enhanced half-life would allow for less frequent dosing of patients being administered the protein. Such proteins are described herein.

SUMMARY OF THE INVENTION

In one aspect, the present invention refers to an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 4 and further comprising an amino acid substitution at position 180; and an amino acid substitution at position 98 of SEQ ID NO:4, wherein, (a) the amino acid residue at position 98 is arginine, and b) the amino acid residue at position 180 is selected from the group consisting of glycine, proline, serine or glutamic acid. In another aspect, the present invention refers to a fusion polypeptide comprising the isolated polypeptide of the present invention, fused to a heterologous amino acid sequence. In another aspect, the present invention refers to a multimer comprising two or more of the fusion polypeptide of the present invention. In another aspect the present invention refers to a pharmaceutical composition comprising the isolated polypeptide of the present invention and a pharmaceutically acceptable formulation agent. In another aspect, the present invention refers to an isolated nucleic acid encoding a polypeptide comprising an amino acid sequence of SEQ ID NO: 4 and further comprising an amino acid substitution at position 180; and an amino acid substitution at position 98 of SEQ ID NO:4, wherein, a) the amino acid residue at position 98 is arginine, and (b) the amino acid residue at position 180 is selected from the group consisting of glycine, proline, serine or glutamic acid. In another aspect, the present invention refers to a vector comprising the nucleic acid molecule the present invention. In another aspect, the present invention refers to a host cell comprising the nucleic acid molecule of the present invention.

Also described herein is an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 4 and further comprising: (a) at least one amino acid substitution at position 180; and (b) at least one amino acid substitution selected from the group consisting of: (i) a substitution at position 171; and (ii) a substitution at position 98; and (c) combinations thereof is disclosed.

In one example the isolated polypeptide comprises a substitution at position 98 and a substitution at position 180 and wherein: (a) the substitution at position 98 is selected from the group consisting of an arginine, cysteine, glutamic acid, glutamine, lysine, and threonine; and (b) the substitution at position 180 is selected from the group consisting of glycine, proline, serine or glutamic acid; and (c)

combinations thereof. In one example the residue at position 98 is arginine and the residue at position 180 is glutamic acid. Also described is a fusion polypeptide comprising the isolated polypeptide fused to a heterologous amino acid sequence. In one example the heterologous amino acid sequence is an Fc domain or fragment thereof, and can comprise the amino acid sequence of SEQ ID NO:11. In another example the polypeptide is fused to the Fc domain via a linker and in yet another example the linker comprises GGGGSGGGGSGGGGS (SEQ ID NO:31). In one specific example the polypeptide comprises SEQ ID NO:47. Also disclosed is a multimer comprising two or more of the fusion polypeptides. A pharmaceutical composition comprising the isolated polypeptide and a pharmaceutically acceptable formulation agent, such as a hydrogel, is also disclosed. In another example, a method of treating a metabolic disorder is provided and in one example comprises administering to a human patient in need thereof a pharmaceutical composition provided herein. In one example the metabolic disorder is diabetes and in another the metabolic disorder is obesity. Nucleic acids encoding the isolated polypeptide are also provided and in one example the nucleic acid comprises SEQ ID NO:46. The nucleic acid can be present in a vector, which can itself be present in a host cell. In still another example the polypeptide comprises: (a) an amino-terminal truncation of no more than 8 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; (b) a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; or (c) an amino-terminal truncation of no more than 8 amino acid residues and a carboxyl-terminal truncation of no

more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal. In still other examples the polypeptide is covalently linked to one or more polymers, such as PEG.

In another example the isolated polypeptide comprises a substitution at position 171 and a substitution at position 180 and wherein: (a) the substitution at position 180 is selected from the group consisting of glycine, proline, serine and glutamic acid; (b) the substitution at position 171 is selected from the group consisting of an alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, serine, threonine, tryptophan, and tyrosine; and (c) combinations thereof. In one example the residue at position 171 is glycine and the residue at position 180 is glutamic acid. Also described is a fusion polypeptide comprising the isolated polypeptide fused to a heterologous amino acid sequence. In one example the heterologous amino acid sequence is an Fc domain or fragment thereof, and can comprise the amino acid sequence of SEQ ID NO:11. In another example the polypeptide is fused to the Fc domain via a linker and in yet another example the linker comprises GGGGSGGGGSGGGGS (SEQ ID NO:31). In one specific example the polypeptide comprises SEQ ID NO:47. Also disclosed is a multimer comprising two or more of the fusion polypeptides. A pharmaceutical composition comprising the isolated polypeptide and a pharmaceutically acceptable formulation agent, such as a hydrogel, is also disclosed. In another example, a method of treating a metabolic disorder is provided and in one example comprises administering to a human patient in need thereof a pharmaceutical composition provided herein. In one example the metabolic disorder is diabetes and in another the metabolic disorder is obesity. Nucleic acids encoding the isolated polypeptide are also provided and in one embodiment the nucleic acid comprises SEQ ID NO:46. The nucleic acid can be present in a vector, which can itself be present in a host cell. In still another example the polypeptide comprises: (a) an amino-terminal truncation of no more than 8 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; (b) a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; or (c) an amino-

terminal truncation of no more than 8 amino acid residues and a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal. In still other examples the polypeptide is covalently linked to one or more polymers, such as PEG.

In yet another example the polypeptide comprises a substitution at position 98, a substitution at position 171 and a substitution at position 180 of SEQ ID NO:4 and wherein: (a) the substitution at position 171 is selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, lysine, serine, threonine, tryptophan, or tyrosine; (b) the substitution at position 98 is selected from the group consisting of arginine, cysteine, glutamic acid, glutamine, lysine, or threonine; and (c) the substitution at position 180 is selected from the group consisting of glycine, proline, serine or glutamic acid; and combinations thereof. In a further example the residue at position 98 is arginine, the residue at position 171 is glycine and the residue at position 180 is a glutamic acid. Also described is a fusion polypeptide comprising the isolated polypeptide fused to a heterologous amino acid sequence. In one example the heterologous amino acid sequence is an Fc domain or fragment thereof, and can comprise the amino acid sequence of SEQ ID NO:11. In another example the polypeptide is fused to the Fc domain via a linker and in yet another example the linker comprises GGGGSGGGGSGGGGS (SEQ ID NO:31). In one specific example the polypeptide comprises SEQ ID NO:47. Also disclosed is a multimer comprising two or more of the fusion polypeptides. A pharmaceutical composition comprising the isolated polypeptide and a pharmaceutically acceptable formulation agent, such as a hydrogel, is also disclosed. In another example, a method of treating a metabolic disorder is provided and in one example comprises administering to a human patient in need thereof a pharmaceutical composition provided herein. In one example the metabolic disorder is diabetes and in another the metabolic disorder is obesity. Nucleic acids encoding the isolated polypeptide are also provided and in one example the nucleic acid comprises SEQ ID NO:46. The nucleic acid can be present in a vector, which can itself be present in a host cell. In still another example the polypeptide comprises: (a) an amino-terminal truncation of no more than 8 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; (b) a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a

mammal; or (c) an amino-terminal truncation of no more than 8 amino acid residues and a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal. In still other exaamples the polypeptide is covalently linked to one or more polymers, such as PEG.

Specific embodiments of the invention will become evident from the following more detailed description of certain embodiments and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1B show the results of an ELK-luciferase activity assay performed on the FGF21 truncation mutants 7-181 and 8-181 (Figure 1A) and the FGF21 truncation mutants 1-172, 1-171, 1-169, and 1-164 (Figure 1B); each panel shows the results obtained for a human FGF21 control.

Figure 2 shows the results of an ELK-luciferase activity assay performed on a human FGF21 control and the FGF21 truncation mutants 3-181, 4-181, 5-181, 7-181, 8-181, 1-180, 1-178, 1-177, 1-176, 1-175, 1-174, 1-173, 1-172, 9-181, and 1-149.

Figure 3 shows the blood glucose levels measured in mice injected with PBS (solid bar), human FGF21 control (open bar), or the FGF21 truncation mutants 8-181 (gray bar) and 9-181 (stippled bar).

Figure 4 shows the percent change in blood glucose levels measured in mice injected with PBS (solid circles), an Fc-FGF21 control (WT) (open circles), or truncated Fc-FGF21 fusion proteins comprising amino acid residues 5-181 (solid triangles) or 7-181 (open triangles).

Figure 5 shows the percent change in blood glucose levels measured in mice injected with PBS (solid circles), an FGF21-Fc control (WT) (open circles), a truncated FGF21-Fc fusion protein comprising residues 1-175 (solid triangles), or a truncated Fc-FGF21 protein comprising amino acid residues 1-171 (open triangles).

Figures 6A-6D show the results of liquid chromatography-mass spectrometry (LC-MS) analysis of a human Fc-(G5)-FGF21 (SEQ ID NO:107) control sample (Figure 6A) and samples of Fc-(G5)-FGF21 drawn from mice at 6 hours (Sample D6; Figure 6B), 24 hours (Sample D24; Figure 6C), and 48 hours (Sample D48; Figure 6D) after injection.

Figures 7A-7D show the results if LC-MS analysis of a mammalian-derived human FGF21-(G3)-Fc (SEQ ID NO:105) control sample (Figure 7A) and samples of

CLAIMS

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 4 and further comprising an amino acid substitution at position 180; and an amino acid substitution at position 98 of SEQ ID NO:4, wherein,
(a) the amino acid residue at position 98 is arginine, and
(b) the amino acid residue at position 180 is selected from the group consisting of glycine, proline, serine or glutamic acid.

2. The isolated polypeptide of claim 1, wherein the residue at position 98 is arginine and the residue at position 180 is glutamic acid.

3. A fusion polypeptide comprising the isolated polypeptide of claim 2, fused to a heterologous amino acid sequence.

4. The fusion polypeptide of claim 3, wherein the heterologous amino acid sequence is an Fc domain or fragment thereof.

5. The fusion polypeptide of claim 4, wherein the Fc domain comprises the amino acid sequence of SEQ ID NO: 11.

6. The fusion polypeptide of claim 5, wherein the polypeptide is fused to the Fc domain via a linker.

7. The fusion polypeptide of claim 6, wherein the linker comprises GGGGSGGGGSGSGGS (SEQ ID NO:31).

8. The fusion polypeptide of claim 7, wherein the polypeptide comprises SEQ ID NO:47.

9. A multimer comprising two or more of the fusion polypeptide of claim 8.

10. A pharmaceutical composition comprising the isolated polypeptide of claim 1 and a pharmaceutically acceptable formulation agent.

11. The pharmaceutical composition of claim 10, wherein the pharmaceutically acceptable formulation agent is a hydrogel.

12. The isolated polypeptide of claim 1, wherein the polypeptide comprises:
(a) an amino-terminal truncation of no more than 8 amino acid residues, Wherein the polypeptide is capable of lowering blood glucose in a mammal;
(b) a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal; or
(c) an amino-terminal truncation of no more than 8 amino acid residues and a carboxyl-terminal truncation of no more than 12 amino acid residues, wherein the polypeptide is capable of lowering blood glucose in a mammal.

13. The isolated polypeptide of claim 1, wherein the polypeptide is covalently linked to one or more polymers.

14. The isolated polypeptide of claim 13, wherein the polymer is PEG.

15. An isolated nucleic acid encoding a polypeptide comprising an amino acid sequence of SEQ ID NO: 4 and further comprising an amino acid substitution at position 180; and an amino acid substitution at position 98 of SEQ ID NO:4, wherein,
(a) the amino acid residue at position 98 is arginine, and
(b) the amino acid residue at position 180 is selected from the group consisting of glycine, proline, serine or glutamic acid.

16. The isolated nucleic acid of claim 15, wherein the nucleic acid comprises SEQ ID NO:46.

17. A vector comprising the nucleic acid molecule claim 16.

18. A host cell comprising the nucleic acid molecule of claim 17.