SUMMARY

[0006] ~~Provided~~Disclosed herein are peptides that are GIP agonist peptides contemplated for use in treating subjects in need thereof, e.g. with diabetes and obesity.

[0007] Native glucagon does not activate the GIP receptor, and normally has about 1% of the activity of native GLP-1 at the GLP-1 receptor. In some ~~embodiments~~examples, the peptides are glucagon analogs comprising a structure based on the amino acid sequence of native human glucagon (SEQ ID NO: 1) but differing at one or more positions as compared to SEQ ID NO: 1, wherein the differences, or modifications, enhance the agonist activity of the analog at the GIP receptor. Such glucagon analogs will have agonist activity at the GIP receptor greater than that of native glucagon and, in some aspects, greater than that of native GIP. In some or any ~~embodiments~~examples, the GIP agonist has a GIP percentage potency of at least 0.1%. In some or any ~~aspects~~examples of the present disclosures, the glucagon analog additionally exhibits agonist activity at one or both of the glucagon receptor and the GLP-1 receptor. Accordingly, GIP agonists, GIP-GLP-1 co-agonists, GIP-glucagon co-agonists, and GIP-GLP-1-glucagon triagonists are ~~provided~~described herein.

[0008] In exemplary ~~embodiments~~examples, the selectivity of the GIP agonist peptide of the present disclosures for the human GLP-1 receptor versus the GIP receptor is less than 100-fold. In some or any ~~embodiments~~examples, the GIP agonist peptide has GIP percentage potency within 20-fold or 10-fold different (higher or lower) of the glucagon percentage potency and/or GLP-1 percentage potency.

[0009] The present disclosures provides an analog of glucagon (SEQ ID NO: 1), comprising

(A) at position 1 an amino acid comprising a side chain of Tyr, His, Thio Ala, or Phe, wherein the alpha carbon of the amino acid at position 1, is attached to a structure selected from the group consisting of:

(i) H_2N-(CH_2)-

(ii) H_2N-

(iii) H(acetyl)N-$(CH_2)_{0-1}$-; and

(iv) H- or CH_3-

(B) an amino acid comprising an acylated or alkylated amino acid at any of positions 9, 10, 12, 16, 20, or 37-43, optionally wherein the non-native acyl or alkyl group is linked to such amino acid via a spacer;

(C) an alpha helix stabilizing amino acid at one or more of positions 16-21, and

(D) up to ten (e.g., up to 1, 2, 3, 4, 5, 6, 7, 8 or 9) additional amino acid modifications relative to SEQ ID NO: 1,

wherein the amino acid at position 1 is an L-stereoisomer or D-stereoisomer,

wherein, optionally, the amino acid at position 1 is other than L-His and L-Tyr,

wherein, when the glucagon analog lacks a hydrophilic moiety, the glucagon analog exhibits an EC50 at the human GIP receptor of less than or about 0.5 nM, and wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

In a first aspect, the present invention refers to an analog of native glucagon, optionally comprising the amino acid sequence of SEQ ID NO: 140, comprising:

A. at position 1 an amino acid comprising a side chain of Tyr, His, Thio Ala, or Phe, wherein the alpha carbon of the amino acid at position 1 is attached to a structure selected from the group consisting of:

(i) H_2N-(CH_2)-;

(ii) H_2N-

(iii) H(acetyl)N-$(CH_2)_{0-1}$-; and

(iv) H- or CH_3-

B. at any of positions 9, 10, 12, 20, or 37-43 an acylated amino acid or alkylated amino acid, optionally wherein the acyl or alkyl group is linked to the amino acid via a spacer;

C. an alpha helix stabilizing amino acid at one or more of positions 16-21, and

D. up to ten additional amino acid modifications relative to native glucagon;

wherein the amino acid at position 1 is an L-stereoisomer or D-stereoisomer,

wherein, optionally, the amino acid at position 1 is other than L-His, L-Tyr, and L-Phe,

wherein, when the glucagon analog lacks a hydrophilic moiety, the glucagon analog exhibits an EC50 at the human GIP receptor of less than or about 0.5 nM, and wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

[0010] Throughout the application, all references to a particular amino acid position by number (e.g.,position 28) refer to the amino acid at that position in native glucagon (SEQ ID NO: 1) or the corresponding amino acid position in any analog thereof. For example, a reference herein to "position 28" would mean the corresponding position 27 for a glucagon analog in which the first amino acid of SEQ ID NO: 1 has been deleted. Similarly, a reference herein to "position 28" would mean the corresponding position 29 for a glucagon analog in which one amino acid has been added before the N-terminus of SEQ ID NO: 1.

[0011] In some ~~embodiments~~examples, the GIP agonist peptides comprise an amino acid sequence of any of SEQ ID NOs: 11-18, 21-23, 25-31, 33-36, 38, 39, 100-139, 141-155, 157-173, 175-194, and 196-200. In some ~~embodiments~~examples, the GIP agonist peptides comprise a structure based on a parent sequence comprising any of SEQ ID NOs: 11-18, 21-23, 25-31, 33-36, 38, 39, 100-139, 141-155, 157-173, 175-194, and 196-200, but differs from the parent sequence at one or more positions, as further described herein. In some ~~embodiments~~examples, the GIP agonist peptides comprise an amino acid sequence of any of SEQ ID NOs: 156, 174, and 195. In some ~~embodiments~~examples, the GIP agonist peptides comprise a structure based on a parent sequence comprising any of SEQ ID NOs: 156, 174, and 195 but differs from the parent sequence at one or more positions, as further described herein. In some ~~embodiments~~examples, the GIP agonist peptides comprise an amino acid sequence of SEQ ID NO: 201 or 202. In some ~~embodiments~~examples, the GIP agonist peptides comprise a structure based on a parent sequence

comprising any of SEQ ID NOs: 201 and 202, but differs from the parent sequence at one or more positions, as further described herein. In exemplary ~~aspects~~examples of the foregoing peptides based on a parent sequence, the amino acid modifications occur at a position other than position 1.

In a second aspect, the present invention refers to a peptide comprising (a) the sequence of

$X_1X_2X_3GTFTSDX_{10}SKYLX_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}FVQWLX_{27}X_{28}X_{29}X_{30}PSSGX_{35}PPPS$

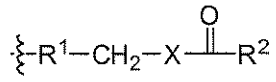
(SEQ ID NO: 140)

wherein:

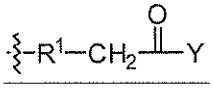
X_1 is selected from the group consisting of: Acetyl L-His, Acetyl L-Tyr, Acetyl L-Thio Ala, Acetyl L-Phe, Acetyl D-His, Acetyl D-Tyr, Acetyl D-Thio Ala, Acetyl D-Phe, Acetyl beta-2-L-His, Acetyl beta-2-L-Tyr, Acetyl beta-2-L-Thio Ala, Acetyl beta-2-L-Phe, Acetyl beta-2-D-His, Acetyl beta-2-D-Tyr, Acetyl beta-2-D-Thio Ala, Acetyl beta-2-D-Phe, Desamino L-His, Desamino L-Tyr, Desamino L-ThioAla, Desamino L-Phe, Desamino D-His, Desamino D-Tyr, Desamino D-ThioAla, Desamino D-Phe, Desamino beta-2-L-His, Desamino beta-2-L-Tyr, Desamino beta-2-L-ThioAla, Desamino beta-2-L-Phe, Desamino beta-2-D-His, Desamino beta-2-D-Tyr, Desamino beta-2-D-ThioAla, Desamino beta-2-D-Phe, D-His, D-Tyr, D-ThioAla, D-Phe, Beta-2-D-His, Beta-2-D-Ala, Beta-2-D-ThioAla, Beta-2-D-Phe, Beta-2-L-His, Beta-2-L-Tyr, Beta-2-L-ThioAla, Beta-2-L-Phe, and L-thioAla.;

X_2 is Ala, Gly, Pro, sarcosine, Ser and Val,

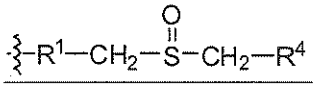
X_3 is Gln, an amino acid comprising a side chain of structure I, II, or III, a hydrophobic amino acid, an acidic amino acid or a basic amino acid, or is an amino acid selected from the group consisting of: Glu, Ala, Leu, Ile, Nle, Val, NorVal, homoserine, Met, methionine sulfoxide, methionine sulfone, acetyl-Orn, acetyl-diaminobutanoic acid, and acetyl-Lys; wherein Structure I, II, and III is:



Structure I



Structure II



Structure III

wherein R^1 is C_{0-3} alkyl or C_{0-3} heteroalkyl; R^2 is NHR^4 or C_{1-3} alkyl; R^3 is C_{1-3} alkyl; R^4 is H or C_{1-3} alkyl; X is NH, O, or S; and Y is NHR^4, SR^3, or OR^3;

X_{10} is Tyr or an amino acid covalently attached to a C12 to C18 acyl or alkyl group;

X_{15} is an acidic amino acid, optionally, Glu or Asp;

X_{16} is any amino acid, optionally, any amino acid other than Gly, Pro, and Ser; e.g., Glu, Ala, alpha, alpha-disubstituted amino acid (e.g., AIB), His, Lys

X_{17} is Arg, His, Gln;

X_{18} is Arg or Ala;

X_{19} is Ala or alpha, alpha disubstituted amino acid;

X_{20} is a selected from group consisting of: alpha, alpha-disubstituted amino acid (e.g., AIB) or Gln or His, Lys, or Ala;

X_{21} is an acidic amino acid, optionally, Asp or Glu;

X_{27} is Leu, Ala, Nle, or Met;

X_{28} is Ala or an acidic amino acid (optionally, Asp or Glu);

X_{29} is aliphatic, e.g., Ala or Leu or Gly or AIB or Val;

X_{30} is small aliphatic amino acid, e.g., Ala or Gly

X_{35} is Ala or a basic amino acid (optionally, Arg or Lys);

wherein, when X_{28} is an acidic amino acid, X35 is a basic amino acid;

wherein, optionally, when X_{28} is Asp, then X_{29} is not Gly

wherein, when X10 is Tyr, the peptide comprises at position 40 an amino acid covalently attached to a C12 to C18 acyl or alkyl group, and, wherein, optionally, the peptide comprises Gly at position 41,

wherein the C-terminal amino acid of the peptide is amidated; or

(b) the sequence SEQ ID NO: 140 with up to 3 amino acid modifications relative to SEQ ID NO: 140, wherein the peptide exhibits agonist activity at the human GIP receptor and the human GLP-1 receptor and/or the human glucagon receptor, optionally, wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

[0012] The ~~invention~~disclosure accordingly provides a peptide comprising, consisting essentially of, or consisting of the sequence of SEQ ID NO: 11. Also provided is a peptide comprising, consisting essentially of, or consisting of the sequence of SEQ ID NO: 12. The ~~invention~~disclosure further provides a peptide comprising, consisting essentially of, or consisting of the sequence of SEQ ID NO: 13. Furthermore provided is a peptide comprising, consisting essentially of, or consisting of the sequence of SEQ ID NO: 15. Also provided is a peptide comprising, consisting essentially of, or consisting of the sequence of SEQ ID NO: 16. The ~~invention~~disclosure additionally provides a peptide comprising, consisting essentially of, or consisting of the sequence of SEQ ID NO: 22. The invention moreover provides a peptide comprising:

a. a sequence of SEQ ID NO: 11 with up to 3 amino acid modifications relative to SEQ ID NO: 11;

b. a sequence of SEQ ID NO: 12 with up to 3 amino acid modifications relative to SEQ ID NO: 12,

c. a sequence of SEQ ID NO: 13 with up to 3 amino acid modifications relative to SEQ ID NO: 13,

d. a sequence of SEQ ID NO: 15 with up to 3 amino acid modifications relative to SEQ ID NO: 15,

e. a sequence of SEQ ID NO: 16 with up to 3 amino acid modifications relative to SEQ ID NO: 16,

f. a sequence of SEQ ID NO: 15 with up to 3 amino acid modifications relative to SEQ ID NO: 22,

wherein the peptide exhibits agonist activity at the human GIP receptor and the human GLP-1 receptor and the peptide has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor. In exemplary ~~aspects~~examples of the peptides comprising any one of the sequences of SEQ ID NOs: 11-13, 15, 16, and 22 with up to 3 (e.g., 1, 2, 3) amino acid modifications, the amino acid modifications occur at a position other than position 1.

[0013] The ~~invention~~disclosure provides a peptide comprising

(a) the sequence of

$X_1X_2X_3GTFTSDX_{10}SKYLX_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}FVQWLX_{27}X_{28}X_{29}X_{30}PSSGX_{35}PPPS$

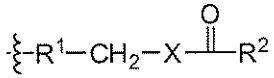
(SEQ ID NO: 35)

wherein:

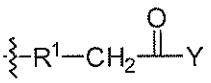
X_1 is selected from the group consisting of: Acetyl L-His, Acetyl L-Tyr, Acetyl L-Thio Ala, Acetyl D-His, Acetyl D-Tyr, Acetyl D-Thio Ala, Acetyl beta-2-L-His, Acetyl beta-2-L-Tyr, Acetyl beta-2-L-Thio Ala, Acetyl beta-2-D-His, Acetyl beta-2-D-Tyr, Acetyl beta-2-D-Thio Ala, Desamino L-His, Desamino L-Tyr, Desamino L-ThioAla, Desamino D-His, Desamino D-Tyr, Desamino D-ThioAla, Desamino beta-2-L-His, Desamino beta-2-L-Tyr, Desamino beta-2-L-ThioAla, Desamino beta-2-D-His, Desamino beta-2-D-Tyr, Desamino beta-2-D-ThioAla, D-His, D-Tyr, D-ThioAla, Beta-2-D-His, Beta-2-D-Ala, Beta-2-D-ThioAla, Beta-2-L-His, Beta-2-L-Tyr, Beta-2-L-ThioAla, and L-thioAla;

X_2 is Ala, Gly, Pro, sarcosine, Ser and Val,

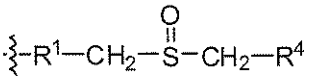
[0014] X_3 is Gln, an amino acid comprising a side chain of structure I, II, or III, a hydrophobic amino acid, an acidic amino acid or a basic amino acid, or is an amino acid selected from the group consisting of: Glu, Ala, Leu, Ile, Nle, Val, NorVal, homoserine, Met, methionine sulfoxide, methionine sulfone, acetyl-Orn, acetyl-diaminobutanoic acid, and acetyl-Lys; wherein structure I, II, and III are:



Structure I



Structure II



Structure III

glucagon receptor, optionally, wherein the glucagon analog has less than 100-fold selectivity for the human GLP-1 receptor versus the GIP receptor.

[0016] In exemplary ~~aspects~~examples of the peptides comprising the sequence of SEQ ID NO: 35 or SEQ ID NO: 140 with up to 3 (e.g., 1, 2, 3) amino acid modifications, the amino acid modifications occur at a position other than position 1.

[0017] Also ~~provided~~disclosed herein are dimers and multimers comprising two or more GIP agonist peptides of the present disclosures. Conjugates comprising a GIP agonist peptide of the present disclosures and a conjugate moiety are additionally provided herein. In some ~~aspects~~examples, the conjugate is a fusion polypeptide comprising the GIP agonist peptide of the present disclosures fused to a heterologous peptide. The present disclosures also provides kits comprising the GIP agonist peptides, dimers, multimers, or conjugates of the present disclosures (or a combination thereof).

[0018] Pharmaceutical compositions comprising any of the GIP agonist peptides, dimers, multimers, or conjugates of the present disclosures (or a combination thereof) and a pharmaceutically acceptable carrier, diluent, or excipient are further provided by the present disclosures. The pharmaceutical compositions are preferably sterile and suitable for parenteral administration. The pharmaceutical compositions are contemplated for use in methods of treating or preventing diabetes or obesity, or medical conditions associated with diabetes or obesity~~.~~. Accordingly, in exemplary embodiments the present disclosure provides a method of reducing weight gain or inducing weight loss, a method of treating or preventing diabetes or obesity, and a method of inducing temporary paralysis of the intestinal tract.

In a further aspect, the present invention thus refers to the use of a pharmaceutical composition of the present invention in the manufacture of a medicament for reducing weight gain or inducing weight loss in a subject in need thereof. In still another aspect, the present invention thus refers to the use of a pharmaceutical composition of the present invention in the manufacture of a medicament for treating diabetes.

Further applications of the peptide analogs and pharmaceutical compositions comprising the same are provided in the following description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figure 1 represents a graph of the total change in body weight (%) as measured on Day 7 of the study.

[0020] Figure 2 represents a graph of the total food intake (as measured on Day 7) of DIO mice subcutaneously injected with a vehicle control or a peptide as shown.

[0021] Figure 3 represents a graph of the fat mass (as measured on Days 0 and 7) of DIO mice subcutaneously injected with a vehicle control or a peptide as shown.

76. The glucagon analog or peptide of claim 75, wherein the glucagon percentage potency is at least 10%.

77. The glucagon analog or peptide of claim 76, wherein the glucagon percentage potency is at least 50%.

78. The glucagon analog or peptide of claim 77, wherein the glucagon percentage potency is more than or about 100%.

79. The glucagon analog or peptide of any one of the preceding claims, wherein the GIP percentage potency is within 10-fold higher or lower than the glucagon percentage potency.

80. A dimer or multimer comprising two or more glucagon analogs or peptides of any one of the preceding claims.

81. A conjugate comprising the glucagon analog or peptide of any one of the preceding claims and a conjugate moiety.

82. The conjugate of claim 81, wherein the glucagon analog is fused to a heterologous peptide analog.

83. A pharmaceutical composition comprising a glucagon analog or peptide of any one of the preceding claims, a dimer or multimer of claim 80, a conjugate of claim 81 or 82, or a combination thereof, and a pharmaceutically acceptable carrier, diluent, or excipient.

84. Use of a pharmaceutical composition of claim 83 in the manufacture of a medicament ~~A method of~~ for reducing weight gain or inducing weight loss in a subject in need thereof~~, comprising administering to a patient in need thereof a pharmaceutical composition of claim 83 in an amount effective to reduce weight gain or induce weight loss~~.

~~85. The method of claim 84, wherein the amount effective to treat obesity in a subject in need thereof.~~

~~86~~85. Use of a pharmaceutical composition of claim 83 in the manufacture of a medicament for ~~A method of~~ treating diabetes~~, comprising administering to a patient in need thereof a pharmaceutical composition of claim 83 in an amount effective to lower blood glucose levels.~~

~~87. A peptide, optionally a glucagon analog, as described herein.~~