

CLAIMS

~~What is claimed is:~~

1. An isolated polypeptide, comprising the amino acid sequence of SEQ ID NO: 90:
X₀PX₂PX₄X₅PX₇X₈X₉X₁₀SPX₁₃X₁₄X₁₅X₁₆RX₁₈X₁₉X₂₀DX₂₂X₂₃HX₂₅X₂₆X₂₇WLTRX₃₂RX₃₄-
(OH/NH₂) (SEQ ID NO: 90), or a pharmaceutically acceptable salt thereof, wherein:

X₀ is absent or K;

X₂ is K;

X₄ is E or K;

X₅ is A or K;

X₇ is G or K

X₈ is E, K, or k;

X₉ is D or K;

X₁₀ is A or K;

X₁₃ is E or K;

X₁₄ is E or K;

X₁₅ is L or W;

X₁₆ is D, E, K, N, Q, S, T, α -methylserine, or homoserine;

X₁₈ is K or Y;

X₁₉ is K or Y;

X₂₀ is A, D, E, K, k, or Dap,

X₂₂ is A, D, K, or L;

X₂₃ is K or R;

X₂₅ is K or Y;

X₂₆ is E, K, or L;

X₂₇ is K or N;

X₃₂ is K or Q;

X₃₄ is F, y, 3-pyridinylalanine, 4-pyridinylalanine, 4-carboxyphenylalanine, 4-fluorophenylalanine, 4-methylphenylalanine, N-methylphenylalanine, homophenylalanine, β -homotyrosine, homotyrosine, or N-methyltyrosine;

wherein when X₀, X₂, X₄, X₅, X₇, X₈, X₁₃, X₂₀, X₂₃, X₂₅, X₂₇, or X₃₂ are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer, and

wherein when X₈ or X₂₀ are k, the D-lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,

provided that the polypeptide comprises at least one residue covalently bound to lipophilic substituent, optionally via a spacer; and

wherein the polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions X₉ and X₁₃ or at positions X₁₆ and X₂₀ or at positions X₂₂ and X₂₆.

~~2. The isolated polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO: 91 PKPEX₅PX₇X₈DASPX₁₃EX₁₅X₁₆RYYX₂₀DX₂₂RHYLNWLTRQRX₃₄(OH/NH₂)(SEQ ID NO: 91), or a pharmaceutically acceptable salt thereof, wherein:~~

~~—— X₅ is A or K;~~

~~—— X₇ is G or K;~~

~~—— X₈ is E, K, or k;~~

~~—— X₁₃ is E or K;~~

~~—— X₁₅ is L or W;~~

~~—— X₁₆ is D, E, K, N, S, α-methylserine, or homoserine;~~

~~—— X₂₀ is A, D, E, K, or k;~~

~~—— X₂₂ is A or L;~~

~~—— X₃₄ is F, 3-pyridinylalanine, 4-pyridinylalanine, 4-carboxyphenylalanine, 4-fluorophenylalanine, 4-methylphenylalanine, N-methylphenylalanine, homophenylalanine, β-homotyrosine, homotyrosine, or N-methyltyrosine;~~

~~—— wherein when X₅, X₇, X₈, X₁₃, or X₂₀ are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer, and~~

~~—— wherein when X₈ or X₂₀ are k, the D-lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,~~

~~provided that the polypeptide comprises at least one residue covalently bound to lipophilic substituent, optionally via a spacer; and~~

~~—— wherein the polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side~~

chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions ~~X₁₆~~ and ~~X₂₀~~.

3. — The isolated polypeptide of claim 1 or claim 2, comprising the amino acid sequence of SEQ ID NO: 92:

~~PKPEX₅PX₇X₈DASPX₁₃EX₁₅X₁₆RYYX₂₀DX₂₂RHYLNWLTRQRX₃₄(OH/NH₂)(SEQ ID NO: 92);~~

or a pharmaceutically acceptable salt thereof, wherein:

—— ~~X₅~~ is A or K;

—— ~~X₇~~ is G or K;

—— ~~X₈~~ is E, K, or k;

—— ~~X₁₃~~ is E or K;

—— ~~X₁₅~~ is L or W;

—— ~~X₁₆~~ is D, E, K, S, α methylserine, or homoserine;

—— ~~X₂₀~~ is A, D, E, K, or k,

—— ~~X₂₂~~ is A or L;

—— ~~X₃₄~~ is F, 3 pyridinylalanine, 4 pyridinylalanine, 4 carboxyphenylalanine, 4 fluorophenylalanine, 4 methylphenylalanine, N methylphenylalanine, homophenylalanine, β homotyrosine, homotyrosine, or N methyltyrosine;

—— wherein when ~~X₅~~, ~~X₇~~, ~~X₈~~, ~~X₁₃~~, or ~~X₂₀~~ are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer, and

—— wherein when ~~X₈~~ or ~~X₂₀~~ are k, the D lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,

provided that the polypeptide comprises at least one residue covalently bound to lipophilic substituent, optionally via a spacer; and

—— wherein the polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions ~~X₁₆~~ and ~~X₂₀~~.

4. — The isolated polypeptide of claim 1 or claim 2, comprising the amino acid sequence of SEQ ID NO: 93:

~~PKPEX₅PX₇X₈DASPX₁₃EX₁₅X₁₆RYYX₂₀DX₂₂RHYLNWLTRQRX₃₄(OH/NH₂)(SEQ ID NO: 93);~~

or a pharmaceutically acceptable salt thereof, wherein:

~~———— X₅ is A or K;~~
~~———— X₇ is G or K;~~
~~———— X₈ is E, K, or k;~~
~~———— X₁₃ is E or K;~~
~~———— X₁₅ is L or W;~~
~~———— X₁₆ is D, E, K, N, S, α -methylserine, or homoserine;~~
~~———— X₂₀ is D, E, K, or k;~~
~~———— X₂₂ is A or L;~~
~~———— X₃₄ is F, 3-pyridinylalanine, 4-pyridinylalanine, 4-carboxyphenylalanine, 4-fluorophenylalanine, 4-methylphenylalanine, N-methylphenylalanine, homophenylalanine, β -homotyrosine, homotyrosine, or N-methyltyrosine;~~
~~———— wherein when X₅, X₇, X₈, X₁₃, or X₂₀ are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer, and~~
~~———— wherein when X₈ or X₂₀ are k, the D-lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,~~
~~provided that the polypeptide comprises at least one residue covalently bound to lipophilic substituent, optionally via a spacer; and~~
~~———— wherein the polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions X₁₆ and X₂₀.~~

5. ~~———— The isolated polypeptide of claim 1 or claim 2, comprising the amino acid sequence of SEQ ID NO: 94:~~

~~PKPEX₅PGX₈DASPX₁₃EWX₁₆RYYX₂₀DX₂₂RHYLNWLTRQX₃₄(OH/NH₂)(SEQ ID NO: 94), or~~
~~a pharmaceutically acceptable salt thereof, wherein:~~

~~———— X₅ is A or K;~~
~~———— X₈ is E, K, or k;~~
~~———— X₁₃ is E or K;~~
~~———— X₁₆ is D, E, K, or N;~~
~~———— X₂₀ is A, D, E, K, or k;~~
~~———— X₂₂ is A or L;~~
~~———— X₃₄ is F or N-methyltyrosine;~~

~~wherein when X₅, X₈, X₁₃, or X₂₀ are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer, and~~

~~wherein when X₈ or X₂₀ are k, the D-lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,~~

~~provided that the polypeptide comprises at least one residue covalently bound to lipophilic substituent, optionally via a spacer; and~~

~~wherein the polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions X₁₆ and X₂₀.~~

62. The isolated polypeptide of ~~any one of claims 1, 2, or 5~~, comprising the amino acid sequence of SEQ ID NO: 95:

PKPEX₅PGK₈DASPX₁₃EWX₁₆RYYX₂₀DLRHYLNWLTRQRF-(OH/NH₂) (SEQ ID NO: 95), or a pharmaceutically acceptable salt thereof, wherein:

X₅ is A or K;

X₁₃ is E or K;

X₁₆ is D, E, K, or N;

X₂₀ is A, D, E, K, or k; and

wherein when X₅, X₁₃, or X₂₀ are K, the lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,

wherein K₈ is optionally covalently bound to a lipophilic substituent, optionally via a spacer, and

wherein when X₂₀ is k, the D-lysine residue is optionally covalently bound to a lipophilic substituent, optionally via a spacer,

provided that the polypeptide comprises at least one residue covalently bound to lipophilic substituent, optionally via a spacer; and

wherein the polypeptide optionally further comprises a lactam bridge formed via an amide bond between the side chains of a lysine and an aspartic acid or between the side chains of a lysine and a glutamic acid, wherein the residues that form the lactam bridge are located at positions X₁₆ and X₂₀.

73. The isolated polypeptide of any one of claims 1-42, wherein when X₁₅ is L, X₂₂ is A.

~~84.~~ The isolated polypeptide of any one of claims ~~1-3, 2, or 5-7~~, wherein when X₁₆ is N, X₂₀ is not A.

~~95.~~ The isolated polypeptide of any one of claims ~~1-5, 7, or 84~~, wherein when X₈ is E, X₅ is K and X₂₀ is K, and the lysine residue at either of X₅ or X₂₀ is covalently bound to a lipophilic substituent, optionally via a spacer.

~~106.~~ The isolated polypeptide of any one of claims ~~1-5 or 7-9~~, wherein X₃₄ is F.

~~117.~~ The isolated polypeptide of any one of claims ~~1-5, 7, 8, or 106~~, wherein X₈ is K covalently bound to a lipophilic substituent, optionally via a spacer.

~~12.~~ The isolated polypeptide of claim ~~6 or claim 11~~, wherein the lysine residue at the 8th position of the polypeptide sequence is covalently bound to a lipophilic substituent, optionally via a spacer.

~~13.~~ The isolated polypeptide of any one of claims ~~1-7 or 10-12~~, wherein X₁₆ is K, X₂₀ is D or E, and the polypeptide comprises a lactam bridge formed via an amide bond between the side chains of the residues located at positions X₁₆ and X₂₀.

~~14.~~ The isolated polypeptide of any one of claims ~~1-7 or 10-12~~, wherein X₁₆ is D or E, X₂₀ is K, and the polypeptide comprises a lactam bridge formed via an amide bond between the side chains of the residues located at positions X₁₆ and X₂₀.

~~15.~~ The isolated polypeptide of any one of claims ~~1-14~~, wherein the lipophilic substituent forms an amide bond between an amino group of the isolated polypeptide and the CO group of the lipophilic substituent.

~~16.~~ The isolated polypeptide of claim ~~15~~, wherein the lipophilic substituent is of Formula I:



Formula I

— wherein

— Z is CH₃ or CO₂H; and

— m is from 4 to 24.

17. — The isolated polypeptide of claim 16, wherein Z is CO_2H .

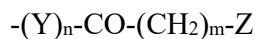
18. — The isolated polypeptide of claim 16 or claim 17, wherein m is from 14 to 20.

19. — The isolated polypeptide of any one of claims 16-18, wherein m is 16, 17, or 18.

20. — The isolated polypeptide of any of claims 1-14, wherein the lipophilic substituent is covalently bound to the isolated polypeptide via a spacer.

21. — The isolated polypeptide of claim 20, wherein the lipophilic substituent, $\text{CO}-(\text{CH}_2)_m\text{Z}$, is linked to an amino group of the isolated polypeptide via the spacer, wherein the spacer forms a bridge between the amino group of the isolated polypeptide and the CO group of the lipophilic substituent.

228. The isolated polypeptide of any one of claims 1-7 ~~20 or claim 21~~, wherein the lipophilic substituent is covalently bound to the isolated polypeptide via a spacer, and wherein the lipophilic substituent and spacer are of Formula II:



Formula II

wherein,

Y is selected from the group consisting of γGlu , Asp, Lys and Gly;

Z is $-\text{CH}_3$ or $-\text{CO}_2\text{H}$;

m is from 4 to 24; and

n is from 1 to 10.

23. — The isolated polypeptide of claim 22, wherein Z is CO_2H .

24. — The isolated polypeptide of claim 22 or claim 23, wherein m is from 14 to 20.

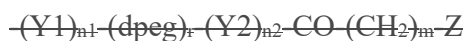
25. — The isolated polypeptide of any one of claims 22-24, wherein m is 16, 17, or 18.

269. The isolated polypeptide of any one of claims 228-25, wherein Y is γGlu , and n is 2.

27. — The isolated polypeptide of any one of claim 22-26, wherein n is from 1 to 5.

28. — The isolated polypeptide of any one of claims 22-27, wherein n is 2.

29. — The isolated polypeptide of claim 20 or claim 21, wherein the lipophilic substituent and spacer are of Formula IV:



Formula IV

wherein

Z is $-CH_3$ or $-CO_2H$;

m is from 4 to 24;

Y1 is selected from the group consisting of γ Glu, Asp, and Gly;

Y2 is selected from the group consisting of γ Glu, Asp, and Gly;

dpeg is $[-CO(CH_2)O(CH_2)_2O(CH_2)NH]-$;

r is from 1 to 8;

n1 is from 0 to 10; and

n2 is from 0 to 10.

30. — The isolated polypeptide of claim 29, wherein Z is $-CO_2H$.

31. — The isolated polypeptide of claim 29 or claim 30, wherein m is from 14 to 20.

32. — The isolated polypeptide of any one of claims 29-31, wherein m is 16, 17, or 18

33. — The isolated polypeptide of any one of claims 29-32, wherein Y1 is γ Glu.

34. — The isolated polypeptide of any one of claims 29-33, wherein n1 is from 0 to 3.

35. — The isolated polypeptide of any one of claims 29-34, wherein Y2 is γ Glu.

36. — The isolated polypeptide of any one of claims 29-35, wherein n2 is from 0 to 3.

37. — The isolated polypeptide of any one of claims 29-36, wherein r is from 1 to 3.

~~38. The isolated polypeptide of any one of claims 1-37, wherein the carboxy terminal amino acid, X₃₄, is X₃₄-(NH₂).~~

~~39~~10. An isolated polypeptide comprising the amino acid sequence of any of SEQ ID NOs: 1 to 78, or a pharmaceutically acceptable salt thereof.

~~40. The isolated polypeptide of claim 39 comprising an amino acid sequence selected from the group consisting of those represented by SEQ ID NOs: 13, 24, 42, and 43.~~

~~41~~11. The isolated polypeptide of claim ~~39~~10 comprising the amino acid sequence of SEQ ID NO: 13, or a pharmaceutically acceptable salt thereof.

~~42~~12. The isolated polypeptide of claim ~~39~~10 comprising the amino acid sequence of SEQ ID NO: 24, or a pharmaceutically acceptable salt thereof.

~~43~~13. The isolated polypeptide of claim ~~39~~10 comprising the amino acid sequence of SEQ ID NO: 42, or a pharmaceutically acceptable salt thereof.

~~44~~14. The isolated polypeptide of claim ~~39~~10 comprising the amino acid sequence of SEQ ID NO: 43, or a pharmaceutically acceptable salt thereof.

~~45~~15. A pharmaceutical composition comprising the isolated polypeptide of any of claims 1 to ~~44~~14, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable adjuvant, carrier, or vehicle.

~~46~~16. A pharmaceutical combination comprising the isolated polypeptide of any of claims 1 to ~~44~~14, or a pharmaceutically acceptable salt thereof, and a GLP-1 receptor agonist.

~~47~~17. An osmotic delivery device, comprising the isolated polypeptide of any of claims 1 to ~~44~~14; ~~the pharmaceutical composition of claim 45, or the pharmaceutical combination of claim 46.~~

~~48. The osmotic delivery device of claim 47, comprising~~

an impermeable reservoir comprising interior and exterior surfaces and first and second open ends;

—— a semi permeable membrane in sealing relationship with the first open end of the reservoir;

—— an osmotic engine within the reservoir and adjacent the semi permeable membrane;

—— a piston adjacent the osmotic engine, wherein the piston forms a movable seal with the interior surface of the reservoir, the piston divides the reservoir into a first chamber and a second chamber, the first chamber comprising the osmotic engine;

—— a suspension formulation, wherein the second chamber comprises the suspension formulation and the suspension formulation is flowable and comprises the isolated polypeptide; and

—— a diffusion moderator inserted in the second open end of the reservoir, the diffusion moderator adjacent the suspension formulation.

4918. ~~A method of treating obesity in a human subject, providing weight loss to the human subject, or suppressing appetite in the human subject, comprising administering to the subject~~ Use of a pharmaceutical composition comprising the isolated polypeptide of any one of claims 1 to 4414, the pharmaceutical composition of claim 4515, the pharmaceutical combination of claim 4616, or the osmotic delivery device of claim 47 or 4817 in the preparation of a medicament for treating obesity in a human subject, providing weight loss to the human subject, or suppressing appetite in the human subject.

5019. ~~A method of treating diabetes in a human subject, comprising administering to the subject~~ Use of a pharmaceutical composition comprising the isolated polypeptide of any one of claims claims 1 to 4414, the pharmaceutical composition of claim 4515, the pharmaceutical combination of claim 4616, or the osmotic delivery device of claim 47 or 4817 in the preparation of a medicament for treating diabetes in a human subject.

51. ~~The method of claim 50, wherein the diabetes is type 1 diabetes.~~

52. ~~The method of claim 50 wherein the diabetes is type 2 diabetes.~~

5320. A method of treating nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH) in a human subject, comprising administering to the subject Use of a pharmaceutical composition comprising the isolated polypeptide of any one of claims ~~claims~~ 1 to 4414, the pharmaceutical composition of claim 4515, the pharmaceutical combination of claim 4616, or the osmotic delivery device of claim 47 or 4817 in the preparation of a medicament for treating nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH) in a human subject.

21. A pharmaceutical composition comprising the isolated polypeptide of any of claims 1-14, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable adjuvant, carrier, or vehicle, wherein the pharmaceutical composition is formulated for injection.

22. A pharmaceutical composition comprising the isolated polypeptide of any of claims 1-14, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable adjuvant, carrier, or vehicle, wherein the pharmaceutical composition is formulated for oral administration.

23. The use of any one of claims 18-20, wherein the pharmaceutical composition is to be administered to the subject via injection, via oral administration, or via an osmotic delivery device.