA	AAT	ACT	ATA	ATG	CAG	CAT	CCG	CGT	CTG	GAA	GAT	GCT	GGT	GTT	TAC	TCT	TGG	TTT
X7	Ser 45% other 3% (no Cys)	AAA	AAT	ACT	ATA	ATG	CAG	CAT	CCG	CGT	CTG	GAA	GAT	GCT	GGT	GTT	TAC	TCT	TGG	TTT
X8	Thr 45% other 3% (no Cys)	AAA	AAT	ACT	ATA	ATG	CAG	CAT	CCG	CGT	CTG	GAA	GAT	GCT	GGT	GTT	TAC	TCT	TGG	TTT
X9	Tyr 45% other 3% (no Cys)	AAA	AAT	ACT	ATA	ATG	CAG	CAT	CCG	CGT	CTG	GAA	GAT	GCT	GGT	GTT	TAC	TCT	TGG	TTT

X11	Rev: Ala 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA
X12	Rev: Arg 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA
X13	Rev: Asn 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA
X14	Rev: Asp 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA
X15	Rev: Gln 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA
X16	Rev: Leu 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA
X17	Rev: Met 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA
X18	Rev: Phe 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA
X19	Rev: Ser 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA
X20	Rev: Thr 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA
X21	Rev: Trp 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA
X22	Rev: Val 45% other 3% (no Cys)	TTT	GTT	CGT	AAT	CAT	CTG	ATG	CCG	TCT	CAG	TTC	ATC	TGC	ACC	AAC	ATA	ACT	CCA	AAA

FIG.3