Endocrinology 143(5):1741-7(2002)). Serum levels of FGF19 in humans are elevated following gastric bypass surgery. Augmented expression and secretion of FGF19 could at least partially explain the diabetes remission experienced following surgery.

[0005] Accordingly, there is a need for alternative treatments of hyperglycemic conditions such as diabetes, prediabetes, insulin resistance, hyperinsulinemia, glucose intolerance or metabolic syndrome, and other disorders and diseases associated with elevated glucose levels, in humans. The invention satisfies this need and provides related advantages.

Summary

[0006] The disclosure is based, in part, on variants of fibroblast growth factor 19 (FGF19) peptide sequences, fusions of fibroblast growth factor 19 (FGF19) and/or fibroblast growth factor 21 (FGF21) peptide sequences and variants of fusions (chimeras) of fibroblast growth factor 19 (FGF19) and/or fibroblast growth factor 21 (FGF21) peptide sequences having one or more activities, such as glucose lowering activity. Such variants and fusions (chimeras) of FGF19 and/or FGF21 peptide sequences include sequences that do not increase or induce hepatocellular carcinoma (HCC) formation or HCC tumorigenesis. Such variants and fusions (chimeras) of FGF19 and/or FGF21 peptide sequences also include sequences that do not induce a substantial elevation or increase in lipid profile.

[0007] In one example, a chimeric peptide sequence includes or consists of: an N-terminal region having at least seven amino acid residues, the N-terminal region having a first amino acid position and a last amino acid position, where the N-terminal region has a DSSPL or DASPH sequence; and a C-terminal region having a portion of FGF19, where the C-terminal region has a first amino acid position and a last amino acid position, where the C-terminal region includes amino acid residues 16-29 of FGF19 (WGDPIRLRHLYTSG), and where the W residue corresponds to the first amino acid position of the C-terminal region.

[0008] In another example, a chimeric peptide sequence includes or consists of: an N-terminal region having a portion of FGF21, where the N-terminal region has a first amino acid position and a last amino acid position, where the N-terminal region has a GQV sequence, and where the V residue corresponds to the last amino acid position of the N-terminal region; and a C-terminal region including a portion of FGF19, the C-terminal region having a first amino acid position and a last amino acid position, where the C-terminal region includes amino acid residues 21-29 of FGF19 (RLRHLYTSG), and where the R residue corresponds to the first position of the C-terminal region.

[0009] In a further example, a chimeric peptide sequence includes or consists of any of: an N-terminal region comprising a portion of SEQ ID NO:100 [FGF21], the N-terminal region

having a first amino acid position and a last amino acid position, wherein the N-terminal region comprises at least 5 (or more) contiguous amino acids of SEQ ID NO:100 [FGF21] including the amino acid residues GQV, and wherein the V residue corresponds to the last amino acid position of the N-terminal region; and a C-terminal region comprising a portion of SEQ ID NO:99 [FGF19], the C-terminal region having a first amino acid position and a last amino acid position, wherein the C-terminal region comprises amino acid residues 21-29 of SEQ ID NO:99 [FGF19], RLRHLYTSG, and wherein the R residue corresponds to the first position of the C-terminal region. In particular examples, the N-terminal region comprises at least 6 contiguous amino acids (or more, e.g., 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-25, 25-30, 30-40, 40-50, 50-75, 75-100 contiguous amino acids) of SEQ ID NO:100 [FGF21] including the amino acid residues GQV.

[0010] In an additional example, a peptide sequence includes or consists of any of: a fibroblast growth factor 19 (FGF19) sequence variant having one or more amino acid substitutions, insertions or deletions compared to a reference or wild type FGF19; a fibroblast growth factor 21 (FGF21) sequence variant having one or more amino acid substitutions, insertions or deletions compared to a reference or wild type FGF21; a portion of an FGF19 sequence fused to a portion of an FGF21 sequence; or a portion of an FGF19 sequence fused to a portion of an FGF21 sequence, wherein the FGF19 and/or FGF21 sequence portion(s) have one or more amino acid substitutions, insertions or deletions compared to a reference or wild type FGF19 and/or FGF21.

[0011] In still further examples, a peptide sequence or a chimeric peptide sequence includes or consists of amino-terminal amino acids 1-16 of SEQ ID NO:100 [FGF21] fused to carboxy-terminal amino acids 21-194 of SEQ ID NO:99 [FGF19], or the peptide sequence has amino-terminal amino acids 1-147 of SEQ ID NO:99 [FGF19] fused to carboxy-terminal amino acids 147-181 of SEQ ID NO:100 [FGF21] (M41), or the peptide sequence has amino-terminal amino acids 1-20 of SEQ ID NO:99 [FGF19] fused to carboxy-terminal amino acids 17-181 of SEQ ID NO:100 [FGF21] (M44), or the peptide sequence has amino-terminal amino acids 1-146 of SEQ ID NO:100 [FGF21] fused to carboxy-terminal amino acids 148-194 of SEQ ID NO:99 [FGF19] (M45), or the peptide sequence has amino-terminal amino acids 1-20 of SEQ ID NO:99 [FGF19] fused to internal amino acids17-146 of SEQ ID NO:100 [FGF21] fused to carboxy-terminal amino acids 148-194 of SEQ ID NO:99 [FGF19] (M46).

[0012] In yet additional examples, a peptide sequence or a chimeric peptide sequence has a WGDPI sequence motif corresponding to the WGDPI sequence of amino acids 16-20 of SEQ ID NO:99 [FGF19], or has a substituted, mutated or absent WGDPI sequence motif corresponding to FGF19 WGDPI sequence of amino acids 16-20 of FGF19, or the WGDPI

sequence motif has one or more amino acids substituted, mutated or absent; or is distinct from an FGF 19 variant sequence having any of GQV, GDI, WGPI, WGDPV, WGDI, GDPI, GPI, WGQPI, WGAPI, AGDPI, WADPI, WGDAI, WGDPA, WDPI, WGDI, WGDP or FGDPI substituted for the FGF19 WGDPI sequence at amino acids 16-20.

[0013] In yet further examples, a peptide sequence or a chimeric peptide sequence has an N-terminal region that includes or consists of amino acid residues VHYG, where the N-terminal region comprises amino acid residues DASPHVHYG, or where the N-terminal region comprises amino acid residues DSSPLVHYG, or where the N-terminal region comprises amino acid residues DSSPLLQ, or where the N-terminal region comprises amino acid residues DSSPLLQFGGQV. In particular examples, the G corresponds to the last position of the N-terminal region, or the Q residue is the last amino acid position of the N-terminal region, or the V residue corresponds to the last position of the N-terminal region..

[0014] In still additional examples, a peptide sequence or a chimeric peptide sequence has an N-terminal region that includes or consists of RHPIP, where R is the first amino acid position of the N-terminal region; or HPIP (e.g., where HPIP are the first 4 amino acid residues of the N-terminal region), where H is the first amino acid position of the N-terminal region; or RPLAF, where R is the first amino acid position of the N-terminal region; or PLAF, where P is the first amino acid position of the N-terminal region; or R, where R is the first amino acid position of the N-terminal region, or has at the N-terminal region any one of the following sequences: MDSSPL, MSDSSPL, SDSSPL, MSSPL or SSPL.

[0015] In other examples, a peptide sequence or a chimeric peptide sequence has, at the first position of the N-terminal region, an “M” residue, an “R” residue, a “S” residue, a “H” residue, a “P” residue, a “L” residue or an “D” residue. In alternative examples, a peptide sequence or a chimeric peptide sequence does not have a “M” residue or an “R” residue at the first amino acid position of the N-terminal region.

[0016] In still other examples, a peptide sequence or a chimeric peptide sequence has at the first and second positions of the N-terminal region an MR sequence, or at the first and second positions of the N-terminal region an RM sequence, or at the first and second positions of the N-terminal region an RD sequence, or at the first and second positions of the N-terminal region an DS sequence, or at the first and second positions of the N-terminal region an MD sequence, or at the first and second positions of the N-terminal region an MS sequence, or at the first through third positions of the N-terminal region an MDS sequence, or at the first through third positions of the N-terminal region an RDS sequence, or at the first through third positions of the N-terminal region an MSD sequence, or at the first through third positions of the N-terminal region an MSS sequence, or at the first through third positions of the N-terminal region an DSS sequence, or at

the first through fourth positions of the N-terminal region an RDSS sequence, or at the first through fourth positions of the N-terminal region an MDSS sequence, or at the first through fifth positions of the N-terminal region an MRDSS sequence, or at the first through fifth positions of the N-terminal region an MSSPL sequence, or at the first through sixth positions of the N-terminal region an MDSSPL sequence, or at the first through seventh positions of the N-terminal region an MSDSSPL sequence.

[0017] In still other examples, a peptide sequence or a chimeric peptide sequence an addition of amino acid residues 30-194 of SEQ ID NO:99 [FGF19] at the C-terminus, resulting in a chimeric polypeptide having at the last position of the C-terminal region that corresponds to about residue 194 of SEQ ID NO:99 [FGF19]. In further other examples, a chimeric peptide sequence or peptide sequence comprises all or a portion of an FGF19 sequence (e.g., SEQ ID NO:99), positioned at the C-terminus of the peptide, or where the amino terminal "R" residue is deleted from the peptide. I

[0018] In more particular examples, a chimeric peptide sequence or peptide sequence includes or consists of any of M1-M98 variant peptide sequences, or a subsequence or fragment of any of the M1-M98 variant peptide sequences. In one aspect, the present disclosure refers to a chimeric peptide comprising or consisting of SEQ ID NO:2, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:69, or SEQ ID NO:70.

[0019] In additional particular examples, a chimeric peptide sequence or peptide sequence has an N-terminal or a C-terminal region from about 20 to about 200 amino acid residues in length. In further particular examples, a chimeric peptide sequence or peptide sequence has at least one amino acid deletion. In still further particular examples, a chimeric peptide sequence or peptide sequence, or a subsequence or fragment thereof, has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acid deletions from the amino terminus, the carboxy-terminus or internally. In a particular non-limiting example, the amino acid substitution, or deletion is at any of amino acid positions 8-20 of FGF19 (AGPHVHYGWGDPI).

[0020] In more particular examples, a chimeric peptide sequence or peptide sequence includes or consists of an amino acid sequence of about 5 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50, 60 to 70, 70 to 80, 80 to 90, 90 to 100 or more amino acids. In more particular examples, a chimeric peptide sequence or peptide sequence includes or consists of an amino acid sequence of about 5 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50, 50 to 60, 60 to 70, 70 to 80, 80 to 90, 90 to 100 or more amino acids of FGF19 or FGF21.

[0021] In further particular examples, chimeric peptide sequences and peptide sequences have particular functions or activities. In one example, a chimeric peptide sequence or peptide sequence maintains or increases an FGFR4 mediated activity. In additional examples, a chimeric peptide sequence or peptide sequence binds to fibroblast growth factor receptor 4 (FGFR4) or activates FGFR4, or does not detectably bind to fibroblast growth factor receptor 4 (FGFR4) or

activate FGFR4, or binds to FGFR4 with an affinity less than, comparable to or greater than FGF19 binding affinity for FGFR4, or activates FGFR4 to an extent or amount less than, comparable to or greater than FGF19 activates FGFR4. In further examples, a chimeric peptide sequence or peptide sequence has reduced hepatocellular carcinoma (HCC) formation compared to FGF19, or an FGF 19 variant sequence having any of GQV, GDI, WGPI, WGDPV, WGDI, GDPI, GPI, WGQPI, WGAPI, AGDPI, WADPI, WGDAI, WGDPA, WDPI, WGDI, WGDP or FGDPI substituted for the WGDPI sequence at amino acids 16-20 of FGF19, and/or has greater glucose lowering activity compared to FGF19, or an FGF 19 variant sequence having any of GQV, GDI, WGPI, WGDPV, WGDI, GDPI, GPI, WGQPI, WGAPI, AGDPI, WADPI, WGDAI, WGDPA, WDPI, WGDI, WGDP or FGDPI substituted for the WGDPI sequence at amino acids 16-20 of FGF19, and/or has less lipid increasing activity compared to FGF19, or an FGF 19 variant sequence having any of GQV, GDI, WGPI, WGDPV, WGDI, GDPI, GPI, WGQPI, WGAPI, AGDPI, WADPI, WGDAI, WGDPA, WDPI, WGDI, WGDP or FGDPI substituted for the WGDPI sequence at amino acids 16-20 of FGF19, and/or has less triglyceride, cholesterol, non-HDL or HDL increasing activity compared to FGF19, or an FGF 19 variant sequence having any of GQV, GDI, WGPI, WGDPV, WGDI, GDPI, GPI, WGQPI, WGAPI, AGDPI, WADPI, WGDAI, WGDPA, WDPI, WGDI, WGDP or FGDPI substituted for the WGDPI sequence at amino acids 16-20 of FGF19, and/or has less lean mass reducing activity compared to FGF21. Such functions and activities can be ascertained in vitro or in vivo, for example, in a *db/db* mouse.

[0022] In still additional examples, chimeric peptide sequences and peptide sequences isolated or purified, and/or chimeric peptide sequences and peptide sequences can be included in compositions. In oneexample, a chimeric peptide sequence or peptide sequence is included in a pharmaceutical composition. Such compositions include combinations of inactive or other active ingredients. In one example, a compositions, such as a pharmaceutical composition includes chimeric peptide sequence or peptide sequence and a glucose lowering agent. In one aspect, the present disclosure refers to a pharmaceutical composition comprising the chimeric peptide as describe herein.

[0023] In yet further examples, nucleic acid molecules encoding the chimeric peptide sequence or peptide sequence are provided. Such molecules can further include an expression control element in operable linkage that confers expression of the nucleic acid molecule encoding the peptide in vitro, in a cell or in vivo, or a vector comprising the nucleic acid molecule (e.g., a viral vector). Transformed and host cells that express the chimeric peptide sequences and peptide sequences are also provided. In one aspect, the present disclosure refers to a nucleic acid molecule encoding the chimeric peptide as described herein, wherein the nucleic acid molecule further comprises an expression control element in operable linkage that confers expression of the nucleic acid molecule encoding the peptide *in vitro*, in a cell, or *in vivo*. In another aspect, the present disclosure refers to a vector comprising the nucleic acid molecule as described herein. In yet another aspect, the present disclosure refers to a transformed or host cell that expresses the chimeric peptide as described herein.

[0024] Uses and methods of treatment that include administration or delivery of any chimeric peptide sequence or peptide sequence are also provided. In particular examples, a use or method of treatment of a subject includes administering an invention chimeric peptide or peptide

sequence to a subject, such as a subject having, or at risk of having, a disease or disorder treatable by an invention peptide sequence, in an amount effective for treating the disease or disorder. In a further example, a method includes administering an invention chimeric peptide or peptide sequence to a subject, such as a subject having a hyperglycemic condition (e.g., diabetes, such as insulin-dependent (type I) diabetes, type II diabetes, or gestational diabetes), insulin resistance, hyperinsulinemia, glucose intolerance or metabolic syndrome, or is obese or has an undesirable body mass. In one aspect, the present disclosure refers to a use of a chimeric peptide as described herein in the manufacture of a medicament for improving glucose metabolism in a subject in need thereof. In another aspect, the present disclosure refers to a use of a chimeric peptide as described herein in the manufacture of a medicament for treating a disorder, wherein the disorder is a hyperglycemic condition, insulin resistance, hyperinsulinemia, glucose intolerance, metabolic syndrome, obesity, undesirable body mass, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), or atherosclerosis.

[0025] In particular examples of the methods and uses, a chimeric peptide sequence or peptide sequence is administered to a subject in an amount effective to improve glucose metabolism in the subject. In more particular examples, a subject has a fasting plasma glucose level greater than 100 mg/dl or has a hemoglobin A1c (HbA1c) level above 6%, prior to administration.

[0026] In further examples, a use or method of treatment of a subject is intended to or results in reduced glucose levels, increased insulin sensitivity, reduced insulin resistance, reduced glucagon, an improvement in glucose tolerance, or glucose metabolism or homeostasis, improved pancreatic function, or reduced triglyceride, cholesterol, IDL, LDL or VLDL levels, or a decrease in blood pressure, a decrease in intimal thickening of the blood vessel, or a decrease in body mass or weight gain.

[0027] Methods of analyzing and/or identifying a chimeric peptide sequence or peptide sequence are also provided, such as chimeric peptide sequences and peptide sequences that have glucose lowering activity without substantial hepatocellular carcinoma (HCC) activity. In one example, a method includes: a) providing a candidate chimeric peptide sequence or peptide sequence; b) administering the candidate peptide sequence to a test animal (e.g., a db/db mouse); c) measuring glucose levels of the animal after administration of the candidate peptide sequence, to determine if the candidate peptide sequence reduces glucose levels. In a particular example, the chimeric peptide sequence or peptide sequence is also analyzed for induction of HCC in the animal (e.g., assessing a hepatic tissue sample from the test animal), or expression of a marker correlating with HCC activity, wherein a candidate peptide having glucose lowering activity and not substantial HCC activity. Such methods identify the candidate as having glucose lowering activity, optionally also without substantial hepatocellular carcinoma (HCC) activity.

Description of Drawings

[0028] **FIG. 1** shows FGF19 and FGF21 protein sequences, and representative variant sequences, namely variant M5, variant M1, variant M2, variant M69, variant M3, variant M48, variant M49, variant M50, variant M51, variant M52, variant M53 and variant M70 peptide

What is Claimed is:

1. A chimeric peptide comprising or consisting of SEQ ID NO:2, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:69, or SEQ ID NO:70.

2. The chimeric peptide of claim 1 comprising or consisting of SEQ ID NO:2.

3. The chimeric peptide of claim 1 comprising or consisting of SEQ ID NO:52.

4. The chimeric peptide of claim 1 comprising or consisting of SEQ ID NO:53.

5. The chimeric peptide of claim 1 comprising SEQ ID NO:70.

6. The chimeric peptide of claim 1 consisting of SEQ ID NO:70.

7. The chimeric peptide of claim 1 comprising SEQ ID NO:69.

8. The chimeric peptide of claim 1 consisting of SEQ ID NO:69.

9. The chimeric peptide of any one of claims 1 to 8, wherein said peptide is fused with an immunoglobulin Fc region.

10. A pharmaceutical composition comprising the chimeric peptide of any one of claims 1 to 9.

11. The pharmaceutical composition of claim 10, wherein the pharmaceutical composition comprises a glucose lowering agent.

12. A nucleic acid molecule encoding the chimeric peptide of any one of claims 1 to 9, wherein the nucleic acid molecule further comprises an expression control element in operable linkage that confers expression of the nucleic acid molecule encoding the peptide *in vitro*, in a cell, or *in vivo*.

13. A vector comprising the nucleic acid molecule of claim 12.

14. The vector of claim 13, wherein the vector is a viral vector.

15. A transformed or host cell that expresses the chimeric peptide of any one of claims 1 to 9.

16. Use of a chimeric peptide as defined in any one of claims 1 to 9 in the manufacture of a medicament for improving glucose metabolism in a subject in need thereof.

17. The use of the chimeric peptide of claim 16, wherein the subject has a hyperglycemic condition, insulin resistance, hyperinsulinemia, glucose intolerance, metabolic syndrome, obesity, undesirable body mass, nonalcoholic fatty liver disease (NAFLD) nonalcoholic steatohepatitis (NASH), or atherosclerosis.

18. The use of the chimeric peptide of claim 17, wherein the hyperglycemic condition comprises diabetes.

19. The use of the chimeric peptide of claim 18, wherein the diabetes is insulin-dependent (type I) diabetes, type II diabetes, gestational diabetes, or prediabetes.

20. The use of the chimeric peptide of claim 16, wherein the subject:
    (i) has a fasting plasma glucose (FPG) level greater than 100 mg/dl;
    (ii) has a FPG level greater than 125 mg/dl;
    (iii) has a FPG level of between 100 and 125 mg/dl; or
    (iv) has a hemoglobin A1c (HbA1c) level above 6%.

21. Use of a chimeric peptide as defined in any one of claims 1 to 9 in the manufacture of a medicament for treating a disorder, wherein the disorder is a hyperglycemic condition, insulin resistance, hyperinsulinemia, glucose intolerance, metabolic syndrome, obesity, undesirable body mass, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), or atherosclerosis.

22. The use of the chimeric peptide of claim 21, wherein the hyperglycemic condition comprises diabetes.

23. The use of the chimeric peptide of claim 22, wherein the diabetes is insulin-dependent (type I) diabetes, type II diabetes, gestational diabetes, or prediabetes.

24. The use of the chimeric peptide of any one of claims 16 to 23, wherein the use results in increased insulin sensitivity, reduced insulin resistance, reduced glucagon, improved glucose tolerance, improved glucose metabolism or homeostasis, improved pancreatic function, reduced triglyceride level, reduced cholesterol level, reduced IDL level, reduced LDL level, reduced VLDL level, reduced blood pressure, reduced intimal thickening of the blood vessel, reduced body mass or weight gain, or reduced glucose levels; wherein optionally the glucose level is reduced by at least 5%.

25. The use of the chimeric peptide of any one of claims 16 to 23, wherein the use further comprises that a supplementary therapy is to be administered.

26. The use of the chimeric peptide of claim 25, wherein said supplementary therapy is to be administered prior to, contemporaneously with or following the administration of the chimeric peptide.

27. The use of the chimeric peptide of claim 25 or 26, wherein the supplementary therapy comprises:

(a) a weight loss surgery, gastric bypass, gastrectonomy, gastric balloon, or gastric sleeve; or

(b) a glucose lowering agent, insulin, GLP1 analogue, biguanide, sulphonylurea, thiazolidinediones, a dipeptidyl peptidase-4 (DPP-4) inhibitor, a bromocriptine formulation, a bile acid sequestrant, metformin, a thiazolidinediones (TZD), a SGLT-2 inhibitor, or any combination thereof.

28. The use of the chimeric peptide of claim 27(b), wherein:

(i) said biguanide or sulphonylurea is tolbutamide, chlorpropamide, acetohexamide, tolazamide, glibenclamide, glipizide or any combination thereof;

(ii) said thiazolidinedione is rosiglitazone or pioglitazone, or combination thereof;

(iii) said bile acid sequestrant is colesevelum; or

(iv) said insulin is bolus insulin, basal insulin, or an analogue thereof.

Abstract

COMPOSITIONS, USES AND METHODS FOR TREATMENT OF METABOLIC DISORDERS AND DISEASES

The invention relates to variants and fusions of fibroblast growth factor 19 (FGF19), variants and fusions of fibroblast growth factor 21 (FGF21), fusions of fibroblast growth factor 19 (FGF19) and/or fibroblast growth factor 21 (FGF21), and variants or fusions of fibroblast growth factor 19 (FGF19) and/or fibroblast growth factor 21 (FGF21) proteins and peptide sequences (and peptidomimetics), having one or more activities, such as glucose lowering activity, and methods for and uses in treatment of hyperglycemia and other disorders.

[Figure 3A]