

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.

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 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, 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, 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 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, or a pharmaceutically acceptable salt thereof.

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.

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 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,
10 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)

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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
20 or L-arginine; X₂₇ is L-leucine, methionine sulfone, or L-norleucine; the amino acid at position

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.

WHAT IS CLAIMED:

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

HX₂QGTX₆TSDX₁₀SX₁₂YLX₁₅X₁₆RX₁₈AQDFVQWLX₂₇DT (SEQ ID NO:20)

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wherein X₂ is D-serine, L-alanine, 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;

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

X₁₆ is L-alanine, 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 a gamma-glutamic acid-
15 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, or a pharmaceutically acceptable salt thereof.

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2. The peptide of claim 1, wherein the peptide has the amino acid sequence
of SEQ ID NO:7, 8, 10, 13, or 17, or a pharmaceutically acceptable salt thereof.

3. The peptide of claim 1, or a pharmaceutically acceptable salt thereof,
wherein the peptide is an co-agonist of the glucagon receptor and the GLP-1 receptor and has a
25 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.

4. The peptide of claim 1, 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 a gamma-glutamic acid-gamma-glutamic acid dipeptide spacer or (ii) para-aminomethyl phenylalanine conjugated to a palmitoyl group by a gamma-glutamic acid-gamma-glutamic acid dipeptide (γ E γ E) spacer; and up to three 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 wherein the serine at position 16 is replaced with L-alanine, or a pharmaceutically acceptable salt thereof.

5. The peptide of claim 4, or a pharmaceutically acceptable salt thereof, wherein the arginine at position 18 is replaced with L-alanine.

6. The peptide of claim 4, or a pharmaceutically acceptable salt thereof, wherein the methionine at position 27 is replaced with L-leucine.

7. The peptide of claim 4, or a pharmaceutically acceptable salt thereof, wherein the asparagine at position 28 is replaced with L-aspartic acid.

8. The peptide of claim 4, or a pharmaceutically acceptable salt thereof, 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.

9. A composition comprising a peptide of any one of the foregoing claims, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

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

11. The use of claim 10, wherein the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

12. The use of claim 11, wherein the diabetes is Type I diabetes, Type II diabetes, or gestational diabetes.

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

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

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15. The use of claim 14, wherein the metabolic disease is diabetes, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or obesity.

16. The use of claim 15, wherein the diabetes is Type I diabetes, Type II
10 diabetes, or gestational diabetes.

17. The peptide of claim 1, wherein the peptide has the amino acid sequence of SEQ ID NO: 7, or a pharmaceutically acceptable salt thereof.

18. The peptide of claim 1, wherein the peptide has the amino acid sequence of SEQ ID NO: 8, or a pharmaceutically acceptable salt thereof.

19. The peptide of claim 1, wherein the peptide has the amino acid sequence of SEQ ID NO: 10, or a pharmaceutically acceptable salt thereof.

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20. The peptide of claim 1, wherein the peptide has the amino acid sequence of SEQ ID NO: 13, or a pharmaceutically acceptable salt thereof.

21. The peptide of claim 1, wherein the peptide has the amino acid sequence of SEQ ID NO: 17, or a pharmaceutically acceptable salt thereof.

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22. The peptide of claims 1, 2, or 4, or as defined in claims 9, 10, 13 or 14, wherein the pharmaceutically acceptable salt is the sodium salt.

23. The use of claim 10 or 14 wherein the metabolic disease is diabetes.

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24. The use of claim 10 or 14 wherein the metabolic disease is obesity.