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(54) Title: EXTENDED GLUCAGON-LIKE PEPTIDE-1 ANALOGS

(57) Abstract: The invention encompasses GLP-1 peptides with modifications at various positions coupled with an extended C-terminus that provides increased stability.

EXTENDED GLUCAGON-LIKE PEPTIDE-1 ANALOGS

A large body of pre-clinical and clinical research data suggests that glucagon-like pepide-1 (GLP-1) shows great promise as a treatment for non-insulin dependent diabetes mellitus (NIDDM) especially when oral agents begin to fail. GLP-1 induces numerous biological effects such as stimulating insulin secretion, inhibiting glucagon secretion, inhibiting gastric emptying, enhancing glucose utilization, and inducing weight loss. Further, pre-clinical studies suggest that GLP-1 may also act to prevent the pancreatic β cell deterioration that occurs as the disease progresses. Perhaps the most salient characteristic of GLP-1 is its ability to stimulate insulin secretion without the associated risk of hypoglycemia that is seen when using insulin therapy or some types of oral therapies that act by increasing insulin expression.

As NIDDM progresses, it becomes extremely important to achieve near normal glycemic control and thereby minimize the complications associated with prolonged hyperglycemia. GLP-1 would appear to be the drug of choice. However, the usefulness of therapy involving GLP-1 peptides has been limited by the fact that GLP-1(1-37) is poorly active, and the two naturally occurring truncated peptides, GLP-1(7-37)OH and GLP-1(7-36)NH_2, are rapidly cleared *in vivo* and have extremely short *in vivo* half-lives.

Further, GLP-1 peptides currently in development cannot be given orally and, like insulin, must be injected. Thus, despite the clear medical advantages associated with therapy involving GLP-1, the short half-life which results in a drug that must be injected one or more times a day has impeded commercial development efforts.

It is known that endogenously produced dipeptidyl-peptidase IV (DPP-IV) inactivates circulating GLP-1 peptides by removing the N-terminal histidine and alanine residues and is a major reason for the short *in vivo* half-life. Thus, recent efforts have focused on the development of GLP-1 peptides that are resistant to DPP-IV degradation. Some of these resistant peptides have modifications at the N-terminus (See U.S. Patent No. 5,705,483), and some are derivatized GLP-1 peptides wherein large acyl groups that prevent DPP-IV from accessing the N-terminus of the peptide are attached to various amino acids (See WO 98/08871).

The present invention, however, provides a different approach to the development of biologically active GLP-1 peptides that persist in the serum for extended periods. The GLP-1 peptides of the present invention are analogs of GLP-1(7-37) wherein various amino acids are added to the C-terminus of the analog. These extended GLP-1 peptides not only have serum half-lives that are much longer than the native molecules but are particularly suited for oral and pulmonary administration due to their resistance to various proteolytic enzymes found in the stomach, intestine, and lungs. Further, many of these extended GLP-1 peptides are more potent than the native molecules. This increased potency coupled with resistance to various proteases facilitates the use of delivery technology associated with limited bioavailability. Thus, the present invention makes possible non-injectable therapy which involves delivering cost-effective amounts of biologically active GLP-1 peptides such that therapeutic serum levels are achieved.

It has now been found that a number of GLP-1 peptides with modifications at various positions coupled with an extended C-terminus show increased stability compared to some DPP-IV resistant GLP-1 molecules such as Val^{8}-GLP-1(7-

37)OH. Many of these extended GLP-1 peptides are more potent as well.

One embodiment of the present invention is a GLP-1 peptide comprising the amino acid sequence of formula 1 (SEQ ID NO:1)

Xaa_7-Xaa_8-Glu-Gly-Thr-Xaa_{12}-Thr-Ser-Asp-Xaa_{16}-Ser-Xaa_{18}-Xaa_{19}-Xaa_{20}-Glu-Xaa_{22}-Gln-Ala-Xaa_{25}-Lys-Xaa_{27}-Phe-Ile-Xaa_{30}-Trp-Leu-Xaa_{33}-Xaa_{34}-Gly-Xaa_{36}-Xaa_{37}-Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}-Xaa_{47}-Xaa_{48}-Xaa_{49}-Xaa_{50}

Formula 1 (SEQ ID NO: 1)

wherein:

Xaa_7 is: L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, α-fluoromethyl-histidine, or α-methyl-histidine;

Xaa_8 is: Ala, Gly, Val, Leu, Ile, Ser, or Thr;

Xaa_{12} is: Phe, Trp, or Tyr;

Xaa_{16} is: Val, Trp, Ile, Leu, Phe, or Tyr;

Xaa_{18} is: Ser, Trp, Tyr, Phe, Lys, Ile, Leu, Val;

Xaa_{19} is: Tyr, Trp, or Phe;

Xaa_{20} is: Leu, Phe, Tyr, or Trp;

Xaa_{22} is: Gly, Glu, Asp, or Lys;

Xaa_{25} is: Ala, Val, Ile, or Leu;

Xaa_{27} is: Glu, Ile, or Ala;

Xaa_{30} is: Ala or Glu;

Xaa_{33} is: Val or Ile;

Xaa_{34} is: Lys, Asp, Arg, or Glu;

Xaa_{36} is: Gly, Pro, or Arg;

Xaa_{37} is: Gly, Pro, or Ser;

Xaa_{38} is: Ser, Pro, or His;

Xaa_{39} is: Ser, Arg, Thr, Trp, or Lys;

Xaa_{40} is: Ser or Gly;

Xaa_{41} is: Ala, Asp, Arg, Glu, Lys, or Gly;

Xaa_{42} is: Pro, Ala, NH_2, or is absent;

Xaa_{43} is: Pro, Ala, NH_2, or is absent;

Xaa_{44} is: Pro, Ala, Arg, Lys, His, NH_2, or is absent;

Xaa_{45} is: Ser, His, Pro, Lys, Arg, Gly, NH_2 or is absent;

Xaa_{46} is: His, Ser, Arg, Lys, Pro, Gly, NH_2 or is absent; and

Xaa_{47} is: His, Ser, Arg, Lys, NH_2 or is absent;

Xaa_{48} is: Gly, His, NH_2 or is absent;

Xaa_{49} is: Pro, His, NH_2 or is absent; and

Xaa_{50} is: Ser, His, Ser-NH_2, His-NH_2 or is absent;

provided that if Xaa_{42}, Xaa_{43}, Xaa_{44}, Xaa_{45}, Xaa_{46}, Xaa_{47}, Xaa_{48}, or Xaa_{49} is absent each amino acid downstream is absent and further provided that the if Xaa_{36} is Arg and Xaa_{37} is Gly or Ser, the GLP-1 peptide does not have the following C-terminal amino acid extension beginning at Xaa_{38}: Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2.

Another embodiment of the present invention is a GLP-1 peptide comprising the amino acid sequence of formula 2 (SEQ ID NO:2)

Xaa_7-Xaa_8-Glu-Gly-Thr-Xaa_{12}-Thr-Ser-Asp-Xaa_{16}-Ser-Xaa_{18}-Xaa_{19}-Xaa_{20}-Glu-Xaa_{22}-Gln-Ala-Xaa_{25}-Lys-Xaa_{27}-Phe-Ile-Xaa_{30}-Trp-Leu-Xaa_{33}-Xaa_{34}-Gly-Xaa_{36}-Xaa_{37}-Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}-Xaa_{47}

Formula 2 (SEQ ID NO: 2)

wherein:

Xaa_7 is: L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, α-fluoromethyl-histidine, or α-methyl-histidine;

Xaa_8 is: Ala, Gly, Val, Leu, Ile, Ser, or Thr;

Xaa_{12} is: Phe, Trp, or Tyr;

Xaa_{16} is: Val, Trp, Ile, Leu, Phe, or Tyr;

Xaa_{18} is: Ser, Trp, Tyr, Phe, Lys, Ile, Leu, or Val;

Xaa_{19} is: Tyr, Trp, or Phe;

Xaa_{20} is: Leu, Phe, Tyr, or Trp;
Xaa_{22} is: Gly, Glu, Asp, or Lys;
Xaa_{25} is: Ala, Val, Ile, or Leu;
Xaa_{27} is: Glu, Ile, or Ala;
Xaa_{30} is: Ala or Glu
Xaa_{33} is: Val or Ile;
Xaa_{34} is: Lys, Asp, Arg, or Glu;
Xaa_{36} is: Gly, Pro, or Arg;
Xaa_{37} is: Gly, Pro, or Ser;
Xaa_{38} is: Ser, Pro, or His;
Xaa_{39} is: Ser, Arg, Thr, Trp, or Lys;
Xaa_{40} is: Ser or Gly;
Xaa_{41} is: Ala, Asp, Arg, Glu, Lys, or Gly;
Xaa_{42} is: Pro, Ala, NH_2, or is absent;
Xaa_{43} is: Pro, Ala, NH_2, or is absent;
Xaa_{44} is: Pro, Ala, Arg, Lys, His, NH_2, or is absent;
Xaa_{45} is: Ser, His, Pro, Lys, Arg, NH_2 or is absent;
Xaa_{46} is: His, Ser, Arg, Lys, NH_2 or is absent; and
Xaa_{47} is: His, Ser, Arg, Lys, NH_2 or is absent;
provided that if Xaa_{42}, Xaa_{43}, Xaa_{44}, Xaa_{45}, Xaa_{46}, or Xaa_{47} is absent each amino acid downstream is absent and further provided that if Xaa_{36} is Arg and Xaa_{37} is Gly or Ser, the GLP-1 peptide does not have the following C-terminal amino acid extension beginning at Xaa_{38}: Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2.

Another embodiment of the present invention is an extended GLP-1 peptide comprising the amino acid sequence of formula 3 (SEQ ID NO:3)

Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Xaa_{16}-Ser-Ser-Tyr-Lys-Glu-Xaa_{22}-Gln-Ala-Xaa_{25}-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Xaa_{33}-Xaa_{34}-Gly-Xaa_{36}-Xaa_{37}-Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}-Xaa_{47}

Formula 3 (SEQ ID NO: 3)

wherein:

Xaa_7 is: L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, α-fluoromethyl-histidine, or α-methyl-histidine;

Xaa_8 is: Gly, Val, Leu, Ile, Ser, or Thr;

Xaa_{16} is: Val, Trp, Ile, Leu, Phe, or Tyr;

Xaa_{22} is: Gly, Glu, Asp, or Lys;

Xaa_{25} is: Ala, Val, Ile, or Leu;

Xaa_{33} is: Val or Ile;

Xaa_{34} is: Lys, Asp, Arg, or Glu;

Xaa_{36} is: Gly, Pro, or Arg;

Xaa_{37} is: Gly, Pro, or Ser;

Xaa_{38} is: Ser, Pro, or His;

Xaa_{39} is: Ser, Arg, Thr, Trp, or Lys;

Xaa_{40} is: Ser or Gly;

Xaa_{41} is: Ala, Asp, Arg, Glu, Lys, or Gly;

Xaa_{42} is: Pro or Ala;

Xaa_{43} is: Pro or Ala;

Xaa_{44} is: Pro, Ala, Arg, Lys, His, NH_2, or is absent;

Xaa_{45} is: Ser, His, Pro, Lys, Arg, NH_2 or is absent;

Xaa_{46} is: His, Ser, Arg, Lys, NH_2 or is absent; and

Xaa_{47} is: His, Ser, Arg, Lys, NH_2 or is absent;

provided that if Xaa_{44}, Xaa_{45}, Xaa_{46}, or Xaa_{47} is absent each amino acid downstream is absent and further provided that if Xaa_{36} is Arg and Xaa_{37} is Gly or Ser, the GLP-1 peptide does not have the following C-terminal amino acid extension beginning at Xaa_{38}: Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2.

Another embodiment of the present invention is an extended GLP-1 peptide comprising the amino acid sequence of formula 4 (SEQ ID NO:4)

Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Lys-Glu-Xaa_{22}-Gln-Ala-Xaa_{25}-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Xaa_{33}-Lys-Gly-Gly-Pro-Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}-Xaa_{47}

Formula 4 (SEQ ID NO:4)

wherein:

Xaa_7 is: L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, α-fluoromethyl-histidine, or α-methyl-histidine;

Xaa_8 is: Gly, Val, Leu, Ile, Ser, or Thr;

Xaa_{22} is: Gly, Glu, Asp, or Lys;

Xaa_{25} is: Ala, Val, Ile, or Leu;

Xaa_{33} is: Val or Ile;

Xaa_{38} is: Ser, Pro, or His;

Xaa_{39} is: Ser, Arg, Thr, Trp, or Lys;

Xaa_{40} is: Ser or Gly;

Xaa_{41} is: Ala, Asp, Arg, Glu, Lys, or Gly;

Xaa_{42} is: Pro or Ala;

Xaa_{43} is: Pro or Ala;

Xaa_{44} is: Pro, Ala, Arg, Lys, His, NH_2, or is absent;

Xaa_{45} is: Ser, His, Pro, Lys, Arg, NH_2 or is absent;

Xaa_{46} is: His, Ser, Arg, Lys, NH_2 or is absent; and

Xaa_{47} is: His, Ser, Arg, Lys, His-NH_2, Ser-NH_2, Arg-NH_2, His-NH_2, NH_2, or is absent;

provided that if Xaa_{44}, Xaa_{45}, Xaa_{46}, or Xaa_{47} is absent each amino acid downstream is absent.

Another embodiment of the present invention is an extended GLP-1 peptide comprising an amino acid sequence of formula 5 (SEQ ID NO:60)

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Gly-Pro-Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}-Xaa_{47}-Xaa_{48}-Xaa_{49}-Xaa_{50}

Formula 5 (SEQ ID NO:60)

Wherein:

Xaa_{38} is: Ser, Pro, or His;

Xaa_{39} is: Ser, Arg, Thr, Trp, or Lys;

Xaa_{40} is: Ser or Gly;

Xaa_{41} is: Ala, Asp, Arg, Glu, Lys; or Gly;

Xaa_{42} is: Pro, Ala, NH_2, or is absent;

Xaa_{43} is: Pro, Ala, NH_2, or is absent;

Xaa_{44} is: Pro, Ala, Arg, Lys, His, NH_2, or is absent;

Xaa_{45} is: Ser, His, Pro, Lys, Arg, Gly, NH_2 or is absent;

Xaa_{46} is: His, Ser, Arg, Lys, Pro, Gly, NH_2 or is absent; and

Xaa_{47} is: His, Ser, Arg, Lys, NH_2 or is absent;

Xaa_{48} is: Gly, His, NH_2 or is absent;

Xaa_{49} is: Pro, His, NH_2 or is absent; and

Xaa_{50} is: Ser, His, Ser-NH_2, His-NH_2 or is absent;

wherein said GLP-1 peptide comprises from one to six further substitutions and provided that if Xaa_{42}, Xaa_{43}, Xaa_{44}, Xaa_{45}, Xaa_{46}, Xaa_{47}, Xaa_{48}, or Xaa_{49} is absent each amino acid downstream is absent

Additional embodiments of formula 1, formula 2, formula 3, formula 4, and formula 5 include GLP-1 peptides that have valine or glycine at position 8 and glutamic acid at position 22.

The present invention also encompasses a method of stimulating the GLP-1 receptor in a subject in need of such stimulation, said method comprising the step of administering to the subject an effective amount of the GLP-1 peptides described herein. Subjects in need of GLP-1 receptor stimulation include those with non-insulin dependent diabetes, stress-induced hyperglycemia, and obesity.

The GLP-1 peptides of the present invention have various amino acid changes relative to the native GLP-1 molecules and have additional amino acids added to the C-terminus beginning at position 37.

Native GLP-1(7-37)OH has the amino acid sequence of SEQ ID NO: 5:

^{7}His-Ala-Glu-^{10}Gly-Thr-Phe-Thr-Ser-^{15}Asp-Val-Ser-Ser-Tyr-^{20}Leu-Glu-Gly-Gln-Ala-^{25}Ala-Lys-Glu-Phe-Ile-^{30}Ala-Trp-Leu-Val-Lys-^{35}Gly-Arg-^{37}Gly (SEQ ID NO: 5)

The native molecule is also amidated *in vivo* such that the glycine residue at position 37 is replaced with an amide group. By custom in the art, the amino terminus of GLP-1(7-37)OH has been assigned residue number 7 and the carboxy-terminus, number 37. The other amino acids in the polypeptide are numbered consecutively, as shown in SEQ ID NO: 4. For example, position 12 is phenylalanine and position 22 is glycine. The same numbering system is used for the extended GLP-1 peptides of the present invention.

The GLP-1 peptides encompassed by the present invention are "extended GLP-1 peptides." Extended GLP-1 peptides have various amino acid substitutions relative to the native GLP-1(7-37) or GLP-1(7-36) molecule and have additional amino acids extending from the C-terminus.

The extended GLP-1 peptides of the present invention have one or more changes selected from the following positions relative to GLP-1(7-37): 7, 8, 12, 16, 18, 19, 20, 22, 25, 27, 30, 33, 34, 36, and 37. In addition, these GLP-1 peptides have at least 4 amino acids added after amino acid residue number 37 (Xaa_{38} through Xaa_{41}). Preferably, at least 6 amino acids are added to the C-terminus. Most preferably between 6 and 10 amino acids are added to the C-terminus. Even more preferably, between 7 and 9 amino acids are added to the C-terminus.

The present invention encompasses extended GLP-1 peptides comprising any combination of the amino acids provided in formula 1 (SEQ ID NO:1), formula 2 (SEQ ID NO:2), formula 3 (SEQ ID NO:3), and formula 4 (SEQ ID NO:4) wherein these extended GLP-1 peptides exhibit

"insulinotropic activity." Insulinotropic activity refers to the ability to stimulate insulin secretion in response to elevated glucose levels, thereby causing glucose uptake by cells and decreased plasma glucose levels. Insulinotropic activity can be assessed by methods known in the art, including using *in vivo* experiments and *in vitro* assays that measure GLP-1 receptor binding activity or receptor activation, e.g., assays employing pancreatic islet cells or insulinoma cells, as described in EP 619,322 to Gelfand, *et al.*, and U.S. Patent No. 5,120,712, respectively. Insulinotropic activity is routinely measured in humans by measuring insulin levels or C-peptide levels.

For the purposes of the present invention an *in vitro* GLP-1 receptor signaling assay is used to determine whether a particular extended GLP-1 peptide will exhibit insulinotropic activity *in vivo*. Extended GLP-1 peptides encompassed by the present invention have an *in vitro* potency that is not less than 1/10 the *in vitro* potency of the DPP-IV resistant GLP-1 analog known as Val^8-GLP-1(7-37)OH. More preferably, the extended GLP-1 peptides of the present invention are as potent or more potent than Val^8-GLP-1(7-37)OH.

"*In vitro* potency" as used herein is the measure of the ability of a peptide to activate the GLP-1 receptor in a cell-based assay. *In vitro* potency is expressed as the "EC_{50}" which is the effective concentration of compound that results in 50% activity in a single dose-response experiment. For the purposes of the present invention, *in vitro* potency is determined using a fluorescence assay that employs HEK-293 Aurora CRE-BLAM cells that stably express the human GLP-1 receptor. These HEK-293 cells have stably integrated a DNA vector having a cAMP response element (CRE) driving expression of the β-lactamase (BLAM) gene. The interaction of a GLP-1 agonist with the receptor initiates a

signal that results in activation of the cAMP response element and subsequent expression of β-lactamase. The β-lactamase CCF2/AM substrate that emits fluorescence when it is cleaved by β-lactamase (Aurora Biosciences Corp.) can then be added to cells that have been exposed to a specific amount of GLP-1 agonist to provide a measure of GLP-1 agonist potency. The assay is further described in Zlokarnik et al. (1998) Science 279:84-88 (See also Example 1). The EC_{50} values for the compounds listed in example 1 were determined using the BLAM assay described above by generating a dose response curve using dilutions ranging from 0.00003 nanomolar to 30 nanomolar. Relative *in vitro* potency values are established by running Val^{8}-GLP-1(7-37)OH as a control and assigning the control a reference value of 1.

Preferably, the extended GLP-1 peptides of the present invention have the amino acid sequence of GLP-1(7-37) modified so that one, two, three, four, five, or six amino acids differ from the amino acid in the corresponding position of GLP-1(7-37) and in addition have at least 4, preferably 6, even more preferably between 6 and 10 amino acids added to the C-terminus.

Preferably, the GLP-1 peptides of the present invention comprise extended GLP-1 analogs wherein the backbone for such analogs or fragments contains an amino acid other than alanine at position 8 (position 8 analogs). The backbone may also include L-histidine, D-histidine, or modified forms of histidine such as desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, α-fluoromethyl-histidine, or α-methyl-histidine at position 7. It is preferable that these position 8 analogs contain one or more additional changes at positions 12, 16, 18, 19, 20, 22, 25, 27, 30, 33, 34, 36, and 37 compared to the corresponding

amino acid of native GLP-1(7-37). It is more preferable that these position 8 analogs contain one or more additional changes at positions 16, 18, 22, 25 and 33 compared to the corresponding amino acid of native GLP-1(7-37).

In a preferred embodiment, the amino acid at position 12 of an extended GLP-1 peptide is selected from the group consisting of tryptophan or tyrosine. It is more preferred that in addition to the substitution at position 12, the amino acid at position 8 is substituted with glycine, valine, leucine, isoleucine, serine, threonine, or methionine and more preferably valine or glycine. It is even more preferred that in addition to the substitutions at position 12 and 8, the amino acid at position 22 is substituted with glutamic acid.

In another preferred embodiment, the amino acid at position 16 of an extended GLP-1 peptide is selected from the group consisting of tryptophan, isoleucine, leucine, phenylalanine, or tyrosine. It is preferred that the amino acid at position 16 is tryptophan. It is more preferred that in addition to the substitutions at position 16, the amino acid at position 8 is substituted with glycine, valine, leucine, isoleucine, serine, threonine, or methionine and more preferably valine or glycine. It is even more preferred that in addition to the substitutions at position 16 and 8, the amino acid at position 22 is substituted with glutamic acid. It is also preferred that in addition to the substitutions at positions 16 and 8, the amino acid at position 33 is substituted with isoluecine. It is also preferred that in addition to the substitutions at position 8, 16, and 22, the amino acid at position 36 is substituted with glycine and the amino acid at position 37 is substituted with proline

In another preferred embodiment, the amino acid at position 18 of an extended GLP-1 peptide is selected from

the group consisting of tryptophan, tyrosine, phenylalanine, lysine, leucine, or isoleucine, preferably tryptophan, tyrosine, and isoleucine. It is more preferred that in addition to the substitution at position 18, the amino acid at position 8 is substituted with glycine, valine, leucine, isoleucine, serine, threonine, or methionine and more preferably valine or glycine. It is even more preferred that in addition to the substitutions at position 18 and 8, the amino acid at position 22 is substituted with glutamic acid. It is also preferred that in addition to the substitutions at positions 18 and 8, the amino acid at position 33 is substituted with isoleucine. It is also preferred that in addition to the substitutions at position 8, 18, and 22, the amino acid at position 36 is substituted with glycine and the amino acid at position 37 is substituted with proline.

In another preferred embodiment, the amino acid at position 19 of an extended GLP-1 peptide is selected from the group consisting of tryptophan or phenylalanine, preferably tryptophan. It is more preferred that in addition to the substitution at position 19, the amino acid at position 8 is substituted with glycine, valine, leucine, isoleucine, serine, threonine, or methionine and more preferably valine or glycine. It is even more preferred that in addition to the substitutions at position 19 and 8, the amino acid at position 22 is substituted with glutamic acid. It is also preferred that in addition to the substitutions at position 8, 19, and 22, the amino acid at position 36 is substituted with glycine and the amino acid at position 37 is substituted with proline.

In another preferred embodiment, the amino acid at position 20 of an extended GLP-1 peptide is selected from the group consisting of phenylalanine, tyrosine, or tryptophan, preferably tryptophan. It is more preferred

that in addition to the substitution at position 20, the amino acid at position 8 is substituted with glycine, valine, leucine, isoleucine, serine, threonine, or methionine and more preferably valine or glycine. It is even more preferred that in addition to the substitutions at position 20 and 8, the amino acid at position 22 is substituted with glutamic acid. It is also preferred that in addition to the substitutions at position 8, 20, and 22, the amino acid at position 36 is substituted with glycine and the amino acid at position 37 is substituted with proline.

In another preferred embodiment, the amino acid at position 25 of an extended GLP-1 peptide is selected from the group consisting of valine, isoleucine, and leucine, preferably valine. It is more preferred that in addition to the substitution at position 25, the amino acid at position 8 is substituted with glycine, valine, leucine, isoleucine, serine, threonine, or methionine and more preferably valine or glycine. It is even more preferred that in addition to the substitutions at position 25 and 8, the amino acid at position 22 is substituted with glutamic acid. It is also preferred that in addition to the substitutions at position 8, 22, and 25, the amino acid at position 36 is substituted with glycine and the amino acid at position 37 is substituted with proline.

In another preferred embodiment, the amino acid at position 27 of an extended GLP-1 peptide is selected from the group consisting of isoleucine or alanine. It is more preferred that in addition to the substitution at position 27, the amino acid at position 8 is substituted with glycine, valine, leucine, isoleucine, serine, threonine, or methionine and more preferably valine or glycine. It is even more preferred that in addition to the substitutions at position 27 and 8, the amino acid at position 22 is

substituted with glutamic acid. It is also preferred that in addition to the substitutions at position 8, 22, and 27, the amino acid at position 36 is substituted with glycine and the amino acid at position 37 is substituted with proline.

In another preferred embodiment, the amino acid at position 33 of an extended GLP-1 peptide is isoleucine. It is more preferred that in addition to the substitution at position 33, the amino acid at position 8 is substituted with glycine, valine, leucine, isoleucine, serine, threonine, or methionine and more preferably valine or glycine. It is even more preferred that in addition to the substitutions at position 33 and 8, the amino acid at position 22 is substituted with glutamic acid. It is also preferred that in addition to the substitutions at position 8, 22, and 33 the amino acid at position 36 is substituted with glycine and the amino acid at position 37 is substituted with proline.

In another preferred embodiment, the amino acid at position 34 is aspartic acid. It is more preferred that in addition to the substitution at position 34, the amino acid at position 8 is substituted with glycine, valine, leucine, isoleucine, serine, threonine, or methionine and more preferably valine or glycine. It is even more preferred that in addition to the substitutions at position 34 and 8, the amino acid at position 22 is substituted with glutamic acid. It is also preferred that in addition to the substitutions at position 8, 22, and 34 the amino acid at position 36 is substituted with glycine and the amino acid at position 37 is substituted with proline.

The C-terminal extension portion fused to the GLP-1 analog backbones discussed above is at least 4 amino acids in length, preferably between 6 and 10 amino acids in length. Preferably, the extended GLP-1 peptides of the

present invention have a serine, proline, or histidine at position 38; a serine, arginine, threonine, tryptophan, or lysine at position 39; a serine or glycine at position 40; an alanine, aspartic acid, arginine, glutamic acid, lysine or glycine at position 41; a proline or alanine at position 42; and a proline or alanine at position 43. Additional amino acids that may be added include a proline, serine, alanine, arginine, lysine, or histidine at position 44; a serine, histidine, proline, lysine or arginine at position 45; a histidine, serine, arginine, or lysine at position 46; and a histidine, serine, arginine, or lysine at position 47. Preferably, histidine is the C-terminal amino acid at either position 42, 43, 44, 45, 46, or 47. Additional amino acids that may be added to the C-terminus also include those specified in formula 1 (SEQ ID NO:1).

It is preferred that when Xaa_{34} is aspartic acid, then Xaa_{41} is arginine or lysine. It is also preferred that Xaa_{39} is serine. It is also preferred that when Xaa_{41} is aspartic acid or arginine, then Xaa_{42}, Xaa_{43}, and Xaa_{44} are all proline. The C-terminal amino acid may be in the typical acid form or may be amidated.

A preferred genus of extended GLP-1 peptides comprise the amino acid sequence of formula 4 (SEQ ID NO:4)

Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Lys-Glu-Xaa_{22}-Gln-Ala-Xaa_{25}-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Xaa_{33}-Lys-Gly-Gly-Pro-Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}-Xaa_{47}

Formula 4 (SEQ ID NO:4)

wherein:

Xaa_7 is: L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, α-fluoromethyl-histidine, or α-methyl-histidine;

Xaa_8 is: Gly, Val, Leu, Ile, Ser, or Thr;

Xaa_{22} is: Gly, Glu, Asp, or Lys;
Xaa_{25} is: Ala, Val, Ile, or Leu;
Xaa_{33} is: Val or Ile;
Xaa_{38} is: Ser, Pro, or His;
Xaa_{39} is: Ser, Arg, Thr, Trp, or Lys;
Xaa_{40} is: Ser or Gly;
Xaa_{41} is: Ala, Asp, Arg, Glu, Lys, or Gly;
Xaa_{42} is: Pro or Ala;
Xaa_{43} is: Pro or Ala;
Xaa_{44} is: Pro, Ala, Arg, Lys, His, NH_2, or is absent;
Xaa_{45} is: Ser, His, Pro, Lys, Arg, NH_2 or is absent;
Xaa_{46} is: His, Ser, Arg, Lys, NH_2 or is absent; and
Xaa_{47} is: His, Ser, Arg, Lys, NH_2 or is absent;
provided that if Xaa_{44}, Xaa_{45}, Xaa_{46}, or Xaa_{47} is absent each amino acid downstream is absent.

Preferred extended GLP-1 peptides are peptides of formula 4 (SEQ ID NO:4) wherein Xaa_7 is L-His, Xaa_8 is Gly or Val, Xaa_{22} is Glu, Xaa_{25} is Val, Xaa_{33} is Ile, Xaa_{38} is Ser, Xaa_{39} is Ser, Xaa_{40} is Gly, Xaa_{41} is Ala, Xaa_{42} is Pro, Xaa_{43} is Pro, Xaa_{44} is Pro, Xaa_{45} is Ser and Xaa_{46} and Xaa_{47} are absent and amidated forms of thereof. Preferred extended GLP-1 peptides also include peptides of formula 4 (SEQ ID NO:4) wherein Xaa_7 is L-His, Xaa_8 is Val, Xaa_{22} is Glu, Xaa_{25} is Ala, Xaa_{33} is Ile, Xaa_{38} is Ser, Xaa_{39} is Ser, Xaa_{40} is Gly, Xaa_{41} is Ala, Xaa_{42} is Pro, Xaa_{43} is Pro, Xaa_{44} is Pro, Xaa_{45} is Ser and Xaa_{46} and Xaa_{47} are absent and amidated forms thereof. Other preferred extended GLP-1 peptides include peptides of formula 4 (SEQ ID NO:4) wherein Xaa_7 is L-His, Xaa_8 is Val, Xaa_{22} is Gly, Xaa_{25} is Ala, Xaa_{33} is Ile, Xaa_{38} is Ser, Xaa_{39} is Ser, Xaa_{40} is Gly, Xaa_{41} is Ala, Xaa_{42} is Pro, Xaa_{43} is Pro, Xaa_{44} is Pro, Xaa_{45} is Ser, and Xaa_{46} and Xaa_{47} are absent and amidated forms thereof.

The present invention encompasses the discovery that specific amino acids added to the C-terminus of a GLP-1

peptide provide specific structural features that protect the peptide from degradation by various proteases yet do not negatively impact the biological activity of the peptide. Further, many of the extended peptides disclosed herein are more potent than DPP-IV resistant GLP-1 analogs such as Val^8-GLP-1(7-37)OH.

Example 1 provides *in vitro* potency data for a representative number of extended GLP-1 peptides. The *in vitro* potency of the tested extended GLP-1 peptides ranged from about the same as Val^8-GLP-1(7-37)OH to greater than 7-fold more potent than Val^8-GLP-1(7-37)OH. Further, example 5 illustrates that extended GLP-1 peptides are also more potent *in vivo*.

Example 2 provides a measure of protease insensitivity for a representative number of extended GLP-1 analogs. The relative proteolytic stability was determined by exposing extended GLP-1 peptides and Val^8-GLP-1(7-37)OH to α-chymotrypsin and then plotting the progress of the enzymatic reaction as described in Example 2. The extended GLP-1 peptides tested ranged from as stable as Val^8-GLP-1(7-37)OH to 5-fold more stable than Val^8-GLP-1(7-37)OH.

The extended GLP-1 peptides of the present invention also have an increased half-life *in vivo* as indicated in example 4. The *in vivo* half-life of these extended peptides is generally longer than the half-life of DPP-IV protected GLP-1 analogs such as Val^8-GLP-1(7-37)OH.

The extended GLP-1 peptides of the present invention are suited for oral administration, nasal administration, pulmonary inhalation or parenteral administration. Parenteral administration can include, for example, systemic administration, such as by intramuscular, intravenous, subcutaneous, or intraperitoneal injection. The GLP-1 compounds can be administered to the subject in conjunction with an acceptable pharmaceutical carrier,

diluent or excipient as part of a pharmaceutical composition for treating various diseases and conditions discussed herein. The pharmaceutical composition can be a solution or a suspension. Suitable pharmaceutical carriers may contain inert ingredients which do not interact with the peptide or peptide derivative. Standard pharmaceutical formulation techniques may be employed such as those described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. Suitable pharmaceutical carriers for parenteral administration include, for example, sterile water, physiological saline, bacteriostatic saline (saline containing about 0.9% mg/ml benzyl alcohol), phosphate-buffered saline, Hank's solution, Ringer's-lactate and the like. Some examples of suitable excipients include lactose, dextrose, sucrose, trehalose, sorbitol, and mannitol.

The GLP-1 compounds may be formulated for administration such that blood plasma levels are maintained in the efficacious range for extended time periods. For example, depot formulations wherein a bioadsorbable polymer is used to provide sustained release over time are also suitable for use in the present invention.

The main barrier to effective oral peptide drug delivery is poor bioavailability due to degradation of peptides by acids and enzymes, poor absorption through epithelial membranes, and transition of peptides to an insoluble form after exposure to the acidic pH environment in the digestive tract. This reduced bioavailability necessitates the use of GLP-1 compounds with increased potency, increased stability, or both. Oral delivery systems for peptides such as those encompassed by the present invention are known in the

art. For example, GLP-1 compounds can be encapsulated using microspheres or other carriers and then delivered orally.

The extended GLP-1 peptides described herein can be used to treat subjects with a wide variety of diseases and conditions. The extended GLP-1 peptides encompassed by the present invention exert their biological effects by acting at a receptor referred to as the "GLP-1 receptor" (see Dillon, J.S. et al. (1993), *Endocrinology*, 133: 1907-1910). Subjects with diseases and/or conditions that respond favorably to GLP-1 receptor stimulation or to the administration of extended GLP-1 peptides can therefore be treated. These subjects are said to "be in need of treatment with extended GLP-1 peptides" or "in need of GLP-1 receptor stimulation".

Included are subjects with non-insulin dependent diabetes, insulin dependent diabetes, stress-induced hyperglycemia, stroke (see WO 00/16797 by Efendic), myocardial infarction (see WO 98/08531 by Efendic), catabolic changes after surgery (see U.S. Patent No. 6,006,753 to Efendic), functional dyspepsia and irritable bowel syndrome (see WO 99/64060 by Efendic). Also included are subjects requiring prophylactic treatment with a GLP-1 peptide, e.g., subjects at risk for developing non-insulin dependent diabetes (see WO 00/07617). Additional subjects include those with impaired glucose tolerance or impaired fasting glucose, subjects with a partial pancreatectomy, subjects having one or more parents with non-insulin dependent diabetes, subjects who have had gestational diabetes and subjects who have had acute or chronic pancreatitis and are at risk for developing non-insulin dependent diabetes.

The extended GLP-1 peptides of the present invention are also useful in treating subjects who are overweight.

Particularly suited are those subjects whose body weight is about 25% above normal body weight for the subject's height and body build. Thus, the extended GLP-1 peptides can also be used to treat obesity (see WO 98/19698 by Efendic).

The extended GLP-1 peptides of the present invention can be used to normalize blood glucose levels, prevent pancreatic β-cell deterioration, induce β-cell proliferation, stimulate insulin gene transcription, up-regulate IDX-1/PDX-1 or other growth factors, improve β-cell function, activate dormant β-cells, differentiate cells into β-cells, stimulate β-cell replication, inhibit β-cell apoptosis, regulate body weight, and induce weight loss.

An "effective amount" of an extended GLP-1 peptide is the quantity which results in a desired therapeutic and/or prophylactic effect without causing unacceptable side-effects when administered to a subject in need of GLP-1 receptor stimulation. A "desired therapeutic effect" includes one or more of the following: 1) an amelioration of the symptom(s) associated with the disease or condition; 2) a delay in the onset of symptoms associated with the disease or condition; 3) increased longevity compared with the absence of the treatment; and 4) greater quality of life compared with the absence of the treatment. For example, an "effective amount" of an extended GLP-1 peptide for the treatment of type 2 diabetes is the quantity that would result in greater control of blood glucose concentration than in the absence of treatment, thereby resulting in a delay in the onset of diabetic complications such as retinopathy, neuropathy or kidney disease. An "effective amount" of an extended GLP-1 peptide for the prevention of diabetes is the quantity that would delay, compared with the absence of treatment, the onset of elevated blood glucose levels that require treatment with drugs such as

sulfonylureas, thiazolidinediones, insulin and/or bisguanidines.

A typical dose range for the extended GLP-1 peptides of the present invention will range from about 1 µg to about 100 mg per day. Preferably, the dose range is about 5 µg to about 1 mg per day. Even more preferably the dose is about 10 µg to about 100 µg per day.

A "subject" is a mammal, preferably a human, but can also be an animal, e.g., companion animals (e.g., dogs, cats, and the like), farm animals (e.g., cows, sheep, pigs, horses, and the like) and laboratory animals (e.g., rats, mice, guinea pigs, and the like).

The extended GLP-1 peptides of the present invention can be prepared using recombinant DNA technology or by using standard methods of solid-phase peptide synthesis techniques. Peptide synthesizers are commercially available from, for example, Applied Biosystems in Foster City CA. Reagents for solid phase synthesis are commercially available, for example, from Midwest Biotech (Fishers, IN). Solid phase peptide synthesizers can be used according to manufacturers instructions for blocking interfering groups, protecting the amino acid to be reacted, coupling, decoupling, and capping of unreacted amino acids.

Typically, an α-*N*-carbamoyl protected amino acid and the *N*-terminal amino acid on the growing peptide chain on a resin is coupled at room temperature in an inert solvent such as dimethylformamide, N-methylpyrrolidone or methylene chloride in the presence of coupling agents such as dicyclohexylcarbodiimide and 1-hydroxybenzotriazole and a base such as diisopropylethylamine. The α-*N*-carbamoyl protecting group is removed from the resulting peptide resin using a reagent such as trifluoroacetic acid or piperidine, and the coupling reaction repeated with the next desired *N*-

protected amino acid to be added to the peptide chain. Suitable amine protecting groups are well known in the art and are described, for example, in Green and Wuts, *"Protecting Groups in Organic Synthesis"*, John Wiley and Sons, 1991, the entire teachings of which are incorporated by reference. Examples include t-butyloxycarbonyl (tBoc) and fluorenylmethoxycarbonyl (Fmoc).

After completion of synthesis, peptides are cleaved from the solid-phase support with simultaneous side-chain deprotection using standard hydrogen fluoride or trifluoroacetic acid cleavage protocols. Crude peptides are then further purified using Reversed-Phase Chromatography on Vydac C18 columns employing linear water-acetonitrile gradients with all solvents containing 0.1% trifluoroacetic acid (TFA). To remove acetonitrile, peptides are lyophilized from a solution containing 0.1 % TFA, acetonitrile and water. Purity can be verified by analytical reversed phase chromatography. Identity of peptides can be verified by mass spectrometry. Peptides can be solubilized in aqueous buffers at neutral pH.

EXAMPLES

Example 1

In vitro potency:

HEK-293 Aurora CRE-BLAM cells expressing the human GLP-1 receptor are seeded at 20,000 to 40,000 cells/well/100 µl into a 96 well black clear bottom plate. The day after seeding, the medium is replaced with plasma free medium. On the third day after seeding, 20 µl of plasma free medium containing different concentrations of GLP-1 agonist is added to each well to generate a dose response curve. Generally, fourteen dilutions containing from 3 nanomolar to 30 nanomolar GLP-1 compound were used to generate a dose response curve from which EC_{50} values could be determined.

After 5 hours of incubation with GLP-1 compound, 20 µl of β-lactamase substrate (CCF2-AM - Aurora Biosciences - product code 100012) was added and incubation was continued for 1 hour at which point the fluorescence was determined on a cytofluor. The following GLP-1 peptides were tested and had EC_{50} values ranging from about the same as to approximately 8-fold greater than the activity of Val^{8}-GLP-1(7-37)OH:

HVEGTFTSDVSSYLEEQAAKEFIAWLVKGRG SEQ ID NO:6

HVEGTFTSDVSSYLEEQAAKEFIAWLIDGGPSSGRPPPS-NH2 SEQ ID NO:7

HVEGTFTSDVSSYLEEQAAKEFIAWLVKGRGSSGDPPPS-NH2 SEQ ID NO:8

HVEGTFTSDVSSYLEEQAAKEFIAWLVKGRPSSGDPPPS-NH2 SEQ ID NO:9

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPPS-NH2 SEQ ID NO:10

HVEGTFTSDVSSYLEEQAAKEFIAWLVKGRPSSGAPPPS-NH2 SEQ ID NO:11

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGAPPPS-NH2 SEQ ID NO:12

HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPS-NH2 SEQ ID NO:13

HVEGTFTSDVSSYLEEQAVKEFIAWLVKGGPSSGAPPPS-NH2 SEQ ID NO:14

HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGDPPPS-NH2 SEQ ID NO:15

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGGSSGDPPPS-NH2 SEQ ID NO:16

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGPGSSGDPPPS-NH2 SEQ ID NO:17

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGSPSGDPPPS-NH2 SEQ ID NO:18

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPS-NH2 SEQ ID NO:19

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPPS SEQ ID NO:20

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDAPPS-NH2 SEQ ID NO:21

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPAPS-NH2 SEQ ID NO:22

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPAS-NH2 SEQ ID NO:23

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDAAAS-NH2 SEQ ID NO:24

HVEGTFTSDWSSYLEGQAAKEFIAWLIKGGPSSGAPPPS SEQ ID NO:25

HVEGTFTSDWSSYLEGQAAKEFIAWLIKGGPSSGAPPPH SEQ ID NO:26

HVEGTFTSDVSSYLEGQAAKEFIAWLIKGGPSSGAPPPS SEQ ID NO:27

HVEGTFTSDVSSYLEGQAAKEFIAWLIKGGPSSGDPPPS SEQ ID NO:28

HVEGTFTSDWSSYLEGQAAKEFIAWLIKGGPSSGAPPPSH SEQ ID NO:29

HVEGTFTSDWSSYLEGQAAKEFIAWLIKGGPHSSGAPPPS SEQ ID NO:30

HVEGTFTSDVSSYLEGQAAKEFIAWLVKGRGSSGAPPPS SEQ ID NO:31

HVEGTFTSDVSSYLEGQAAKEFIAWLVKGGPSSGAPPPS SEQ ID NO:32

HVEGTFTSDVSSYLEEQAAKEFIAWLVKGGPSSGAPPPS SEQ ID NO:33

HVEGTFTSDVSSYLEEQAAKEFIAWLVKGRGSSGAPPPS SEQ ID NO:34

HVEGTFTSDVSSYLEEQAVKEFIAWLIKGRGSSGAPPPS SEQ ID NO:35

HVEGTFTSDWSSYLEEQAAKEFIAWLIKGRGSSGAPPPS SEQ ID NO:36

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGRGHSSGAPPPS SEQ ID NO:37
HVEGTFTSDVSSYLEEQAAKEFIAWLVKGRGHSSGAPPPS SEQ ID NO:38
HVEGTFTSDWSSYLEEQAAKEFIAWLIKGGPHSSGAPPPSH SEQ ID NO:39
HVEGTFTSDWSSYLEEQAAKEFIAWLIKGGPSSGAPPPSH SEQ ID NO:40
HVEGTFTSDVSWYLEGQAVKEFIAWLIKGGPHSSGAPPPS SEQ ID NO:41
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPS SEQ ID NO:42
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPSH SEQ ID NO:43
HVEGTFTSDWSSYLEEQAVKEFIAWLIKGGPSSGAPPPS SEQ ID NO:44
HVEGTFTSDWSSYLEEQAVKEFIAWLIKGGPSSGAPPPSH SEQ ID NO:45
HVEGTFTSDWSSYLEEQAVKEFIAWLIKGGPHSSGAPPPS SEQ ID NO:46
HVEGTFTSDWSKYLEEQAVKEFIAWLIKGGPSSGAPPPSH SEQ ID NO:47
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPRG SEQ ID NO:48
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPRG-NH2 SEQ ID NO:49
HVEGTFTSDVSSYLEEQAAKEFIAWLVKGGPSSGAPPPS-NH2 SEQ ID NO:50
HVEGTFTSDVSSYLEEQAAKEFIAWLVDGGPSSGRPPPS-NH2 SEQ ID NO:51
HVEGTFTSDVSSYLEEQAAKEFIAWLVDGGPSSGRPPPS SEQ ID NO:52
HVEGTFTSDVSSYLEEQAAKEFIAWLVDGGPSSGKPPPS SEQ ID NO:53
HVEGTFTSDVSSYLEEQAAKEFIAWLVDGGPSSGRG SEQ ID NO:54
HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGAPPPS SEQ ID NO:55
HVEGTFTSDVSSYLEEQAAKEFIAWLVKGGPSWGAPPPS SEQ ID NO:56
HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGAPPPGPS SEQ ID NO:57
HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGAPPPGPSGPS SEQ ID NO:58

Example 2

Proteolytic stability:

The relative susceptibility of various extended GLP-1 peptides to α-chymotrypsin was assessed in a reaction mixture with the control peptide Val^8-GLP-1(7-37)OH. A 10 mM phosphate/citrate solution, pH 7.4, was prepared containing GLP-1 peptides at a concentration of 100 μM. A 10 μl aliquot of this solution was then incubated at 4°C in a 200 ul 10 mM phosphate/citrate solution, pH 7.4, containing 10 mM $CaCl_2$. Alpha-Chymotrypsin (SIGMA, C-3142 lot 89F8155) was then added to a final concentration of 250 ng/ml. A 20 μl aliquot was injected onto an analytical Zorbax 300SB-C8 (4.6 mm i.d. x 50 mm) column at a 1 ml/min

flowrate in 10% acetonitrile/0.075% TFA before addition of the enzyme, as well as 20, 40, 60, 80, and 100 minutes following addition of the enzyme. Peaks were separated with a gradient of 10 to 90% acetonitrile/0.075% TFA over 15 min. The progress of the enzymatic reaction was followed by plotting loss of peak area of the starting material over time. The rate of proteolytic degradation was calculated from the initial rate of cleavage (timepoint 0 and 20 min) and directly compared to the rate of cleavage of the control peptide Val^8-GLP-1(7-37)OH. The following extended GLP-1 peptides were tested and had stability rates ranging from about the same as to greater than 5-fold more stable than Val^8-GLP-1(7-37)OH:

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPPS-NH2 SEQ ID NO:10

HVEGTFTSDVSSYLEEQAAKEFIAWLIDGGPSSGRPPPS-NH2 SEQ ID NO:7

HVEGTFTSDVSSYLEEQAAKEFIAWLVKGRGSSGDPPPS-NH2 SEQ ID NO:8

HVEGTFTSDVSSYLEEQAAKEFIAWLVKGRPSSGDPPPS-NH2 SEQ ID NO:9

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGAPPPS-NH2 SEQ ID NO:12

HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPS-NH2 SEQ ID NO:13

HVEGTFTSDVSSYLEEQAVKEFIAWLVKGGPSSGAPPPS-NH2 SEQ ID NO:14

HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGDPPPS-NH2 SEQ ID NO:15

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGGSSGDPPPS-NH2 SEQ ID NO:16

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGPGSSGDPPPS-NH2 SEQ ID NO:17

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGSPSGDPPPS-NH2 SEQ ID NO:18

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPS-NH2 SEQ ID NO:19

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPPS SEQ ID NO:20

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDAPPS-NH2 SEQ ID NO:21

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPAPS-NH2 SEQ ID NO:22

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPAS-NH2 SEQ ID NO:23

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDAAAS-NH2 SEQ ID NO:24

HVEGTFTSDWSSYLEGQAAKEFIAWLIKGGPSSGAPPPS SEQ ID NO:25

HVEGTFTSDWSSYLEGQAAKEFIAWLIKGGPSSGAPPPH SEQ ID NO:26

HVEGTFTSDVSSYLEGQAAKEFIAWLIKGGPSSGAPPPS SEQ ID NO:27

HVEGTFTSDVSSYLEGQAAKEFIAWLVKGGPSSGAPPPS SEQ ID NO:32

HVEGTFTSDVSSYLEEQAAKEFIAWLVKGGPSSGAPPPS SEQ ID NO:33

HVEGTFTSDVSSYLEEQAAKEFIAWLVKGRGHSSGAPPPS SEQ ID NO:38

HVEGTFTSDWSSYLEEQAAKEFIAWLIKGGPHSSGAPPPSH SEQ ID NO:39

HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPSH SEQ ID NO:43

HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPRG SEQ ID NO:48
HVEGTFTSDVSSYLEEQAAKEFIAWLVDGGPSSGRG SEQ ID NO:54

Example 3

Physical stability:

Extended GLP-1 peptides were analyzed with respect to their potential to aggregate in solution. In general, peptides in solution were stirred at elevated temperature in a suitable buffer while recording turbidity at 350 nm as a function of time. Time to the onset of aggregation was measured to quantify the potential of a given GLP molecule to aggregate under these stressed conditions.

A GLP-1 peptide was first dissolved under alkaline conditions (pH 10.5) for 30 minutes to dissolve any pre-aggregated material. The solution was then adjusted to pH 7.4 and filtered. Specifically, 4 mg of a lyophilized GLP-1 compound was dissolved in 3 ml of 10 mM phosphate/10 mM citrate. The pH was adjusted to 10.0-10.5 and held for 30 minutes. The solution was adjusted with HCl to pH 7.4 and filtered through a suitable filter, for example a Millex GV syringe filter (Millipore Corporation, Bedford, MA). This solution was then diluted to a final sample containing 0.3 mg/mL protein in 10 mM citrate, 10 mM phosphate, 150 mM NaCl, and adjusted to pH 7.4 to 7.5. The sample was incubated at 37°C in a quartz cuvette. Every five minutes the turbidity of the solution was measured at 350 nm on an AVIV Model 14DS UV-VIS spectrophotometer (Lakewood, NJ). For 30 seconds prior to and during the measurement the solution was stirred using a magnetic stir bar from Starna Cells, Inc. (Atascadero, CA). An increase in OD at 350 nm indicates aggregation of the GLP-peptide. The time to aggregation was approximated by the intersection of linear fits to the pre-growth and growth phase according to the

method of Drake (Arvinte T, Cudd A, and Drake AF. (1993) *J. Biol. Chem.* 268, 6415-6422).

The cuvette was cleaned between experiments with a caustic soap solution (e.g., Contrad-70). The following extended GLP-1 peptides were tested and were stable in solution for at least 55 hours compared to Val^8-GLP-1(7-37)OH which was stable for about 1 hour:

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPPS-NH2 SEQ ID NO:10
HVEGTFTSDVSSYLEEQAAKEFIAWLIDGGPSSGRPPPS-NH2 SEQ ID NO:7
HVEGTFTSDVSSYLEEQAAKEFIAWLVKGRGSSGDPPPS-NH2 SEQ ID NO:8
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGDPPPS-NH2 SEQ ID NO:15
HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPPS SEQ ID NO:20
HVEGTFTSDWSSYLEGQAAKEFIAWLIKGGPSSGAPPPS SEQ ID NO:25
HVEGTFTSDWSSYLEGQAAKEFIAWLIKGGPSSGAPPPH SEQ ID NO:26
HVEGTFTSDVSSYLEGQAAKEFIAWLIKGGPSSGAPPPS SEQ ID NO:27
HVEGTFTSDWSSYLEGQAAKEFIAWLIKGGPHSSGAPPPS SEQ ID NO:30
HVEGTFTSDVSSYLEGQAAKEFIAWLVKGGPSSGAPPPS SEQ ID NO:32
HVEGTFTSDVSSYLEEQAAKEFIAWLVKGGPSSGAPPPS SEQ ID NO:33
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPS SEQ ID NO:42
HVEGTFTSDWSSYLEEQAAKEFIAWLIKGGPHSSGAPPPSH SEQ ID NO:39
HVEGTFTSDWSSYLEEQAVKEFIAWLIKGGPSSGAPPPS SEQ ID NO:44
HVEGTFTSDVSSYLEEQAAKEFIAWLVDGGPSSGRG SEQ ID NO:54
HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGAPPPS SEQ ID NO:55
HVEGTFTSDVSSYLEGQAAKEFIAWLVKGRGSSGAPPPS SEQ ID NO:31
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGRGSSGAPPPS SEQ ID NO:35
HVEGTFTSDWSSYLEEQAAKEFIAWLIKGRGSSGAPPPS SEQ ID NO:36

Example 4

Pharmacokinetics of an extended GLP-1 peptide:

Pharmacokinetic parameters were determined for the following 5 different extended GLP-1 peptides:

Compound 1: HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPPS-NH_2 SEQ ID NO:10

Compound 2: HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPPS SEQ ID NO:20

Compound 3: HVEGTFTSDVSSYLEEQAAKEFIAWLVKGRGSSGAPPPS

SEQ ID NO:34

Compound 4: HVEGTFTSDVSSYLEGQAAKEFIAWLVKGRGSSGAPPPS

SEQ ID NO:31

Compound 5: HVEGTFTSDWSSYLEEQAAKEFIAWLIKGRGSSGAPPPS

SEQ ID NO:36

Parameters were determined following a single intravenous dose of 10μg/kg in four different rats. Data are listed in Tables 1 and 2

Table 1: PK parameters for extended GLP-1 peptides following a single intravenous injection of 10 µg/kg to Sprague Dawley rats.

Compound Administered	Subject	Cmax (ng/mL)	$AUC_{0\text{-}last}$ (ng*min/mL)	t1/2 (min)	Cl (mL/min/kg)	V_{ss} (mL/kg)
1	Rat1	22.8	448.5	10.9	22.3	265.7
	Rat2	22.4	469.4	10.1	21.3	270.4
	Rat3	24.6	504.8	13.1	19.8	272.7
	Rat4	24.1	478.8	10.1	20.9	246.3
	Mean	**23.5**	**475.4**	**11.0**	**21.1**	**263.8**
	S.D	1.0	23.3	1.4	41.0	12.0
2	Rat1	9.8	512.8	35.8	19.5	973.9
	Rat2	16.9	312.2	12.3	32.0	355.7
	Rat3	21.4	430.0	10.6	23.3	283.3
	Rat4	21.1	409.1	14.4	24.4	313.4
	Mean	**17.3**	**416.0**	**18.3**	**24.8**	**481.6**
	S.D	5.4	82.4	11.8	5.3	329.6
3	Rat1	23.6	466.8	11.6	21.4	263.3
	Rat2	22.7	465.6	11.7	21.5	280.5
	Rat3	24.3	492.5	12.0	20.3	262.4
	Rat4	21.1	409.1	14.4	24.4	313.4
	Mean	23.3	477.5	12.1	21.0	277.2
	S.D.	0.9	13.4	0.6	0.6	18.8
4	Rat1	25.4	560.0	12.0	17.9	258.6
	Rat2	24.9	486.7	11.9	20.5	251.1
	Rat3	20.0	424.9	11.2	23.5	315.9
	Rat4	24.0	461.5	11.0	21.7	249.2
	Mean	23.6	483.3	11.5	20.9	268.7
	S.D.	2.5	57.2	0.5	2.4	31.7
5	Rat1	35.9	747.1	11.9	13.4	179.2
	Rat2	25.9	525.9	11.6	19.0	243.6
	Rat3	39.7	852.0	13.2	11.7	171.6
	Rat4	29.6	546.3	10.9	18.3	188.1
	Mean	32.8	667.9	11.9	15.6	195.6
	S.D.	6.2	158.2	1.0	3.6	32.7

Abbreviations: , kg = killigram, µg = microgram, min = minute, ng = nanogram, mL = milliliter, C_{max} = maximum plasma concentration, AUC = area under the concentration curve, t1/2 = plasma half life, Cl = clearance, V = volume of distribution based on the terminal phase, SD = Standard Deviation.

Table 2: Plasma Concentrations (pg/mL) of extended GLP-1 peptides following a single intravenous administration of 10 µg/kg to Sprague Dawley rats.

		Time (min)				
Compound	Rat#	5	15	30	60	120
1	1	22778	8322	2894	467	<150[a]
	2	22369	11008	3119	478	<150 [a]
	3	24565	10027	3115	857	<150 [a]
	4	24119	9088	3279	415	<150 [a]
	Mean	**23458**	**9611**	**3102**	**554**	**NC**
	SD	**1051**	**1163**	**158**	**204**	**NC**
2	1	9827	7529	4990	3073	1045
	2	16926	4364	1876	<150 [a]	<150 [a]
	3	21404	9191	2622	459	<150 [a]
	4	21092	6199	2318	671	<150 [a]
	Mean	**17312**	**6821**	**2952**	**1051**	**NC**
	SD	**5392**	**2045**	**1393**	**1377**	**NC**
3	1	23585	8604	2996	566	<150 [a]
	2	22666	8467	3477	589	<150 [a]
	3	24349	9240	3282	670	<150 [a]
	4	22377	8930	4010	802	<150 [a]
	Mean	**23244**	**8810**	**3441**	**657**	**NC**
	SD	**899**	**346**	**428**	**107**	**NC**
4	1	25386	12141	4366	869	<150 [a]
	2	24853	9246	2782	631	<150 [a]
	3	20017	9576	2894	564	<150 [a]
	4	23963	8475	2680	480	<150 [a]
	Mean	**23555**	**9860**	**3181**	**636**	**NC**
	SD	**2430**	**1589**	**795**	**167**	**NC**
5	1	35938	16063	4773	1089	<150 [a]
	2	25899	10678	3361	686	<150 [a]
	3	39673	15595	6757	1450	<150[a]
	4	29602	8169	2991	458	<150 [a]
	Mean	**32778**	**12626**	**4471**	**921**	**NC**
	SD	**6190**	**3842**	**1707**	**439**	**NC**

NC = not calculated; µg = microgram; pg = picogram; mL = milliliter; kg = kilogram; min = minute.

[a] Less than the lower limit of quantitation (a value of zero was used for the purpose of calculations).

Example 5

In vivo activity of extended GLP-1 peptides:

Several different extended and non-extended GLP-1 peptides were tested for activity in a hyperglycemic clamp study in dogs. Glucose was infused for 200 minutes to maintain constant levels. For the first 80 minutes dogs were infused intravenously with vehicle to establish a baseline insulin concentration. For the next 60 minutes, GLP-1 peptides were administered at a rate of 1 pmol/kg/min. For the final 60 minutes the infusion rate of each GLP-1 compound was increased to 3 pmol/kg/min. Blood samples were taken periodically for the determination of insulin and GLP-1 peptide concentrations. Insulin change values were calculated as the difference between the value at time *t* and the average value during the last 20 minutes of the control period (60-80) minutes and are presented in Table 3. Areas under the insulin change curves were calculated using the trapezoidal rule over the last 30 minutes of each infusion period. GLP-1 peptide concentrations are presented in Table 4. Values listed are the means ± standard error of the mean (SEM).

Table 3: Pharmacodynamics from dog hyperglycemic (150 mg/dL) clamp studies

Compound	**n**	Pharmacodynamics **(insulin change AUC; mU•min/mL)**	
		Compound Infusion Rate (pmol/kg/min)	
		1	3
Vehicle	5	0.4 ± 0.1	0.4 ± 0.2
HVEGTFTSDVSSYLEGQAAKEFIAWLVKGRG SEQ ID NO:59 (No Cex)	5	1.1 ± 0.7	2.2 ± 1.2
HVEGTFTSDVSSYLEEQAAKEFIAWLIKG**GPSSGDPPPS**-NH2 SEQ ID NO:10	5	2.8 ± 1.0	5.5 ± 2.1

HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPPS SEQ ID NO:20	5	1.5 ± 0.5	3.7 ± 1.7
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPS-NH2 SEQ ID NO:13	5	1.4 ± 0.3	4.0 ±0.9
HVEGTFTSDVSSYLEEQAAKEFIAWLVKGGPSSGAPPPS SEQ ID NO:33	5	1.9 ± 0.5	4.3 ± 1.5
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGRGSSGAPPPS SEQ ID NO:35	5	1.1 ± 0.1	3.7 ± 0.9
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPS SEQ ID NO:42	6	2.3 ± 0.6	6.1 ± 1.5
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPSH SEQ ID NO:43	5	1.2 ± 0.5	4.6 ± 1.9
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPRG SEQ ID NO:48	5	2.2 ± 0.6	4.0 ± 1.4
HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGAPPPS SEQ ID NO:55	7	2.2 ± 0.5	5.2 ± 1.4

Table 4: GLP-1 peptide concentration from dog hyperglycemic (150 mg/dL) clamp studies after 60 minutes of compound infusion.

Compound	n	Compound Concentration (t = 60'; pM)	
		Compound Infusion Rate (pmol/kg/min)	
		1	3
Vehicle	5	------	------
HVEGTFTSDVSSYLEGQAAKEFIAWLVKGRG SEQ ID NO:59 (No Cex)	5	166 ± 23	410 ± 25
HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPPS-NH2 SEQ ID NO:10	5	268 ± 45	977 ± 135
HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGDPPPS SEQ ID NO:20	5	204 ± 24	755 ± 72
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPS-NH2 SEQ ID NO:13	5	366 ± 59	1316 ± 211
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGRGSSGAPPPS SEQ ID NO:35	5	267 ± 31	1036 ± 103
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPS SEQ ID NO:42	6	276 ± 36	1114 ± 139
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPSH SEQ ID NO:43	5	306 ± 20	1057 ± 34
HVEGTFTSDVSSYLEEQAVKEFIAWLIKGGPSSGAPPPRG SEQ ID NO:48	5	227 ± 40	1092 ± 106
HVEGTFTSDVSSYLEEQAAKEFIAWLIKGGPSSGAPPPS SEQ ID NO:55	7	246 ± 17	766 ± 46

CLAIMS

What is claimed is:

1. An extended GLP-1 peptide comprising an amino acid sequence of the formula:

Xaa_7-Xaa_8-Glu-Gly-Thr-Xaa_{12}-Thr-Ser-Asp-Xaa_{16}-Ser-Xaa_{18}-Xaa_{19}-Xaa_{20}-Glu-Xaa_{22}-Gln-Ala-Xaa_{25}-Lys-Xaa_{27}-Phe-Ile-Xaa_{30}-Trp-Leu-Xaa_{33}-Xaa_{34}-Gly-Xaa_{36}-Xaa_{37}-Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}-Xaa_{47}

Formula 1 (SEQ ID NO: 1)

wherein:

Xaa_7 is: L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, α-fluoromethyl-histidine, or α-methyl-histidine;

Xaa_8 is: Ala, Gly, Val, Leu, Ile, Ser, or Thr;

Xaa_{12} is: Phe, Trp, or Tyr;

Xaa_{16} is: Val, Trp, Ile, Leu, Phe, or Tyr;

Xaa_{18} is: Ser, Trp, Tyr, Phe, Lys, Ile, Leu, Val;

Xaa_{19} is: Tyr, Trp, or Phe;

Xaa_{20} is: Leu, Phe, Tyr, or Trp;

Xaa_{22} is: Gly, Glu, Asp, or Lys;

Xaa_{25} is: Ala, Val, Ile, or Leu;

Xaa_{27} is: Glu, Ile, or Ala;

Xaa_{30} is: Ala or Glu

Xaa_{33} is: Val or Ile;

Xaa_{34} is: Lys, Asp, Arg, or Glu;

Xaa_{36} is: Gly, Pro, or Arg;

Xaa_{37} is: Gly, Pro, or Ser;

Xaa_{38} is: Ser, Pro, or His;

Xaa_{39} is: Ser, Arg, Thr, Trp, or Lys;

Xaa_{40} is: Ser or Gly;

Xaa_{41} is: Ala, Asp, Arg, Glu, Lys, or Gly;

Xaa_{42} is: Pro, Ala, NH_2, or is absent;
Xaa_{43} is: Pro, Ala, NH_2, or is absent;
Xaa_{44} is: Pro, Ala, Arg, Lys, His, NH_2, or is absent;
Xaa_{45} is: Ser, His, Pro, Lys, Arg, NH_2 or is absent;
Xaa_{46} is: His, Ser, Arg, Lys, NH_2 or is absent; and
Xaa_{47} is: His, Ser, Arg, Lys, NH_2 or is absent;
provided that if Xaa_{42}, Xaa_{43}, Xaa_{44}, Xaa_{45}, Xaa_{46}, or Xaa_{47} is absent each amino acid downstream is absent and further provided that if Xaa_{36} is Arg and Xaa_{37} is Gly or Ser, the GLP-1 peptide does not have the following C-terminal amino acid extension beginning at Xaa_{38}: Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2.

2. The GLP-1 peptide of Claim 1 wherein the first 31 amino acids of the peptide do not differ from GLP-1(7-37) by more than 6 amino acids.

3. The GLP-1 peptide of Claim 2 wherein the first 31 amino acids of the peptide do not differ from GLP-1(7-37) by more than 5 amino acids.

4. The GLP-1 peptide of Claim 3 wherein the first 31 amino acids of the peptide do not differ from GLP-1(7-37) by more than 4 amino acids.

5. The GLP-1 peptide of Claim 4 wherein the first 31 amino acids of the peptide do not differ from GLP-1(7-37) by more than 3 amino acids.

6. The GLP-1 peptide of any one of Claims 1 through 5 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, and Xaa_{22} is Glu.

7. The GLP-1 peptide of any one of Claims 1 through 5 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, and Xaa_{16} is Trp.

8. The GLP-1 peptide of Claim 7 wherein Xaa_{22} is Glu.

9. The GLP-1 peptide of any one of Claims 1 through 5 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, and Xaa_{25} is Val.

10. The GLP-1 peptide of Claim 9 wherein Xaa_{22} is Glu.

11. The GLP-1 peptide of any one of Claims 1 through 5 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, and Xaa_{33} is Ile.

12. The GLP-1 peptide of Claim 11 wherein Xaa_{22} is Glu.

13. The GLP-1 peptide of any one of Claims 1 through 5 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, and Xaa_{34} is Asp.

14. The GLP-1 peptide of Claim 13 wherein Xaa_{22} is Glu.

15. The GLP-1 peptide of Claim 13 wherein Xaa_{41} is Arg.

16. The GLP-1 peptide of any one of Claims 1 through 5 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, Xaa_{36} is Gly, and Xaa_{37} is Pro.

17. The GLP-1 peptide of any one of Claims 1 through 5 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, and Xaa_{18} is Trp.

18. The GLP-1 peptide of any one of Claims 1 through 5 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, and Xaa_{20} is Trp.

19. The GLP-1 peptide of any one of Claims 1 through 18 wherein the C-terminal amino acid is amidated.

20. The GLP-1 peptide of any one of Claims 1 through 5 wherein the C-terminal amino acid is His.

21. An extended GLP-1 peptide comprising the amino acid sequence of the formula

Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Xaa_{16}-Ser-Ser-Tyr-Lys-Glu-Xaa_{22}-Gln-Ala-Xaa_{25}-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Xaa_{33}-Xaa_{34}-Gly-Xaa_{36}-Xaa_{37}-Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}-Xaa_{47}

Formula 3 (SEQ ID NO: 3)

wherein:

Xaa_7 is: L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, α-fluoromethyl-histidine, or α-methyl-histidine;

Xaa_8 is: Gly, Val, Leu, Ile, Ser, or Thr;

Xaa_{16} is: Val, Trp, Ile, Leu, Phe, or Tyr;

Xaa_{22} is: Gly, Glu, Asp, or Lys;

Xaa_{25} is: Ala, Val, Ile, or Leu;

Xaa_{33} is: Val or Ile;

Xaa_{34} is: Lys, Asp, Arg, or Glu;

Xaa_{36} is: Gly, Pro, or Arg;

Xaa_{37} is: Gly, Pro, or Ser;

Xaa_{38} is: Ser, Pro, or His;

Xaa_{39} is: Ser, Arg, Thr, Trp, or Lys;

Xaa_{40} is: Ser or Gly;

Xaa_{41} is: Ala, Asp, Arg, Glu, Lys, or Gly;

Xaa_{42} is: Pro, Ala, NH_2 or is absent;
Xaa_{43} is: Pro, Ala, NH_2 or is absent;
Xaa_{44} is: Pro, Ala, Arg, Lys, His; NH_2, or is absent;
Xaa_{45} is: Ser, His, Pro, Lys, Arg, NH_2 or is absent;
Xaa_{46} is: His, Ser, Arg, Lys, NH_2 or is absent; and
Xaa_{47} is: His, Ser, Arg, Lys, NH_2 or is absent;
provided that if Xaa_{42}, Xaa_{43}, Xaa_{44}, Xaa_{45}, Xaa_{46}, or Xaa_{47} is absent each amino acid downstream is absent and further provided that if Xaa_{36} is Arg and Xaa_{37} is Gly or Ser, the GLP-1 peptide does not have the following C-terminal amino acid extension beginning at Xaa_{38}: Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2.

22. The GLP-1 peptide of Claim 21 wherein
Xaa_7 is L-histidine;
Xaa_8 is Gly or Val;
Xaa_{16} is Phe, Trp, Tyr, Ile, or Leu;
Xaa_{22} is Glu; and
Xaa_{25} is Ala;
Xaa_{33} is Ile;
Xaa_{36} is Gly; and
Xaa_{37} is Pro.

23. The GLP-1 compound of Claim 22 wherein Xaa_{16} is Trp.

24. The GLP-1 compound of Claim 22 wherein Xaa_{16} is Phe.

25. The GLP-1 compound of Claim 22 wherein Xaa_{16} is Tyr.

26. The GLP-1 compound of Claim 22 wherein
Xaa_7 is L-histidine;
Xaa_8 is Gly or Val;
Xaa_{16} is Val;
Xaa_{22} is Glu; and
Xaa_{33} is Ile.

27. An extended GLP-1 peptide comprising the amino acid sequence of the formula

Xaa_7-Xaa_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Lys-Glu-Xaa_{22}-Gln-Ala-Xaa_{25}-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Xaa_{33}-Lys-Gly-Gly-Pro-Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}-Xaa_{47}

Formula 4 (SEQ ID NO:4)

wherein:
Xaa_7 is: L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, α-fluoromethyl-histidine, or α-methyl-histidine;
Xaa_8 is: Gly, Val, Leu, Ile, Ser, or Thr;
Xaa_{22} is: Gly, Glu, Asp, or Lys;
Xaa_{25} is: Ala, Val, Ile, or Leu;
Xaa_{33} is: Val or Ile;
Xaa_{38} is: Ser, Pro, or His;
Xaa_{39} is: Ser, Arg, Thr, Trp, or Lys;
Xaa_{40} is: Ser or Gly;
Xaa_{41} is: Ala, Asp, Arg, Glu, Lys, or Gly;
Xaa_{42} is: Pro or Ala;
Xaa_{43} is: Pro or Ala;
Xaa_{44} is: Pro, Ala, Arg, Lys, His, NH_2, or is absent;
Xaa_{45} is: Ser, His, Pro, Lys, Arg, NH_2 or is absent;
Xaa_{46} is: His, Ser, Arg, Lys, NH_2 or is absent; and
Xaa_{47} is: His, Ser, Arg, Lys, NH_2 or is absent;

provided that if Xaa_{44}, Xaa_{45}, Xaa_{46}, or Xaa_{47} is absent each amino acid downstream is absent.

28. The GLP-1 peptide of Claim 27 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, and Xaa_{22} is Glu.

29. The GLP-1 peptide of Claim 27 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, Xaa_{22} is Glu, and Xaa_{33} is Ile.

30. The GLP-1 peptide of Claim 27 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, Xaa_{22} is Glu, Xaa_{25} is Val, and Xaa_{33} is Ile.

31. The GLP-1 peptide of Claim 27 wherein Xaa_7 is L-histidine, Xaa_8 is Val or Gly, Xaa_{22} is Glu, Xaa_{33} is Ile, Xaa_{38} is Ser, Xaa_{39} is Ser, Xaa_{40} is Gly, Xaa_{41} is Ala, Xaa_{42} is Pro, Xaa_{43} is Pro, Xaa_{44} is Pro, Xaa_{45} is Ser, and Xaa_{46} is absent.

32. The GLP-1 peptide of Claim 31 wherein Xaa_8 is Val.

33. An extended GLP-1 peptide comprising an amino acid sequence of the formula

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Gly-Pro-Xaa_{38}-Xaa_{39}-Xaa_{40}-Xaa_{41}-Xaa_{42}-Xaa_{43}-Xaa_{44}-Xaa_{45}-Xaa_{46}-Xaa_{47}-Xaa_{48}-Xaa_{49}-Xaa_{50}

Formula 5 (SEQ ID NO:60)

Wherein:

Xaa_{38} is: Ser, Pro, or His;

Xaa_{39} is: Ser, Arg, Thr, Trp, or Lys;

Xaa_{40} is: Ser or Gly;

Xaa_{41} is: Ala, Asp, Arg, Glu, Lys, or Gly;
Xaa_{42} is: Pro, Ala, NH_2, or is absent;
Xaa_{43} is: Pro, Ala, NH_2, or is absent;
Xaa_{44} is: Pro, Ala, Arg, Lys, His, NH_2, or is absent;
Xaa_{45} is: Ser, His, Pro, Lys, Arg, Gly, NH_2 or is absent;
Xaa_{46} is: His, Ser, Arg, Lys, Pro, Gly, NH_2 or is absent; and
Xaa_{47} is: His, Ser, Arg, Lys, NH_2 or is absent;
Xaa_{48} is: Gly, His, NH_2 or is absent;
Xaa_{49} is: Pro, His, NH_2 or is absent; and
Xaa_{50} is: Ser, His, Ser-NH_2, His-NH_2 or is absent;
wherein said GLP-1 peptide comprises from one to six further substitutions and provided that if Xaa_{42}, Xaa_{43}, Xaa_{44}, Xaa_{45}, Xaa_{46}, Xaa_{47}, Xaa_{48}, or Xaa_{49} is absent each amino acid downstream is absent.

34. The GLP-1 peptide of Claim 33, wherein the further substitution is selected from the group consisting of at least one of the following substitutions:

a) His at position 7 is substituted with D-histidine, desamino-histidine, 2-amino-histidine, β-hydroxy-histidine, homohistidine, α-fluoromethyl-histidine, or α-methyl-histidine;
b) Ala at position 8 is substituted with Gly, Val, Leu, Ile, Ser, or Thr;
c) Phe at position 12 is substituted with Trp, or Tyr;
d) Val at position 16 is substituted with Trp, Ile, Leu, Phe, or Tyr;
e) Ser at position 18 is substituted with Trp, Tyr, Phe, Lys, Ile, Leu, or Val;
f) Tyr at position 19 is substituted with Trp or Phe;

g) Leu at position 20 is substituted with Phe, Tyr, or Trp;

h) Gly at position 22 is substituted with Glu, Asp, or Lys;

i) Ala at position 25 is substituted with Val, Ile, or Leu

j) Glu at position 27 is substituted with Ile or Ala;

k) Ala at position 30 is substituted with Glu;

l) Val at position 33 is substituted with Ile; and

m) Lys at position 34 is substituted with Asp, Arg or Glu

35. The GLP-1 peptide of Claim 34 wherein the further substitution is selected from the group consisting of:

a) Ala at position 8 is substituted with Val or Gly;

b) Gly at position 22 is substituted with Glu; and

c) Val at position 33 is substituted with Ile

36. A method of stimulating the GLP-1 receptor in a subject in need of blood glucose normalization, said method comprising the step of administering to the subject an effective amount of the GLP-1 peptide of any one of Claims 1 through 35.

37. The method of Claim 36 wherein the subject is being treated for non-insulin dependent diabetes.

38. A method of treating a subject prophylactically for non-insulin dependent diabetes comprising the step of administering to the subject an effective amount of a GLP-1 peptide of any one of Claims 1 through 35.

39. A method of reducing or maintaining body weight in a subject in need thereof, comprising administering to the subject an effective amount of a GLP-1 compound of any one of Claims 1 through 35.

40. A method of treating obesity in a subject in need thereof, comprising administering to the subject an effective amount of a GLP-1 compound of any one of Claims 1 through 35.

41. A method of treating stroke, myocardial infarction, stress-induced hyperglycemia, or irritable bowel syndrome in a subject in need thereof, comprising administering to the subject an effective amount of a GLP-1 compound of any one of Claims 1 through 35.

42. The use of a GLP-1 compound of any one of Claims 1-35 in the manufacture of a medicament for the treatment of non-insulin dependent diabetes, obesity, stroke, myocardial infarction, stress-induced hyperglycemia, or irritable bowel syndrome.

43. The use of Claim 42 wherein the medicament is used to treat non-insulin dependent diabetes.

44. The use of claim 42 wherein the medicament is used to treat obesity.

45. A process of making a pharmaceutical formulation comprising mixing a GLP-1 peptide of any one of Claims 1 through 35 with a pharmaceutical carrier.

46. A pharmaceutical formulation comprising a GLP-1 compound of any one of Claims 1 through 35 and a pharmaceutical carrier.

5

X-15133.ST25.Sequence Listingtxt.txt
SEQUENCE LISTING

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<120> EXTENDED GLUCAGON-LIKE PEPTIDE-1 ANALOGS

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20 25 30

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20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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<400> 14

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 15
<211> 39
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<222> (39)..(39)
<223> Ser at position 39 is amidated

<400> 15

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Asp Pro Pro Pro Ser
35

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<220>
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<220>
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<222> (39)..(39)
<223> Ser at position 39 is amidated

<400> 16

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Gly Ser
20 25 30

Ser Gly Asp Pro Pro Pro Ser
35

<210> 17
<211> 39
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<220>
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<220>
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<222> (39)..(39)
<223> Ser at position 39 is amidated

<400> 17

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Pro Gly Ser
20 25 30

Ser Gly Asp Pro Pro Pro Ser
35

<210> 18
<211> 39
<212> PRT
<213> Artificial

<220>
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<220>
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<222> (39)..(39)
<223> Ser at position 39 is amidated

<400> 18

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Ser Pro
20 25 30

Ser Gly Asp Pro Pro Pro Ser
35

<210> 19
<211> 38
<212> PRT
<213> Artificial

<220>
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<220>
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<222> (38)..(38)
<223> Ser at position 38 is amidated

<400> 19

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Asp Pro Pro Ser
35

<210> 20
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 20

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Asp Pro Pro Pro Ser
35

<210> 21
<211> 39
<212> PRT
<213> Artificial

<220>
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<220>
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<222> (39)..(39)
<223> Ser at position 39 is amidated

<400> 21

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Asp Ala Pro Pro Ser
35

<210> 22
<211> 39
<212> PRT
<213> Artificial

<220>
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<220>

<221> MOD_RES
<222> (39)..(39)
<223> Ser at position 39 is amidated

<400> 22

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Asp Pro Ala Pro Ser
35

<210> 23
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<220>
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<220>
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<222> (39)..(39)
<223> Ser at position 39 is amidated

<400> 23

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Asp Pro Pro Ala Ser
35

<210> 24
<211> 39
<212> PRT
<213> Artificial

<220>
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<222> (39)..(39)
<223> Ser at position 39 is amidated

<400> 24

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu

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1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Asp Ala Ala Ala Ser
35

<210> 25
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 25

His Val Glu Gly Thr Phe Thr Ser Asp Trp Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 26
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 26

His Val Glu Gly Thr Phe Thr Ser Asp Trp Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro His
35

<210> 27
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 27

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 28
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 28

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Asp Pro Pro Pro Ser
35

<210> 29
<211> 40
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 29

His Val Glu Gly Thr Phe Thr Ser Asp Trp Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser His
35 40

<210> 30
<211> 40
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 30

His Val Glu Gly Thr Phe Thr Ser Asp Trp Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro His
20 25 30

Ser Ser Gly Ala Pro Pro Pro Ser
35 40

<210> 31
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 31

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 32
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 32

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 33
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 33

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 34
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 34

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 35
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 35

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Arg Gly Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 36
<211> 39
<212> PRT

<213> Artificial

<220>
<223> synthetic construct

<400> 36

His Val Glu Gly Thr Phe Thr Ser Asp Trp Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Arg Gly Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 37
<211> 40
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 37

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Arg Gly His
20 25 30

Ser Ser Gly Ala Pro Pro Pro Ser
35 40

<210> 38
<211> 40
<212> PRT
<213> Artificial

<220>
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<400> 38

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly His
20 25 30

Ser Ser Gly Ala Pro Pro Pro Ser
35 40

<210> 39

<211> 41
<212> PRT
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<220>
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<400> 39

His Val Glu Gly Thr Phe Thr Ser Asp Trp Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro His
20 25 30

Ser Ser Gly Ala Pro Pro Pro Ser His
35 40

<210> 40
<211> 40
<212> PRT
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<220>
<223> synthetic construct

<400> 40

His Val Glu Gly Thr Phe Thr Ser Asp Trp Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser His
35 40

<210> 41
<211> 40
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 41

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Trp Tyr Leu Glu Gly
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro His
20 25 30

Ser Ser Gly Ala Pro Pro Pro Ser
35 40

<210> 42
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 42

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 43
<211> 40
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 43

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser His
35 40

<210> 44
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 44

His Val Glu Gly Thr Phe Thr Ser Asp Trp Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser

35

<210> 45
<211> 40
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 45

His Val Glu Gly Thr Phe Thr Ser Asp Trp Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser His
35 40

<210> 46
<211> 40
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 46

His Val Glu Gly Thr Phe Thr Ser Asp Trp Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro His
20 25 30

Ser Ser Gly Ala Pro Pro Pro Ser
35 40

<210> 47
<211> 40
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 47

His Val Glu Gly Thr Phe Thr Ser Asp Trp Ser Lys Tyr Leu Glu Glu
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser His
35 40

<210> 48
<211> 40
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 48

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Arg Gly
35 40

<210> 49
<211> 40
<212> PRT
<213> Artificial

<220>
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<220>
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<222> (40)..(40)
<223> Gly at position 40 is amidated

<400> 49

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Val Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Arg Gly
35 40

<210> 50
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<220>

<221> MOD_RES
<222> (39)..(39)
<223> Ser at position 39 is amidated

<400> 50

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 51
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<220>
<221> MOD_RES
<222> (39)..(39)
<223> Ser at position 39 is amidated

<400> 51

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Asp Gly Gly Pro Ser
20 25 30

Ser Gly Arg Pro Pro Pro Ser
35

<210> 52
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 52

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Asp Gly Gly Pro Ser
20 25 30

Ser Gly Arg Pro Pro Pro Ser
35

<210> 53
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 53

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Asp Gly Gly Pro Ser
20 25 30

Ser Gly Lys Pro Pro Pro Ser
35

<210> 54
<211> 36
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 54

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Asp Gly Gly Pro Ser
20 25 30

Ser Gly Arg Gly
35

<210> 55
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 55

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser

20 25 30

Ser Gly Ala Pro Pro Pro Ser
35

<210> 56
<211> 39
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 56

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Pro Ser
20 25 30

Trp Gly Ala Pro Pro Pro Ser
35

<210> 57
<211> 41
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 57

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Gly Pro Ser
35 40

<210> 58
<211> 44
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 58

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ile Lys Gly Gly Pro Ser
20 25 30

Ser Gly Ala Pro Pro Pro Gly Pro Ser Gly Pro Ser
35 40

<210> 59
<211> 31
<212> PRT
<213> Artificial

<220>
<223> synthetic construct

<400> 59

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
20 25 30

<210> 60
<211> 44
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<220>
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<222> (32)..(32)
<223> Xaa is Ser, Pro, or His

<220>
<221> MISC_FEATURE
<222> (33)..(33)
<223> Xaa is Ser, Arg, Thr, Trp, or Lys

<220>
<221> MISC_FEATURE
<222> (34)..(34)
<223> Xaa is Ser or Gly

<220>
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<222> (35)..(35)
<223> Xaa is Ala, Asp, Arg, Glu, Lys, Gly or a Modified Residue

<220>
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<222> (35)..(35)
<223> AMIDATION

<220>
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<222> (36)..(36)
<223> Xaa is Pro, Ala, Absent or a Modified Residue

<220>
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<222> (36)..(36)
<223> AMIDATION

<220>
<221> MISC_FEATURE
<222> (37)..(37)
<223> Xaa is Pro, Ala, Absent or a Modified Residue

<220>
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<222> (37)..(37)
<223> AMIDATION

<220>
<221> MISC_FEATURE
<222> (38)..(38)
<223> Xaa is Pro, Ala, Arg, Lys, His, Absent or a Modified Residue

<220>
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<223> AMIDATION

<220>
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<222> (39)..(39)
<223> Xaa is Ser, His, Pro, Lys, Arg, Gly, Absent or a Modified Residue

<220>
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<222> (39)..(39)
<223> AMIDATION

<220>
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<222> (40)..(40)
<223> Xaa is His, Ser, Arg, Lys, Pro, Gly, Absent or a Modified Residue

<220>
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<222> (40)..(40)
<223> AMIDATION

<220>
<221> MISC_FEATURE
<222> (41)..(41)

<223> Xaa is His, Ser, Arg, Lys, Absent or a Modified Residue

<220>
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<222> (41)..(41)
<223> AMIDATION

<220>
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<222> (42)..(42)
<223> Xaa is Gly, His, Absent or a Modified Residue

<220>
<221> MOD_RES
<222> (42)..(42)
<223> AMIDATION

<220>
<221> MISC_FEATURE
<222> (43)..(43)
<223> Xaa is Pro, His, Absent or a Modified Residue

<220>
<221> MOD_RES
<222> (43)..(43)
<223> AMIDATION

<220>
<221> MISC_FEATURE
<222> (44)..(44)
<223> Xaa is Ser, His, Absent or a Modified Residue

<220>
<221> MOD_RES
<222> (44)..(44)
<223> AMIDATION

<400> 60

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Gly Pro Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35 40