

54: 2390-2395 (2005)). Robust weight loss was observed at the end of the study in treated subjects (1.9%, placebo-corrected). Plasma levels of OXM were similar to that observed in the infusion study (peak concentration about 950 pM). The absence of any tachyphylaxis and a low incidence of mild and transient nausea (about 3%) despite the relatively high doses necessitated by the poor in vivo stability of OXM (plasma $t_{1/2}$ < 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)). 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.

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-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 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 relatively balanced activity or potency at the GLP-1 receptor and GCG receptor.

BRIEF SUMMARY

The disclosure provides peptides that are agonists of the glucagon (GCG) receptor and the glucagon-like peptide 1 (GLP-1) receptor and have a relatively balanced activity or

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

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

In one aspect, the present disclosure provides a peptide comprising or consisting of the amino acid sequence

HX₂QGTX₆TSDX₁₀SX₁₂YLX₁₅X₁₆RX₁₈AQDFVQWLX₂₇DT (SEQ ID NO:20)

Wherein X₂ is D-serine, L-alanine, α-aminoisobutyric acid (Aib), or 1-amino-cyclobutane carboxylic acid; X₆ is L-phenylalanine, para-fluoro phenylalanine or threo-β-phenylserine; X₁₀ is L-lysine or para-aminomethyl phenylalanine; X₁₂ is L-lysine or L-leucine; X₁₅ is L-glutamic acid, L-leucine, or L-aspartic acid; X₁₆ is L-alanine, Aib, L-glutamic acid, or L-valine; X₁₈ is L-alanine or L-arginine; X₂₇ is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer; and, the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

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

In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12.

In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.00, 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, or 1.10.

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

HX₂QGTX₆TSDX₁₀SX₁₂YLX₁₅X₁₆RX₁₈AQDFVQWLX₂₇DT (SEQ ID NO:20)

Wherein X₂ is D-serine, L-alanine, α-aminoisobutyric acid (Aib), or 1-amino-cyclobutane carboxylic acid; X₆ is L-phenylalanine, para-fluoro phenylalanine or threo-β-phenylserine; X₁₀ is L-lysine or para-aminomethyl phenylalanine; X₁₂ is L-lysine or L-leucine; X₁₅ is L-glutamic acid or L-leucine; X₁₆ is L-alanine, Aib, L-glutamic acid, or L-valine; X₁₈ is L-alanine or L-arginine; X₂₇ is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position

10 is conjugated to a palmitoyl group by either a gamma-glutamic acid spacer (γ E) or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γ E γ E) spacer; the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; and, a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

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

In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0 $\pm$ 0.12.

In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.00, 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, or 1.10.

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

HX₂QGTX₆TSDX₁₀SX₁₂YLX₁₅X₁₆RX₁₈AQDFVQWLX₂₇DT (SEQ ID NO:20)

Wherein X₂ is D-serine, L-alanine, α -aminoisobutyric acid (Aib), or 1-amino-cyclobutane carboxylic acid; X₆ is L-phenylalanine, para-fluoro phenylalanine or threo- β -phenylserine; X₁₀ is L-lysine or para-aminomethyl phenylalanine; X₁₂ is L-lysine or L-leucine; X₁₅ is L-glutamic acid or L-leucine; X₁₆ is L-alanine, Aib, L-glutamic acid, or L-valine; X₁₈ is L-alanine or L-arginine; X₂₇ is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid (γ E) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γ E γ E) spacer; the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; and, a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

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

In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12.

In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.00, 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, or 1.10.

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

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

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

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

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

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

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

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

5 In another aspect, the disclosure provides a composition comprising a peptide of any one of the foregoing peptides and a pharmaceutically acceptable carrier.

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

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

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

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

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

20 The present disclosure, in another aspect, provides for the use of any one of the aforementioned peptides or any one of the foregoing composition for manufacture of a medicament for the treatment of metabolic disease.

The present disclosure further provides for the use of any one of the aforementioned compositions for manufacture of a medicament for the treatment of metabolic disease.

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

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

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

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

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

In particular embodiments, the composition comprising the co-agonist peptide is
10 administered at a time prior to the time the composition comprising the insulin or insulin analog is administered. In another embodiment, 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 embodiment, the composition comprising the co-agonist peptide is administered at the same time as the composition comprising the insulin or
15 insulin analog is administered.

In particular embodiments, the insulin analog is insulin detemir, insulin glargine, insulin levemir, insulin glulisine, or insulin lispro.

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

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

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

25 The present disclosure, in another aspect, provides a composition comprising any one of the aforementioned peptides; an insulin or insulin analog; and, a pharmaceutically acceptable carrier.

The present disclosure, in another aspect, provides for the use of a composition comprising any one of the aforementioned peptides; an insulin or insulin analog; and, a
30 pharmaceutically acceptable carrier for the treatment of a metabolic disease.

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

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

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

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

In particular embodiments, the present disclosure provides a peptide consisting of the amino acid sequence

25
HX₂QGTX₆TSDX₁₀SX₁₂YLX₁₅X₁₆RX₁₈AQDFVQWLX₂₇DT (SEQ ID NO:20)

Wherein X₂ is D-serine, L-alanine, α -aminoisobutyric acid (Aib), or 1-amino-cyclobutane carboxylic acid; X₆ is L-phenylalanine, para-fluoro phenylalanine or threo- β -phenylserine; X₁₀
30 is L-lysine or para-aminomethyl phenylalanine; X₁₂ is L-lysine or L-leucine; X₁₅ is L-glutamic

acid or L-leucine; X₁₆ is L-alanine, Aib, L-glutamic acid, or L-valine; X₁₈ is L-alanine or L-arginine; X₂₇ is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer; and, the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

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

In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12.

In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.00, 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, or 1.10.

The present disclosure further provides a composition consisting of a peptide having the amino acid sequence

HX₂QGTKX₆TSDX₁₀SX₁₂YLY₁₅X₁₆RX₁₈AQDFVQWLX₂₇DT (SEQ ID NO:20)

Wherein X₂ is D-serine, L-alanine, α-aminoisobutyric acid (Aib), or 1-amino-cyclobutane carboxylic acid; X₆ is L-phenylalanine, para-fluoro phenylalanine or threo-β-phenylserine; X₁₀ is L-lysine or para-aminomethyl phenylalanine; X₁₂ is L-lysine or L-leucine; X₁₅ is L-glutamic acid or L-leucine; X₁₆ is L-alanine, Aib, L-glutamic acid, or L-valine; X₁₈ is L-alanine or L-arginine; X₂₇ is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid spacer (γE) or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer; the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; and, a

pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

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

In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12.

In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.00, 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, or 1.10.

The present disclosure further provides a pharmaceutical composition consisting of a peptide having the amino acid sequence

HX₂QGTX₆TSDX₁₀SX₁₂Y LX₁₅X₁₆RX₁₈AQDFVQWLX₂₇DT (SEQ ID NO:20)

Wherein X₂ is D-serine, L-alanine, α-aminoisobutyric acid (Aib), or 1-amino-cyclobutane carboxylic acid; X₆ is L-phenylalanine, para-fluoro phenylalanine or threo-β-phenylserine; X₁₀ is L-lysine or para-aminomethyl phenylalanine; X₁₂ is L-lysine or L-leucine; X₁₅ is L-glutamic acid or L-leucine; X₁₆ is L-alanine, Aib, L-glutamic acid, or L-valine; X₁₈ is L-alanine or L-arginine; X₂₇ is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid (γE) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γEγE) spacer; the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; and, a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

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

In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0±0.12.

5 In particular embodiments, the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.00, 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, or 1.10.

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

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

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

The present disclosure further provides a composition consisting of (i) a peptide
30 consisting of the amino acid sequence of native human glucagon as shown in SEQ ID NO:1 but in which the L-serine at position 2 is replaced with a D-serine; the tyrosine at position 10 is

replaced with (i) L-lysine conjugated to a palmitoyl group by either a gamma-glutamic acid (γ E) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γ E γ E) spacer or (ii) para-aminomethyl phenylalanine conjugated to a palmitoyl group by either a gamma-glutamic acid (γ E) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γ E γ E) spacer; and up to
5 six additional amino acid substitutions; wherein the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0 $\pm$ 0.12, and wherein the peptide optionally includes a
10 protecting group that, if present, is joined to the C-terminal carboxy group of the peptide, and (ii) a pharmaceutically acceptable carrier; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

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

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

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

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

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

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

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

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

15 The present disclosure further provides for the use of any one of the aforementioned peptides for manufacture of a medicament for the treatment of obesity metabolic disease.

 The present disclosure further provides for the use of any one of the aforementioned compositions for manufacture of a medicament for the treatment of obesity
20 metabolic disease.

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

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

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

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

Further provided is method for treating a metabolic disease in a patient or individual consisting of: administering to the patient or individual an effective amount of any one

of the aforementioned compositions consisting of a co-agonist peptide and administering to the patient or individual an effective amount of a composition consisting of an insulin or insulin analog to treat the metabolic disease in the patient or individual.

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

In particular embodiments, the insulin analog is insulin detemir, insulin glargine, insulin levemir, insulin glulisine, or insulin lispro.

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

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

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

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

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

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

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

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

5 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.

10 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 or one or more co-agonist peptides 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 humans and companion animals such as cats and dogs.

15 As used herein, the term "pharmaceutically acceptable carrier" includes any carrier suitable for administering to an individual, for example any of the standard pharmaceutical carriers, including but not limited to 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
20 government or listed in the U.S. Pharmacopeia for use in animals, including humans. In general, "pharmaceutically acceptable carrier" 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.
25 The term "carrier" refers to a diluent, adjuvant, excipient, or 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.

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
30 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

WHAT IS CLAIMED:

1. A peptide comprising or consisting of the amino acid sequence

HX₂QGTX₆TSDX₁₀SX₁₂YLX₁₅X₁₆RX₁₈AQDFVQWLX₂₇DT (SEQ ID NO:20)

5

Wherein X₂ is D-serine, L-alanine, α -aminoisobutyric acid (Aib), or 1-amino-cyclobutane carboxylic acid;

X₆ is L-phenylalanine, para-fluoro phenylalanine or threo- β -phenylserine;

X₁₀ is L-lysine or para-aminomethyl phenylalanine;

10 X₁₂ is L-lysine or L-leucine;

X₁₅ is L-glutamic acid, L-leucine, or L-aspartic acid

X₁₆ is L-alanine, Aib, L-glutamic acid, or L-valine;

X₁₈ is L-alanine or L-arginine;

X₂₇ is L-leucine, methionine sulfone, or L-norleucine;

15 the amino acid at position 10 is conjugated to a palmitoyl group by either a gamma-glutamic acid (γ E) spacer or a gamma-glutamic acid-gamma-glutamic acid dipeptide (γ E γ E) spacer; and, the peptide optionally includes a protecting group that, if present, is joined to the C-terminal carboxy group of the peptide; with the proviso that the peptide does not have the amino acid sequence of SEQ ID NO:21.

20

2. The peptide of claim 1, wherein the peptide has the amino acid sequence of SEQ ID NO:7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, or 19.

3. The peptide of claim 1, wherein the peptide is an co-agonist of the

25 glucagon receptor and the GLP-1 receptor and has a ratio of EC₅₀ at the glucagon receptor to EC₅₀ at the GLP-1 receptor of about 0.88 to about 1.25, about 0.90 to about 1.25, about 0.90 to about 1.10, about 0.90 to about 1.00, or about 1.0 $\pm$ 0.12.

30

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

5. The peptide of claim 4, wherein the serine at position 16 is replaced with L-alanine.

6. The peptide of claim 4, wherein the arginine at position 18 is replaced with L-alanine.

7. The peptide of claim 4, wherein the methionine at position 27 is replaced with L-leucine.

8. The peptide of claim 4, wherein the asparagine at position 28 is replaced with L-aspartic acid.

9. The peptide of claim 4, wherein the serine at position 16 is replaced with L-alanine, the arginine at position 18 is replaced with L-alanine, the methionine at position 27 is replaced with L-leucine, and the asparagine at position 28 is replaced with L-aspartic acid.

10. A composition comprising a peptide of any one of the foregoing claims and a pharmaceutically acceptable carrier.

11. Use of the peptide of claim 1, 2, 3, 4, 5, 6, 7, 8, or 9 or the composition of
5 claim 10 for manufacture of a medicament for the treatment of a metabolic disease.

12. The use of claim 11, wherein the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

10 13. The use of claim 12, wherein the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

14. A composition comprising a peptide of claim 1, 2, 3, 4, 5, 6, 7, 8, or 9; an insulin or insulin analog; and, a pharmaceutically acceptable carrier.

15. Use of a composition comprising a peptide of claim 1, 2, 3, 4, 5, 6, 7, 8, or 9; an insulin or insulin analog; and, a pharmaceutically acceptable carrier for the manufacture of a medicament for the treatment of a metabolic disease.

5 16. The use of claim 15, wherein the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

17. The use of claim 16, wherein the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

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