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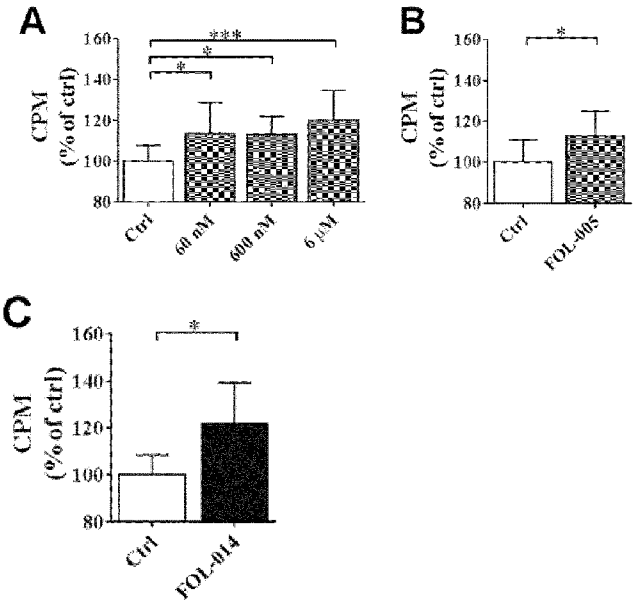
(54) Title: PEPTIDES FOR TREATMENT OF DIABETES

Fig. 1



(57) Abstract: The present disclosure concerns agents and their use in the treatment of endocrine, nutritional and/or metabolic diseases in a mammal. The disclosure furthermore concerns novel peptides.

[Continued on next page]

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Peptides for treatment of diabetes

Technical field

The present disclosure relates to peptides useful for treatment of diabetes and associated disorders.

Background

The peptide hormone insulin, which is produced by β-cells in the islets of Langerhans in the pancreas, is released in response to increasing blood glucose levels. Thus, glucose is removed from the blood by insulin dependent stimulation of glucose transporters located in the cell membranes of the target tissue, e.g. adipose tissue, skeletal muscle and liver. Insulin exerts its biological effects by binding to and activating the membrane-bound insulin receptor (IR), thereby initiating a cascade of intracellular signalling events, which regulate multiple biological processes such as glucose and lipid metabolism.

Currently, the treatment of diabetes, both type 1 and type 2 diabetes, relies primarily on insulin treatment. A complement to insulin treatment is long-acting glucagon-like peptide-1 (GLP-1) receptor agonists, i.e. derivatives that act on the same receptor as GLP-1. GLP-1 is a metabolic hormone that stimulates insulin secretion. Besides increasing insulin secretion from the pancreas in a glucose-dependent manner, GLP-1 is known to increase insulin-sensitivity in both α- and β-cells; to increase β-cell mass and insulin expression, post-translational modification, and secretion; and to decrease glucagon secretion from the pancreas. Other medications used complementary to insulin treatment for the purpose of lowering plasma glucose levels include DPP-IV inhibitors, Metformin, SGLT-2 inhibitors and sulfonylurea.

Certain drawbacks are associated with long term use of insulin, such as weight gain and increased risks of cancer and hypoglycaemia. Thus, there is a growing demand in the field for novel non-insulin compounds capable of, not only treating diabetes, by addressing insulin resistance and hyperglycemia, but also reducing associated and consequential complications.

Identification of novel compounds that can restore glucose metabolism and treat diabetes and related disorders is thus highly relevant. Multiple approaches can be contemplated, albeit none of which are obvious to the person of skill in the art.

Summary

The present inventors have found peptides which stimulate β–cell proliferation, have the ability to rescue β–cell from apoptosis induced by glucotoxic conditions, and stimulate insulin secretion from rat INS-1 β–cells as well as isolated mouse pancreatic islets. Furthermore, the present inventors found that in a glucose tolerance test, the peptides lowered plasma glucose levels *in vivo* and delayed onset of diabetes disease in BB *lyp/lyp* rats, a model for type 1 diabetes. Hence, the peptides of the present disclosure are suitable for use in the treatment of endocrine, nutritional and metabolic diseases and disorders.

In one aspect, the present disclosure relates to an agent comprising or consisting of:

a) a peptide or peptide analog, wherein the peptide or peptide analog comprises an amino acid sequence of the general formula:

$KX_2LAX_5X_6X_7X_8IX_{10}LX_{12}YGIK$ **(SEQ ID NO: 140)**

wherein:

X_2 is C, P or G;

X_5 is E or G;

X_6 is C, D or I;

X_7 is D, I, S or G;

X_8 is S, D or G;

X_{10} is E or G;

X_{12} is S or T;

with the proviso that if X_{12} is T then the peptide comprises no more than 25 amino acids; and

with the proviso that if X_2 is P, X_5 is E, X_6 is I, X_7 is D, X_8 is S, X_{10} is E and X_{12} is S, the peptide comprises no more than 85 amino acid residues.

or a biologically active fragment and/or variant thereof, wherein said biologically active fragment and/or variant is selected from the group consisting of

CLAEIDSC (SEQ ID NO: 142), CFKPLAEIDSIECSYGIK (SEQ ID NO: 143), KPLAEGDIELSYGIK (SEQ ID NO: 147), KPLAEIELSYGIK (SEQ ID NO: 148), KCLAEIDSCELSYGIK (SEQ ID NO: 155), and CFKPLAEIDSIEC (SEQ ID NO: 156);

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c).

In one aspect, the present disclosure relates to an agent comprising:

a) a peptide or peptide analog comprising or consisting of the amino acid sequence GDPNDGRGDSVVYGLR (SEQ ID NO: 137), VDTYDGGISVVYGLR (SEQ ID NO: 138), and VDTYDGDGSVVYGLR (SEQ ID NO: 139). VDVPEGDISLAYGLR (SEQ ID NO: 157), LDGLVRAYDNISPVG (SEQ ID NO: 158), GDPNGDISVVYGLR (SEQ ID NO: 159), VDVPNGDISLAYRLR (SEQ ID NO: 160) VDVPEGDISLAYRLR (SEQ ID NO: 161), V(beta-D)TYDGDISVVYGLR (SEQ ID NO:167), VDTY(beta-D)GDISVVYGLR (SEQ ID NO: 168), VDTYDG(beta-D)ISVVYGLR (SEQ ID NO:169);

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c).

In one aspect, the present disclosure relates to a composition comprising the agent described herein above.

In one aspect, the present disclosure relates to an agent or a composition comprising said agent, for use as a medicament.

In one aspect, the present disclosure relates to an agent comprising:

a) (i) a peptide or a peptide analog, wherein the peptide or the peptide analog comprises or consists of an amino acid sequence of the general formula:

$KX_2LAX_5X_6X_7X_8IX_{10}LX_{12}YGIK$ **(SEQ ID NO: 140)**

wherein:

X_2 is C, P or G;

X_5 is E or G;

X_6 is C, D or I;

X_7 is D, I, S or G;

X_8 is S, D or G;

X_{10} is E or G;

X_{12} is S or T;

with the proviso that if X_{12} is T, the peptide comprises no more than 25 amino acid residues;

(ii) a peptide, wherein the peptide comprises an amino acid sequence of the general formula: $VDZ_3Z_4Z_5GZ_7Z_8SZ_{10}Z_{11}YGLR$ **(SEQ ID NO: 68)**

wherein:

Z_3 is T or V;

Z_4 is Y or P;

Z_5 is D or N;

Z_7 is D or G;

Z_8 is I or G;

Z_{10} is V or L;

Z_{11} is V or A;

(iii) a peptide, wherein the peptide comprises or consists of an amino acid sequence selected from the group consisting of KCLAECDSIELSYGIK (SEQ ID NO: 141), CLAEIDSC (SEQ ID NO: 142), CFKPLAEIDSIECSYGIK (SEQ ID NO: 143), KPLAEIELSYGIK (SEQ ID NO: 148), KCLAEIDSCELSYGIK (SEQ ID NO: 155) and CFKPLAEIDSIEC (SEQ ID NO: 156);

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c).

for use in the treatment of an endocrine disease, a nutritional disease and/or a metabolic disease in a mammal.

In one aspect, the present disclosure concerns a method for treating an endocrine disease a nutritional disease and/or a metabolic disease, the method comprising administering a therapeutically effective amount of an agent described herein, to an individual in need thereof.

In one aspect, the present disclosure concerns the use of an agent as described herein for the manufacture of a medicament for the treatment of an endocrine disease a nutritional disease and/or a metabolic disease.

In one aspect, the present disclosure concerns a method for delaying onset of diabetes, the method comprising administering a therapeutically effective amount of an agent described herein, to an individual in need thereof.

In one aspect, the present disclosure concerns a method for decreasing blood glucose levels, the method comprising administering a therapeutically effective amount of an agent described herein, to an individual in need thereof.

In one aspect, the present disclosure concerns a method, e.g. an in vitro method, for improving beta cell morphology, the method comprising administering a therapeutically effective amount of an agent described herein, to an individual in need thereof.

In one aspect, the present disclosure concerns a method for improving beta cell viability, the method comprising administering a therapeutically effective amount of an agent described herein, to an individual in need thereof.

In one aspect, the present disclosure concerns the use of agent described herein for the preparation of a diagnostic composition for the diagnosis of a disease, disorder or damage of the pancreas in an individual.

Description of Drawings

Figure 1. *FOL-005 and FOL-014 induced proliferation of β-cells*
Addition of increasing concentrations of FOL-005 in solution induced increasing proliferation of INS-1 cells after 48 hours (Fig. 1A). Wells coated with FOL-005 and blocked with Bovine Serum Albumin (BSA) induced more proliferation of β-cells compared to only BSA coated control (ctrl) wells (Fig. 1B). Wells pre-coated with FOL-014 and blocked with BSA induced more proliferation compared to only BSA coated wells (Fig. 1C). Data is presented as counts per minute (CPM) relative unstimulated control (ctrl) cells. Mean ± SD are presented for 10-12 different observations in each group.

Figure 2. *FOL-005 protected β-cells against glucotoxicity*
INS-1 cells incubated during 48h in 20 mM glucose displayed more apoptotic cells (Annexin V positive) compared to cells incubated at 5 mM glucose. Addition of FOL-005 to cells incubated with 20 mM glucose reduced the level of apoptotic cells compared to 20 mM glucose alone (Fig. 2A). Apoptosis measured by caspase-3 activity was increased in INS-1 cells at 20 mM compared to 5 mM glucose. Addition of FOL-005 diminished the rate of glucotoxicity-induced caspse-3 activity (Fig. 2B). Mean ± SD are presented for 4–8 different observations in each group.

Figure 3. *Insulin secretion was increased from islets and β-cells following FOL-005 stimulation*
FOL-005 stimulated β-cell and islet insulin secretion. Insulin release from INS-1 cells was increased after FOL-005 (6 µM) stimulation in-non glucose containing media compared to non-stimulated control (ctrl) and to a scrambled control peptide (FOL-015) (Fig. 3A). FOL-005 stimulated insulin release from INS-1 at both low (5 mM) and high (20 mM) glucose (Fig. 3B). Isolated mouse pancreatic islets stimulated with FOL-005 (6 µM) or GLP-1 (100 nM) secreted more insulin compared to unstimulated control islets (Fig. 3C). Mean ± SD are presented for 5–6 different observations in each group.

Figure 4. *Insulin secretion was increased from islets and β-cells following FOL-014 stimulation*
FOL-014 stimulated insulin secretion from β-cells and pancreatic islets. INS-1 cells stimulated with FOL-014 (6 µM) secreted more insulin compared to unstimulated control cells (Fig. 4A). Isolated mouse pancreatic islets stimulated with FOL-014 (6 µM) secreted more insulin compared to control islets (Fig. 4B). Addition of GLP-1 (100 nM) or FOL-014 (0.6 µM) had no effect on insulin secretion. Mean ± SD are presented for 5–6 different observations in each group.

Figure 5. *The effect of FOL-014 on insulin secretion was dose dependent*. Stimulation of INS-1 cells by increasing doses of FOL-014 resulted in a significant increase in insulin secretion for all concentrations tested. The insulin secretion increased in a linear fashion in the presence of FOL-014 ranging from 0.6 nM to 60 nM. Higher concentrations appeared to result in a less pronounced effect on insulin secretion. Furthermore, FOL-014 induced insulin secretion was comparable to the effect of 100 nM GLP-1. Bars represent mean values and standard error of the mean (SEM).

Figure 6. *The effect on insulin secretion of FOL-014 was glucose concentration dependent.* The insulin secretion from untreated or FOL-014 exposed INS-1 cells was measured in the presence of increasing glucose concentrations. At glucose levels 5.5 mM or higher, the insulin secretion was significantly higher in the FOL-014 treated cells, as compared to untreated control cells. Bars represent mean values and standard error of the mean (SEM).

Figure 7. *FOL-005 and FOL-014 dosed together with native GLP-1 elicited an additive effect on insulin secretion*. The insulin release from INS-1 cells was measured following combination treatment of GLP-1 together with FOL-005 and FOL-014 (all three peptides in a concentration of 100 nM), respectively and compared with the effect of each peptide alone. The combination of GLP-1 and FOL-014 significantly increased the insulin secretion as compared with each peptide alone. An increase was also observed for the combination of FOL-005 and GLP-1. Bars represent mean values and standard error of the mean (SEM).

Figure 8. *FOL-014 affected insulin and glucagon secretion in pancreatic islets*. Two different concentrations of FOL-014 were tested and compared with the effect of 100 nM GLP-1 on isolated mouse islets in low (2.8 mM) (A, C) and high (16.7mM) (B, D) concentrations of glucose. In the low glucose samples, the presence of FOL-014 did not increase insulin secretion, but reduced glucagon secretion as compared with control and GLP-1. In the high glucose samples, 600 nM FOL-014 and GLP-1, but not 6 µM FOL-014, significantly increased insulin secretion (B), and GLP-1 as well as both concentrations of FOL-014 efficiently reduced glucagon secretion (D). Bars represent mean values and standard error of the mean (SEM).

Figure 9. *FOL-014 lowered plasma glucose levels in vivo following a glucose injection*. An intraperitoneal glucose tolerance test (IPGTT) was performed on wild type C57bl/6 mice. FOL-014 dosed at 200 nmol/kg significantly lowered the plasma glucose levels as compared to the control at 15 minutes, 30 minutes and 45 minutes (P=0.0027). At the 30 nmol/kg dose, FOL-014 lowered the glucose levels with a significant effect at 45 minutes after the glucose injection. The dotted line corresponds to mean non-fasting glucose levels. Data represents mean values and standard error of the mean (SEM). Statistical analysis was performed using student's t-test.

Figure 10. *FOL-014 delayed the onset of type-1 diabetes in BB lyp/lyp rats*. BB *lyp/lyp* rats treated with FOL-014 showed a significant delay in the onset of diabetes defined as plasma glucose < 11.1 mmol/l. Age of onset of diabetes for each rat was depicted in (A) with a significant difference between untreated and treated groups. The percentage of animals developing type 1 diabetes each day was depicted in (B) with a significant difference between groups. Error bars in (A) represent standard error of the mean (SEM).

Figure 11. *The effect on insulin secretion of peptide analogues derived from FOL-005 or FOL-014*. Novel peptide analogues were tested in two separate INS-1 cell lines (A and B) for their ability to induce insulin secretion under high glucose (16.7 mM) conditions. The effect was compared with that of native GLP-1, FOL-005 and FOL-014 as well as the effect of high glucose alone. Analogues inducing insulin release below the average of the high glucose control were considered non-functional (not shown). The level of insulin secretion is depicted in black, filled bars for the novel analogues, and in contrasting patterns for the comparators. Bars represent mean values and standard error of the mean (SEM).

Figure 12. *FOL-005 and FOL-014 displayed specific distribution patterns following injection in mouse*. Following subcutaneous administration of ^{3}H-FOL-005, the highest overall levels of radioactivity were present in pancreas and at the injection site, 1 hour (A) and 2 hours (B) after injection. Accumulation of the ^{3}H-FOL-005 is also visible in liver, kidney, salivary glands. Using Pearl Trilogy Small Animal Imaging System *in vivo* bio-distribution and tissue localization of Cy7.5 labelled FOL-005 (C) and FOL-014 (D) in NMRI nude mice via subcutaneous injection was investigated. Following initial control imaging, a dose of 10 nmol per mouse was administered and live imaging was performed at 5min, 20min, 50min, 60min, 2hrs, 4hrs, 6hrs, 24hrs and 48 hrs. High accumulation of both peptides was evident in the pancreatic region as well as at the injection site.

Detailed description

The disclosure is as defined in the claims.

In one aspect, the present disclosure concerns a peptide or a peptide analog comprising an amino acid sequence of the general formula:

a) $KX_2LAX_5X_6X_7X_8IX_{10}LX_{12}YGIK$ **(SEQ ID NO: 140)**

wherein:

X_2 is C, P or G;

X_5 is E or G;

X_6 is C, D or I;

X_7 is D, I, S or G;

X_8 is S, D or G;

X_{10} is E or G;

X_{12} is S or T;

with the proviso that if X_{12} is T, the peptide comprises no more than 25 amino acids; and

with the proviso that if X_2 is P, X_5 is E, X_6 is I, X_7 is D, X_8 is S, X_{10} is E and X_{12} is S, the peptide comprises no more than 85 amino acid residues;

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c).

In one embodiment, the present disclosure concerns a peptide or a peptide analog comprising an amino acid sequence of the general formula:

$KX_2LAX_5X_6X_7X_8IX_{10}LSYGIK$ **(SEQ ID NO: 162)**

wherein:

X_2 is C, P or G;

X_5 is E or G;

X_6 is C, I or absent;

X_7 is D, G or absent;

X_8 is S, G or absent;

X_{10} is E or G;

wherein absent means that the amino acid X_5 is coupled to the amino acid X_{10}

In one embodiment, the present disclosure concerns a peptide comprising an amino acid sequence of the general formula:

$KX_2LAX_5IX_{10}LSYGIK$ **(SEQ ID NO: 163)**

wherein:

X_2 is C, P or G;

X_5 is E or G;

X_{10} is E or G.

In one embodiment, the present disclosure concerns an agent comprising:

a) a peptide, wherein the peptide is selected from the group consisting of:

i) a peptide comprising or consisting of the amino acid sequence of SEQ ID NO: 136, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, and 156;

ii) a biologically active sequence variant of any one of the peptides of i), wherein any one amino acid has been altered for another proteinogenic or non-proteinogenic amino acid, with the proviso that no more than five amino acids are so altered;

iii) a biologically active fragment of the peptide of any one of i) or ii), wherein the fragment comprises at least 10 consecutive amino acids of any one of i) or ii);

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c).

In one embodiment, the present disclosure concerns an agent comprising:

a) a peptide, wherein the peptide comprises or consists of an amino acid sequence selected from the group consisting of GDPNDGRGDSVVYGLR (SEQ ID NO: 137), VDTYDGGISVVYGLR (SEQ ID NO: 138), and VDTYDGDGSVVYGLR (SEQ ID NO: 139). VDVPEGDISLAYGLR (SEQ ID NO: 157), LDGLVRAYDNISPVG (SEQ ID NO: 158), GDPNGDISVVYGLR (SEQ ID NO: 159), VDVPNGDISLAYRLR (SEQ ID NO:

160) VDVPEGDISLAYRLR (SEQ ID NO: 161);

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c).

In one embodiment, the present disclosure concerns a peptide comprising an amino acid sequence of the general formula:

$VDVPZ_5GDISLAYZ_{13}LR$ **(SEQ ID NO: 164)**

wherein:

Z_5 is E or N;

Z_{13} is R or G.

In one embodiment, the present disclosure concerns a peptide comprising an amino acid sequence of the general formula:

$VDTYDGZ_7Z_8SVVYGLR$ **(SEQ ID NO: 165)**

wherein:

Z_7 is D or G;

Z_8 is I or G.

In one embodiment, the present disclosure concerns a peptide comprising an amino acid sequence of the general formula:

$GDPNZ_5Z_6Z_7Z_8Z_9SVVYGLR$ **(SEQ ID NO: 166)**

wherein:

Z_5 is D or G;

Z_6 is D or G

Z_7 is I or R;

Z_8 is G or absent;

Z_9 is D or absent.

The term 'absent' as used herein, e.g. "X_6 is C, I or absent" is to be understood as that the amino acid residues directly adjacent to the absent amino acid are directly linked to each other by a conventional amide bond.

The term “peptide analog” described herein refers to a peptide comprising or consisting of a non-naturally occurring peptide.

The term ‘amino acid’ as used herein includes the standard twenty genetically-encoded amino acids and their corresponding stereoisomers in the ‘D’ form (as compared to the natural ‘L’ form), omega-amino acids and other naturally-occurring amino acids, unconventional amino acids (e.g., α,α-disubstituted amino acids, N-alkyl amino acids, etc.) and chemically derivatized amino acids (see below).

When an amino acid is being specifically enumerated, such as ‘alanine’ or ‘Ala’ or ‘A’, the term refers to both L-alanine and D-alanine unless explicitly stated otherwise. Other unconventional amino acids may also be suitable components for peptides of the present disclosure, as long as the desired functional property is retained by the peptide. For the peptides shown, each encoded amino acid residue, where appropriate, is represented by a single letter designation, corresponding to the trivial name of the conventional amino acid.

Chemical derivatives of one or more amino acids may be achieved by reaction with a functional side group. Such derivatives include, for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, *p*-toluene sulphonyl groups, carboxybenzoxy groups, *t*-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters and hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. Also included as chemical derivatives are those peptides which contain naturally occurring amino acid derivatives of the twenty standard amino acids. For example: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine and ornithine for lysine. Derivatives also include peptides containing one or more additions or deletions as long as the requisite activity is maintained. Other included modifications are amidation, amino terminal acylation (*e.g.* acetylation or thioglycolic acid amidation), terminal carboxylamidation (*e.g.* with ammonia or methylamine), and the like terminal modifications.

Some of the peptides of the disclosure shares amino acid sequence similarity with a sub-region of naturally occurring osteopontin proteins. In some embodiments, said peptide may be regarded as an active fragment of a naturally-occurring osteopontin protein or a variant of such as a fragment.

Some of the peptides of the disclosure shares amino acid sequence similarity with a sub-region of naturally occurring tenascin proteins. In some embodiments, said peptide may be regarded as an active fragment of a naturally-occurring tenascin protein or a variant of such as a fragment.

By “fragment”, at least 5 contiguous amino acids of the amino acid sequence are included, for example at least 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 contiguous amino acids of the amino acid sequence. Thus, the fragment may be 15 or fewer amino acids in length, for example 14, 13, 12, 11, 10, 9, 8, 7, 6 or 5 amino acids in length

In one embodiment, said peptide is of no more than no more than 85, such as no more than 80, such as no more than 75, such as no more than 70, such as no more than 65, such as no more than 60, such as nor more than 55, such as no more than 50, such as no more than 55, such as no more than 40 amino acids, such as no more than 35, such as no more than 30, such as no more than 28, such as no more than 26, such as no more than 24, such as no more than 22, such as no more than 20, such as no more than 19, such as no more than 18, such as no more than 17, such as no more than 16, such as no more than 15, such as no more than 14, such as no more than 13, such as no more than 12, such as no more than 11, such as no more than 10 amino acids in length.

In another embodiment, said peptide is between 5 and 30 amino acids in length, such as between 5 and 20, such as between 8 and 20, such as between 8 and 16, such as between 10 and 15 amino acids in length.

In yet another embodiment, said fragment comprises 15 or fewer amino acids in length, such as fewer than 14 amino acids, such as fewer than 13 amino acids, such as fewer than 12 amino acids, such as fewer than 11 amino acids, such as fewer than 10 amino acids, such as fewer than 9 amino acids, such as fewer than 8 amino acids, such as

fewer than 7 amino acids, such as fewer than 6 amino acids, such as fewer than 5 amino acids in length.

The term "variant" refers to a peptide that does not share 100% amino acid sequence identity with the parent peptide, i.e. one or more amino acids must be mutated. "Mutated" refers to altering an amino acid at a specified position in the parent peptide. For example, an amino acid at a specified position may be deleted, altered, substituted or may be the site of an insertion/addition of one or more amino acids. It will be appreciated by persons skilled in the art that the substitutions may be conservative or non-conservative.

In one embodiment, said peptide variant comprises or consists of a sequence wherein no more than five amino acids are altered for another proteinogenic or non-proteinogenic amino acid, such as no more than 4 amino acids, such as no more than 3 amino acids, such as no more than 2 amino acids, such as no more than 1 amino acid is altered. In one embodiment, one or more amino acids are conservatively substituted. "Conservatively substituted" refers to a substitution of one amino acid with another with similar properties (size, hydrophobicity, *etc*), such that the function of the peptide is not significantly altered. Thus, by "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

In another embodiment, said peptide comprises or consists of one or more additional amino acids, inserted at the N- and/or C-terminus and/or internally within the sequence. In one embodiment, at least 2 additional amino acids, such as at least 3, such as at least 4, such as at least 5, such as at least 6, such as at least 7, such as at least 8, such as at least 9, such as at least 10, such as at least 15 or such as at least 20 additional amino acids are inserted. The additional amino acids may be the amino acids from the corresponding positions of the wildtype human osteopontin (SEQ ID NO: 66) or from the corresponding positions of the wildtype murine osteopontin (SEQ ID NO: 134). The term "corresponding positions" of the wildtype osteopontin we mean that the additional amino acids are the same as those present in the equivalent position in the above wildtype osteopontin (if one imagines that the amino acid sequence of SEQ ID NO:1 replaces the sequence underlined in italics in SEQ ID NO:66

In another embodiment, the peptide is selected from the group consisting of SEQ ID NO: 1, 136, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 67, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 135, 137, 138, 139, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 167, 168 and 169;

i. 15-amino acid peptides:

VDTYDGDISVVYGLR	**SEQ ID NO: 1**
VDTYDGDISVVYGLS	**SEQ ID NO: 2**

ii. 14-amino acid peptides:

VDTYDGDISVVYGL	**SEQ ID NO: 3**
DTYDGDISVVYGLR	**SEQ ID NO: 4**
TYDGDISVVYGLRS	**SEQ ID NO: 5**

iii. 13-amino acid peptides:

VDTYDGDISVVYG	**SEQ ID NO: 6**
DTYDGDISVVYGL	**SEQ ID NO: 7**
TYDGDISVVYGLR	**SEQ ID NO: 8**
YDGDISVVYGLRS	**SEQ ID NO: 9**

iv. 12-amino acid peptides:

VDTYDGDISVVY	**SEQ ID NO: 10**
DTYDGDISVVYG	**SEQ ID NO: 11**
TYDGDISVVYGL	**SEQ ID NO: 12**
YDGDISVVYGLR	**SEQ ID NO: 13**
DGDISVVYGLRS	**SEQ ID NO: 14**

v. 11-amino acid peptides:

VDTYDGDISVV	**SEQ ID NO: 15**
DTYDGDISVVY	**SEQ ID NO: 16**
TYDGDISVVYG	**SEQ ID NO: 17**
YDGDISVVYGL	**SEQ ID NO: 18**
DGDISVVYGLR	**SEQ ID NO: 19**
GDISVVYGLRS	**SEQ ID NO: 20**

vi. 10-amino acid peptides:

VDTYDGDISV	**SEQ ID NO: 21**
DTYDGDISVV	**SEQ ID NO: 22**
TYDGDISVVY	**SEQ ID NO: 23**
YDGDISVVYG	**SEQ ID NO: 24**
DGDISVVYGL	**SEQ ID NO: 25**
GDISVVYGLR	**SEQ ID NO: 26**
DISVVYGLRS	**SEQ ID NO: 27**

vii. 9-amino acid peptides:

VDTYDGDIS	**SEQ ID NO: 28**
DTYDGDISV	**SEQ ID NO: 29**
TYDGDISVV	**SEQ ID NO: 30**
YDGDISVVY	**SEQ ID NO: 31**
DGDISVVYG	**SEQ ID NO: 32**
GDISVVYGL	**SEQ ID NO: 33**
DISVVYGLR	**SEQ ID NO: 34**
ISVVYGLRS	**SEQ ID NO: 35**

viii. 8-amino acid peptides:

VDTYDGDI	**SEQ ID NO: 36**
DTYDGDIS	**SEQ ID NO: 37**
TYDGDISV	**SEQ ID NO: 38**
YDGDISVV	**SEQ ID NO: 39**
DGDISVVY	**SEQ ID NO: 40**
GDISVVYG	**SEQ ID NO: 41**
DISVVYGL	**SEQ ID NO: 42**
ISVVYGLR	**SEQ ID NO: 43**

ix. 7-amino acid peptides:

VDTYDGD	**SEQ ID NO: 44**
DTYDGDI	**SEQ ID NO: 45**
TYDGDIS	**SEQ ID NO: 46**
YDGDISV	**SEQ ID NO: 47**
DGDISVV	**SEQ ID NO: 48**
GDISVVY	**SEQ ID NO: 49**
DISVVYG	**SEQ ID NO: 50**
ISVVYGL	**SEQ ID NO: 51**

x. 6-amino acid peptides:

DTYDGD	**SEQ ID NO: 52**
TYDGDI	**SEQ ID NO: 53**
YDGDIS	**SEQ ID NO: 54**
DGDISV	**SEQ ID NO: 55**
GDISVV	**SEQ ID NO: 56**
DISVVY	**SEQ ID NO: 57**

	ISVVYG	**SEQ ID NO: 58**

xi. 5-amino acid peptides:

TYDGD	**SEQ ID NO: 59**
YDGDI	**SEQ ID NO: 60**
DGDIS	**SEQ ID NO: 61**
GDISV	**SEQ ID NO: 62**
DISVV	**SEQ ID NO: 63**
ISVVY	**SEQ ID NO: 64**
SVVYG	**SEQ ID NO: 65**

xii. 16-amino acid peptide:

VDTYDGRGDSVVYGLR	**SEQ ID NO: 67**

xiii. 15-amino acid peptides:

VDVPNGDISLAYGLR	**SEQ ID NO: 69**
DVPNGDISLAYGLRS	**SEQ ID NO: 70**

xiv. 14-amino acid peptides:

VDVPNGDISLAYGL	**SEQ ID NO: 71**
DVPNGDISLAYGLR	**SEQ ID NO: 72**
VPNGDISLAYGLRS	**SEQ ID NO: 73**

xv. 13-amino acid peptides:

VDVPNGDISLAYG	**SEQ ID NO: 74**
DVPNGDISLAYGL	**SEQ ID NO: 75**
VPNGDISLAYGLR	**SEQ ID NO: 76**
PNGDISLAYGLRS	**SEQ ID NO: 77**

xvi. 12-amino acid peptides:

VDVPNGDISLAY	**SEQ ID NO: 78**
DVPNGDISLAYG	**SEQ ID NO: 79**
VPNGDISLAYGL	**SEQ ID NO: 80**
PNGDISLAYGLR	**SEQ ID NO: 81**
NGDISLAYGLRS	**SEQ ID NO: 82**

xvii. 11-amino acid peptides:

VDVPNGDISLA	**SEQ ID NO: 83**
DVPNGDISLAY	**SEQ ID NO: 84**
VPNGDISLAYG	**SEQ ID NO: 85**
PNGDISLAYGL	**SEQ ID NO: 86**
NGDISLAYGLR	**SEQ ID NO: 87**
GDISLAYGLRS	**SEQ ID NO: 88**

xviii. 10-amino acid peptides:

VDVPNGDISL	SEQ ID NO: 89
DVPNGDISLA	SEQ ID NO: 90
VPNGDISLAY	SEQ ID NO: 91
PNGDISLAYG	SEQ ID NO: 92
NGDISLAYGL	SEQ ID NO: 93
GDISLAYGLR	SEQ ID NO: 94
DISLAYGLRS	SEQ ID NO: 95

xix. 9-amino acid peptides:

VDVPNGDIS	SEQ ID NO: 96
DVPNGDISL	SEQ ID NO: 97
VPNGDISLA	SEQ ID NO: 98
PNGDISLAY	SEQ ID NO: 99
NGDISLAYG	SEQ ID NO: 100
GDISLAYGL	SEQ ID NO: 101
DISLAYGLR	SEQ ID NO: 102
ISLAYGLRS	SEQ ID NO: 103

xx. 8-amino acid peptides:

VDVPNGDI	SEQ ID NO: 104
DVPNGDIS	SEQ ID NO: 105
VPNGDISL	SEQ ID NO: 106
PNGDISLA	SEQ ID NO: 107
NGDISLAY	SEQ ID NO: 108
GDISLAYG	SEQ ID NO: 109
DISLAYGL	SEQ ID NO: 110
ISLAYGLR	SEQ ID NO: 111

xxi. 7-amino acid peptides:

VDVPNGD	SEQ ID NO: 112
DVPNGDI	SEQ ID NO: 113
VPNGDIS	SEQ ID NO: 114
PNGDISL	SEQ ID NO: 115
NGDISLA	SEQ ID NO: 116
GDISLAY	SEQ ID NO: 117
DISLAYG	SEQ ID NO: 118
ISLAYGL	SEQ ID NO: 119

xxii. 6-amino acid peptides:

DVPNGD	SEQ ID NO: 120
VPNGDI	SEQ ID NO: 121
PNGDIS	SEQ ID NO: 122
NGDISL	SEQ ID NO: 123
GDISLA	SEQ ID NO: 124
DISLAY	SEQ ID NO: 125
ISLAYG	SEQ ID NO: 126

xxiii. 5-amino acid peptides:

VPNGD	**SEQ ID NO: 127**
PNGDI	**SEQ ID NO: 128**
NGDIS	**SEQ ID NO: 129**
GDISL	**SEQ ID NO: 130**
DISLA	**SEQ ID NO: 131**
ISLAY	**SEQ ID NO: 132**
SLAYG	**SEQ ID NO: 133**

xxiv. 16-amino acid peptides:

KPLAEIDSIELSYGIK	**SEQ ID NO: 136**
GDPNDGRGDSVVYGLR	**SEQ ID NO: 137**

xxv. 15--amino acid peptides:

VDTYDGGISVVYGLR	**SEQ ID NO: 138**
VDTYDGDGSVVYGLR	**SEQ ID NO: 139**

xxvi. 16-amino acid peptides:

KCLAECDSIELSYGIK	**SEQ ID NO: 141**

xxvii. 8--amino acid peptides:

CLAEIDSC	**SEQ ID NO: 142**

xxviii. 18-amino acid peptides:

CFKPLAEIDSIECSYGIK	**SEQ ID NO: 143**

xxix. 16--amino acid peptides:

KPLAEDISIELSYGIK	**SEQ ID NO: 144**
KPLAEISDIELSYGIK	**SEQ ID NO: 145**
KPLAEIGDIELSYGIK	**SEQ ID NO: 146**

xxx. 15-amino acid peptides:

KPLAEGDIELSYGIK	**SEQ ID NO: 147**

xxxi. 13--amino acid peptides:

KPLAEIELSYGIK	**SEQ ID NO: 148**

xxxii. 16--amino acid peptides:

KPLAEIDSIELTYGIK	**SEQ ID NO: 149**
KPLAEIDGIELSYGIK	**SEQ ID NO: 150**

KPLAEIDGIELTYGIK	**SEQ ID NO: 151**
KPLAEIGSIELSYGIK	**SEQ ID NO: 152**
KGLAEIDSIELSYGIK	**SEQ ID NO: 153**
KPLAGIDSIGLSYGIK	**SEQ ID NO: 154**
KCLAEIDSCELSYGIK	**SEQ ID NO: 155**

xxxiii. 13--amino acid peptides:

CFKPLAEIDSIEC	**SEQ ID NO: 156**

xxxiv. 15-amino acid peptides:

VDVPEGDISLAYGLR	**SEQ ID NO: 157**
LDGLVRAYDNISPVG	**SEQ ID NO: 158**

xxxv. 14-amino acid peptides:

GDPNGDISVVYGLR	**SEQ ID NO: 159**

xxxvi. 15-amino acid peptides:

VDVPNGDISLAYRLR	**SEQ ID NO: 160**
VDVPEGDISLAYRLR	**SEQ ID NO: 161**
V(beta-D)TYDGDISVVYGLR	**SEQ ID NO: 167**
VDTY(beta-D)GDISVVYGLR	**SEQ ID NO: 168**
VDTYDG(beta-D)ISVVYGLR	**SEQ ID NO: 169**

In one embodiment said peptide is derived from osteopontin, such as a mammalian osteopontin variant and/or fragment.

In one embodiment, said peptide is non-naturally occurring, such as a peptide comprising non-proteinogenic amino acid residues.

In some embodiments, said peptide is further conjugated to a moiety, which may be selected from the group consisting of PEG, monosaccharides, fluorophores, chromophores, radioactive compounds, and cell-penetrating peptides. In one embodiment, the fluorophore is selected from the group consisting of Lucifer yellow, biotin, 5,6-carboxyltetramethylrhodamine (*TAMRA*), indodicarbocyanine (C5) Alexa Fluor® 488, Alexa Fluor® 532, Alexa Fluor® 647, ATTO 488, ATTO 532, 6-carboxyfluorescein (6-FAM), Alexa Fluor® 350, DY-415, ATTO 425, ATTO 465, Bodipy® FL, fluorescein isothiocyanate, Oregon Green® 488, Oregon Green® 514, Rhodamine Green™, 5'-Tetrachloro-Fluorescein, ATTO 520, 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluoresceine, Yakima Yellow™ dyes, Bodipy® 530/550, hexachloro-

fluorescein, Alexa Fluor® 555, DY-549, Bodipy® TMR-X, cyanine phosphoramidites (cyanine 3, cyanine 3.5, cyanine 5, cyanine 5.5, cyanine 7.5), ATTO 550, Rhodamine Red™, ATTO 565, Carboxy-X-Rhodamine, Texas Red (Sulforhodamine 101 acid chloride), LightCycler® Red 610, ATTO 594, DY-480-XL, DY-610, ATTO 610, LightCycler® Red 640, Bodipy 630/650, ATTO 633, Bodipy 650/665, ATTO 647N, DY-649, LightCycler® Red 670, ATTO 680, LightCycler® Red 705, DY-682, ATTO 700, ATTO 740, DY-782, IRD 700, IRD 800, CAL Fluor® Gold 540 nm, CAL Fluor® Gold 522 nm, CAL Fluor® Gold 544 nm , CAL Fluor® Orange 560 nm, CAL Fluor® Orange 538 nm, CAL Fluor® Orange 559 nm, CAL Fluor® Red 590 nm, CAL Fluor® Red 569 nm, CAL Fluor® Red 591 nm, CAL Fluor® Red 610 nm, CAL Fluor® Red 590 nm, CAL Fluor® Red 610 nm, CAL Fluor® Red 635 nm, Quasar® 570 nm, Quasar® 548 nm, Quasar® 566 nm (Cy 3), Quasar® 670 nm, Quasar® 647 nm, Quasar® 670 nm, Quasar® 705 nm, Quasar® 690 nm, Quasar® 705 nm (Cy 5.5), Pulsar® 650 Dyes, SuperRox® Dyes.).

In another embodiment, said peptide is further modified such as being glycosylated or by PEGylation, amidation, esterification, acylation, acetylation and/or alkylation.

In one embodiment, said peptide comprises or consists of tandem repeats, which may comprise or consist of the amino acid sequence of any one or more of the sequences as described herein.

In one embodiment, said peptide is cyclic. The cyclic structure may be achieved by any suitable method of synthesis. Thus, heterodetic linkages may include, but are not limited to formation via disulphide, cysteine, alkylene or sulphide bridges.

In a further embodiment, the peptide comprises or consists of a fusion. For example, the peptide may comprise a fusion of the amino acid sequence of SEQ ID NO: 1 or 136.

The term ‘fusion’ of a peptide relates to an amino acid sequence corresponding to, for example, SEQ ID NO: 1 or 136 (or a fragment or variant thereof) fused to any other peptide. For example, the said peptide may be fused to a polypeptide such as glutathione-S-transferase (GST) or protein A in order to facilitate purification of said peptide. Examples of such fusions are well known to those skilled in the art. Similarly,

the said peptide may be fused to an oligo-histidine tag such as His6 or to an epitope recognised by an antibody such as the well-known Myc tag epitope. Fusions to any variant or derivative of said peptide are also included in the scope of the disclosure.

Alternatively, the fused portion may be a lipophilic molecule or peptide domain that is capable of promoting cellular uptake of the polypeptide, as known to those skilled in the art.

Novel peptides

In one embodiment, the present disclosure relates to a peptide comprising or consisting of an amino acid sequence selected from the group consisting of KPLAEIDSIELSYGIK (SEQ ID NO: 136), GDPNDGRGDSVVYGLR (SEQ ID NO: 137), VDTYDGGISVVYGLR (SEQ ID NO: 138), and VDTYDGDGSVVYGLR (SEQ ID NO: 139), VDVPEGDISLAYGLR (SEQ ID NO: 157), LDGLVRAYDNISPVG (SEQ ID NO: 158), GDPNGDISVVYGLR (SEQ ID NO: 159), VDVPNGDISLAYRLR (SEQ ID NO: 160) VDVPEGDISLAYRLR (SEQ ID NO: 161), or a variant or fragment thereof.

In another embodiment, the present disclosure relates to a peptide comprising or consisting of an amino acid sequence selected from the group consisting of KCLAECDSIELSYGIK (SEQ ID NO: 141), CLAEIDSC (SEQ ID NO: 142), CFKPLAEIDSIECSYGIK (SEQ ID NO: 143), KPLAEDISIELSYGIK (SEQ ID NO: 145), KPLAEIGDIELSYGIK (SEQ ID NO: 146), KPLAEGDIELSYGIK (SEQ ID NO: 147), KPLAEIELSYGIK (SEQ ID NO: 148), KPLAEIDSIELTYGIK (SEQ ID NO: 149), KPLAEIDGIELSYGIK (SEQ ID NO: 150), KPLAEIDGIELTYGIK (SEQ ID NO: 151), KPLAEIGSIELSYGIK (SEQ ID NO: 152), KGLAEIDSIELSYGIK (SEQ ID NO: 153), KPLAGIDSIGLSYGIK (SEQ ID NO: 154), KCLAEIDSCELSYGIK (SEQ ID NO: 155) and CFKPLAEIDSIEC (SEQ ID NO: 156), or a variant or fragment thereof.

In one embodiment, the present disclosure relates to the agent comprising a peptide, wherein the peptide comprises or consists of the amino acid sequence KPLAEIDSIELSYGIK (SEQ ID NO: 136), or a variant or fragment thereof.

In one embodiment, the present disclosure relates to the agent comprising a peptide, wherein the peptide comprises or consists of the amino acid sequence KPLAGIDSIGLSYGIK (SEQ ID NO: 154), or a variant or fragment thereof.

In one embodiment, the present disclosure relates to the agent comprising a peptide, wherein the peptide comprises or consists of the amino acid sequence KGLAEIDSIELSYGIK (SEQ ID NO: 153), or a variant or fragment thereof.

In one embodiment, the present disclosure relates to the agent comprising a peptide, wherein the peptide comprises or consists of the amino acid sequence KCLAECDSIELSYGIK (SEQ ID NO: 141), or a variant or fragment thereof.

In one embodiment, the present disclosure relates to the agent comprising a peptide, wherein the peptide comprises or consists of the amino acid sequence KPLAEIDGIELTYGIK (SEQ ID NO: 151), or a variant or fragment thereof.

In one embodiment, the present disclosure relates to the agent comprising a peptide, wherein the peptide comprises or consists of the amino acid sequence KPLAEIGSIELSYGIK (SEQ ID NO: 152), or a variant or fragment thereof.

In one embodiment, the present disclosure relates to the agent comprising a peptide, wherein the peptide comprises or consists of the amino acid sequence KPLAEIELSYGIK (SEQ ID NO: 148), or a variant or fragment thereof.

In one embodiment, the present disclosure relates to an agent comprising:

b) a peptide or peptide analog comprising or consisting of the amino acid sequence GDPNDGRGDSVVYGLR (SEQ ID NO: 137), VDTYDGGISVVYGLR (SEQ ID NO: 138), and VDTYDGDGSVVYGLR (SEQ ID NO: 139). VDVPEGDISLAYGLR (SEQ ID NO: 157), LDGLVRAYDNISPVG (SEQ ID NO: 158), GDPNGDISVVYGLR (SEQ ID NO: 159), VDVPNGDISLAYRLR (SEQ ID NO: 160) VDVPEGDISLAYRLR (SEQ ID NO: 161), V(beta-D)TYDGDISVVYGLR (SEQ ID NO:167), VDTY(beta-D)GDISVVYGLR (SEQ ID NO: 168), VDTYDG(beta-D)ISVVYGLR (SEQ ID NO:169);

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c).

In some embodiments, said variant comprises or consists of a sequence wherein any one amino acid has been altered for another proteinogenic or non-proteinogenic amino acid, with the proviso that no more than five amino acids are so altered, such as no more than 4 amino acids, such as no more than 3 amino acids, such as no more than 2 amino acids, such as no more than 1 amino acid is altered. In some embodiments, one or more amino acids are conservatively substituted.

In some embodiments, said peptide comprises or consists of one or more additional amino acids, inserted at the N- and/or C-terminus and/or internally within the sequence. In one embodiment, at least 2 additional amino acids, such as at least 3, such as at least 4, such as at least 5, such as at least 6, such as at least 7, such as at least 8, such as at least 9, such as at least 10, such as at least 15 or such as at least 20 additional amino acids are inserted.

In some embodiments, said peptide is no more than 85, such as no more than 80, such as no more than 75, such as no more than 70, such as no more than 65, such as no more than 60, such as nor more than 55, such as no more than 50, such as no more than 55, such as no more than 40 amino acids, such as no more than 35, such as no more than 30, such as no more than 28, such as no more than 26, such as no more than 24, such as no more than 22, such as no more than 20, such as no more than 19, such as no more than 18, such as no more than 17, such as no more than 16, such as no more than 15, such as no more than 14, such as no more than 13, such as no more than 12, such as no more than 11, such as no more than 10 amino acids in length.

In some embodiments, said peptide is further conjugated to a moiety, which may be selected from the group consisting of PEG, monosaccharides, fluorophores, chromophores, radioactive compounds, and cell-penetrating peptides.

In one embodiment, said peptide is further modified such as being glycosylated or by PEGylation, amidation, esterification, acylation, acetylation and/or alkylation.

In some embodiments, said peptide comprises or consists of tandem repeats, which may comprise or consist of the amino acid sequence of any one or more of the sequences as described herein above.

In one embodiment, said peptide is cyclic. The cyclic structure may be achieved by any suitable method of synthesis. Thus, heterodetic linkages may include, but are not limited to formation via, cysteine, disulphide, alkylene or sulphide bridges.

Indications

The agents of the present disclosure are suitable for use in the treatment of endocrine, nutritional and metabolic diseases and disorders.

In one embodiment, the mammal in need of treatment of an endocrine disease, a nutritional disease and/or a metabolic disease is a human.

In some embodiments, the endocrine disease, nutritional disease and/or metabolic disease is selected from the group consisting of diabetes mellitus, type 1 diabetes mellitus, type 2 diabetes mellitus, malnutrition-related diabetes mellitus, disorders of glucose regulation and pancreatic internal secretion, insulin resistance syndrome, impaired glucose tolerance, hyperglycemia, hyperinsulinemia, and any combinations thereof.

In some embodiments, the endocrine disease, nutritional disease and/or metabolic disease is selected from the group consisting of diabetes mellitus, disorders of the thyroid gland, disorders of glucose regulation and pancreatic internal secretion, disorders of endocrine glands, malnutrition, nutritional deficiencies, obesity, hyperalimentation, and metabolic disorders.

In one embodiment, diabetes mellitus is selected from the group consisting of type 1 diabetes mellitus, type 2 diabetes mellitus, malnutrition-related diabetes mellitus, specified diabetes mellitus, and unspecified diabetes mellitus.

In one embodiment, disorders of glucose regulation and pancreatic internal secretion are selected from the group consisting of nondiabetic hypoglycaemic coma and disorders of pancreatic internal secretion.

In one embodiment, disorders of obesity and hyperalimentation are selected from the group consisting of localized adiposity, hyperalimentation, and sequelae of hyperalimentation.

In one embodiment, disorders of nutritional deficiencies are selected from the group consisting of disorders of aromatic amino-acid metabolism, disorders of branched-chain amino-acid metabolism and fatty-acid metabolism, disorders of amino-acid metabolism, lactose intolerance, disorders of carbohydrate metabolism, disorders of sphingolipid metabolism, disorders of lipid storage disorders, disorders of glycosaminoglycan metabolism, disorders of glycoprotein metabolism, disorders of lipoprotein metabolism, lipidaemias, disorders of purine and pyrimidine metabolism, disorders of porphyrin and bilirubin metabolism, disorders of mineral metabolism , cystic fibrosis, amyloidosis, volume depletion , disorders of fluid, electrolyte and acid-base balance, and postprocedural endocrine and metabolic disorders.

Compositions

In one aspect, the present disclosure relates to a composition comprising the agent described herein.

In one aspect, the present disclosure relates to an agent selected from the group consisting of:

a) a peptide or a peptide analog selected from the group consisting of

(i) a peptide comprising or consisting of an amino acid sequence of the general formula:

$KX_2LAX_5X_6X_7X_8IX_{10}LX_{12}YGIK$ **(SEQ ID NO: 140)**

wherein:

X_2 is C, P or G;

X_5 is E or G;

X_6 is C, D or I;

X_7 is D, I, S or G;

X_8 is S, D or G;

X_{10} is E or G;

X_{12} is S or T

with the proviso that if X_{12} is T, the peptide comprises no more than 25 amino acid residues; and

(ii) a peptide comprising or consisting of an amino acid sequence of the general formula:

$VDZ_3Z_4Z_5GZ_7Z_8SZ_{10}Z_{11}YGLR$ **(SEQ ID NO: 68)**

wherein:

Z_3 is T or V;
Z_4 is Y or P;
Z_5 is D or N;
Z_7 is D or G;
Z_8 is I or G;
Z_{10} is V or L;
Z_{11} is V or A; and

(iii) a peptide comprising or consists of an amino acid sequence selected from the group consisting of KCLAECDSIELSYGIK (SEQ ID NO: 141), CLAEIDSC (SEQ ID NO: 142), CFKPLAEIDSIECSYGIK (SEQ ID NO: 143), KPLAEIELSYGIK (SEQ ID NO: 148), KCLAEIDSCELSYGIK (SEQ ID NO: 155) and CFKPLAEIDSIEC (SEQ ID NO: 156);

b) a polynucleotide encoding upon expression, the peptide of a);
c) a vector comprising the polynucleotide of b); and
d) a cell comprising the polynucleotide of b), or the vector of c);

for use in the treatment of an endocrine disease, a nutritional disease and/or a metabolic disease in a mammal.

In one aspect, the present disclosure relates to a composition for use in treatment of an endocrine disease, a nutritional disease and/or a metabolic disease, comprising an agent described herein. In one embodiment, said composition is a pharmaceutical composition.

In one embodiment, the agent further comprises a second active ingredient. Said second active ingredient may be selected from the group consisting of insulin, glucagon-like peptide-1 (GLP-1), biguanides, forskolin compounds, sulfonylurea, a dipeptidyl peptidase-4 (DPP4) inhibitor, an alpha-glucosidase inhibitor, a thiazolidinedione, a meglitidine and a sodium-glucose cotransporter-2 (SGLT2) inhibitor.

Other methods

In one aspect, the present disclosure concerns a method of treating an endocrine disease, a nutritional disease and/or a metabolic disease, the method comprising administering an agent described herein to a subject in need thereof.

In one aspect, the present disclosure concerns the use of an agent for the manufacture of a medicament for use in treatment of an endocrine disease, a nutritional disease and/or a metabolic disease in a mammal.

In one aspect, the present disclosure concerns a polynucleotide encoding upon expression the peptide as described herein. In one aspect, the present disclosure concerns a vector comprising said polynucleotide encoding upon expression the peptide as described herein. In one aspect, the present disclosure concerns a cell comprising said polynucleotide or said vector encoding upon expression the peptide as described herein

In one aspect, the present disclosure concerns a method for increasing insulin secretion, the method comprising administering a therapeutically effective amount of a peptide described herein, to an individual in need thereof. In one embodiment, said method is an in vitro method.

In one aspect, the present disclosure concerns a method for decreasing blood glucose levels, the method comprising administering a therapeutically effective amount of a peptide described herein, to an individual in need thereof. In one embodiment, said method is an in vitro method. In one embodiment, insulin secretion is increased. In another embodiment, cellular uptake of glucose is increased. In yet another embodiment, insulin production is increased. In another embodiment glucagon production is decreased.

In one aspect, the present disclosure concerns a method, e.g. an in vitro method, for improving β- cell morphology, the method comprising administering a therapeutically effective amount of a peptide described herein, to an individual in need thereof.

In one aspect, the present disclosure concerns a method for improving β-cell viability, the method comprising administering a therapeutically effective amount of a peptide described herein, to an individual in need thereof.

In one aspect, the present disclosure concerns a method for delaying onset of diabetes and diabetes associated disorders and disease, the method comprising administering a

therapeutically effective amount of a peptide described herein, to an individual in need thereof.

In one embodiment of the present disclosure, the agent may further comprise a detectable moiety. For example, a detectable moiety may comprise or consist of a radioisotope, such as a radioisotope selected from the group consisting of ^{99m}Tc, ^{111}In, ^{67}Ga, ^{68}Ga, ^{72}As,^{89}Zr, ^{123}I and ^{201}Tl. The binding moieties may thus be coupled to nanoparticles that have the capability of multi-imaging (for example, SPECT, PET, MRI, Optical, or Ultrasound). Alternatively, the detectable moiety may comprise or consist of a paramagnetic isotope, such as a paramagnetic isotope is selected from the group consisting of ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr and ^{56}Fe.

In the case that the agent comprises a detectable moiety, then the detectable moiety may be detectable by an imaging technique such as SPECT, PET, MRI, optical or ultrasound imaging.

In one aspect, the present disclosure concerns the use of agent described herein for the preparation of a diagnostic composition for the diagnosis of a disease, disorder or damage of the pancreas in an individual.

Items

1. An agent comprising:

a) a peptide, wherein the peptide or peptide analog comprises an amino acid sequence of the general formula:

$KX_2LAX_5X_6X_7X_8IX_{10}LX_{12}YGIK$ **(SEQ ID NO: 140)**

wherein:

X_2 is C, P or G;

X_5 is E or G;

X_6 is C, D or I;

X_7 is D, I, S or G;

X_8 is S, D or G;

X_{10} is E or G;

X_{12} is S or T;

with the proviso that if X_{12} is T, the peptide comprises no more than 25 amino acid residues; and

with the proviso that if X_2 is P, X_5 is E, X_6 is I, X_7 is D, X_8 is S, X_{10} is E and X_{12} is S, the peptide comprises no more than 85 amino acid residues;

or a biologically active fragment and/or variant of SEQ ID NO: 140;

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c).

2. An agent comprising a peptide, wherein the peptide comprises an amino acid sequence of the general formula:

$VDVPZ_5GDISLAYZ_{13}LR$ **(SEQ ID NO: 164)**

wherein:

Z_5 is E or N;

Z_{13} is R or G.

3. An agent comprising a peptide, wherein the peptide comprises an amino acid sequence of the general formula:

VDTYDGZ$_7$Z$_8$SVVYGLR **(SEQ ID NO: 165)**

wherein:

Z$_7$ is D or G;

Z$_8$ is I or G.

4. An agent comprising a peptide, wherein the peptide comprises an amino acid sequence of the general formula:

GDPNZ$_5$Z$_6$Z$_7$Z$_8$Z$_9$SVVYGLR **(SEQ ID NO: 166)**

wherein:

Z$_5$ is D or G;

Z$_6$ is D or G

Z$_7$ is I or R;

Z$_8$ is G or absent;

Z$_9$ is D or absent.

5. The agent according to item 2 to 4, wherein the agent comprising:

a) a peptide, wherein the peptide comprises or consists of an amino acid sequence selected from the group consisting of GDPNDGRGDSVVYGLR (SEQ ID NO: 137), VDTYDGGISVVYGLR (SEQ ID NO: 138), and VDTYDGDGSVVYGLR (SEQ ID NO: 139). VDVPEGDISLAYGLR (SEQ ID NO: 157), LDGLVRAYDNISPVG (SEQ ID NO: 158), GDPNGDISVVYGLR (SEQ ID NO: 159), VDVPNGDISLAYRLR (SEQ ID NO: 160) VDVPEGDISLAYRLR (SEQ ID NO: 161);

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c).

6. The agent according to item 1, wherein the peptide comprises or consists of an amino acid sequence of the general formula:

KX$_2$LAX$_5$X$_6$X$_7$X$_8$IX$_{10}$LSYGIK **(SEQ ID NO: 162)**

wherein:

X$_2$ is C, P or G;

X$_5$ is E or G;

X_6 is C, I or absent;
X_7 is D, G or absent;
X_8 is S, G or absent;
X_{10} is E or G.

7. The agent according to item 6, wherein the peptide comprises an amino acid sequence of the general formula:
$KX_2LAX_5IX_{10}LSYGIK$ **(SEQ ID NO: 163)**
wherein:
X_2 is C, P or G;
X_5 is E or G;
X_{10} is E or G.

8. An agent comprising:
a) a peptide or peptide analog comprising or consisting of the amino acid sequence GDPNDGRGDSVVYGLR (SEQ ID NO: 137), VDTYDGGISVVYGLR (SEQ ID NO: 138), and VDTYDGDGSVVYGLR (SEQ ID NO: 139). VDVPEGDISLAYGLR (SEQ ID NO: 157), LDGLVRAYDNISPVG (SEQ ID NO: 158), GDPNGDISVVYGLR (SEQ ID NO: 159), VDVPNGDISLAYRLR (SEQ ID NO: 160) VDVPEGDISLAYRLR (SEQ ID NO: 161), V(beta-D)TYDGDISVVYGLR (SEQ ID NO:167), VDTY(beta-D)GDISVVYGLR (SEQ ID NO: 168), VDTYDG(beta-D)ISVVYGLR (SEQ ID NO:169);

b) a polynucleotide encoding upon expression, the peptide of a);
c) a vector comprising the polynucleotide of b); and
d) a cell comprising the polynucleotide of b), or the vector of c).

9. The agent according to any one of the preceding items, wherein the agent comprises non-naturally occurring, e.g. non-proteinogenic, amino acid residues.

10. The agent according to any one of the preceding items, wherein the agent is conjugated to a moiety.

11. The agent according to any one of the preceding items, wherein the moiety is selected from the group consisting of polyethylene glycol (PEG), monosaccharides,

fluorophores, chromophores, radioactive compounds, and cell-penetrating peptides.

12. The agent according to any one of the preceding items, wherein the agent is further modified such as being glycosylated or by PEGylation, amidation, esterification, acylation, acetylation and/or alkylation.

13. The agent according to any one of the preceding items, wherein the agent comprises or consists of tandem repeats.

14. The agent according to any one of the preceding items, wherein the tandem repeats comprise or consist of the amino acid sequence of any one or more of the sequences as described in the preceding items.

15. The agent according to any of the preceding items, wherein the agent is fused to another polypeptide.

16. The agent according to any one of the preceding items, wherein the said polypeptide is selected from the group consisting of glutathione-S-transferase (GST) and protein A.

17. The agent according to any of the preceding items, wherein the agent is fused to a tag.

18. The agent according to any one of the preceding items, wherein the said tag is an oligo-histidine tag.

19. The agent according to any of the preceding items, wherein the agent is cyclic, such as wherein the peptide is cyclic.

20. The agent according to any of the preceding items, wherein the peptide or peptide analog is capable of forming at least one intramolecular cysteine bridge, e.g. to form a cyclic or partially cyclic peptide.

21. The agent according to any of the preceding items, wherein the peptide or peptide analog comprises or consists of an amino acid sequence selected from the group consisting of KCLAECDSIELSYGIK (SEQ ID NO: 141), CLAEIDSC (SEQ ID NO: 142), CFKPLAEIDSIECSYGIK (SEQ ID NO: 143), KPLAEDISIELSYGIK (SEQ ID

NO: 145), KPLAEIGDIELSYGIK (SEQ ID NO: 146), KPLAEGDIELSYGIK (SEQ ID NO: 147), KPLAEIELSYGIK (SEQ ID NO: 148), KPLAEIDSIELTYGIK (SEQ ID NO: 149), KPLAEIDGIELSYGIK (SEQ ID NO: 150), KPLAEIDGIELTYGIK (SEQ ID NO: 151), KPLAEIGSIELSYGIK (SEQ ID NO: 152), KGLAEIDSIELSYGIK (SEQ ID NO: 153), KPLAGIDSIGLSYGIK (SEQ ID NO: 154), KCLAEIDSCELSYGIK (SEQ ID NO: 155) and CFKPLAEIDSIEC (SEQ ID NO: 156), or a variant or fragment thereof.

22. The agent according to any of the preceding items, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KPLAEIDSIELSYGIK (SEQ ID NO: 136), or a variant or fragment thereof.

23. The agent according to any of the preceding items, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KPLAGIDSIGLSYGIK (SEQ ID NO: 154), or a variant or fragment thereof.

24. The agent according to any of the preceding items, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KGLAEIDSIELSYGIK (SEQ ID NO: 153), or a variant or fragment thereof.

25. The agent according to any of the preceding items, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KCLAECDSIELSYGIK (SEQ ID NO: 141), or a variant or fragment thereof.

26. The agent according to any of the preceding items, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KPLAEIDGIELTYGIK (SEQ ID NO: 151), or a variant or fragment thereof.

27. The agent according to any of the preceding items, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KPLAEIGSIELSYGIK (SEQ ID NO: 152), or a variant or fragment thereof.

28. The agent according to any of the preceding items, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KPLAEIELSYGIK (SEQ ID NO: 148), or a variant or fragment thereof.

29. The agent according to any one of the preceding items, wherein the variant comprises or consists of a sequence wherein any one amino acid has been altered for another proteinogenic or non-proteinogenic amino acid, with the proviso that no more than five amino acids are so altered.

30. The agent according to any one of the preceding items, wherein the variant comprises or consists of a sequence wherein no more than five amino acids are altered for another proteinogenic or non-proteinogenic amino acid, such as no more than 4 amino acids, such as no more than 3 amino acids, such as no more than 2 amino acids, such as no more than 1 amino acid is altered.

31. The agent according to any one of the preceding items, wherein one or more amino acids are conservatively substituted.

32. The agent according to any one of the preceding items, wherein the peptide or peptide analog comprises or consists of one or more additional amino acids, inserted at the N- and/or C-terminus and/or internally within the sequence.

33. The agent according to any one of the preceding items, wherein the peptide or peptide analog comprises 1 additional amino acid conjugated to either N- or C-terminal.

34. The agent according to any of the preceding items, wherein the agent comprises no more than 85, such as no more than 80, such as no more than 75, such as no more than 70, such as no more than 65, such as no more than 60, such as nor more than 55, such as no more than 50, such as no more than 55, such as no more than 40 amino acids, such as no more than 35, such as no more than 30, such as no more than 28, such as no more than 26, such as no more than 24, such as no more than 22, such as no more than 20, such as no more than 19, such as no more than 18, such as no more than 17, such as no more than 16, such as no more than 15, such as no more than 14, such as no more than 13, such as no more than 12, such as no more than 11, such as no more than 10 amino acids.

35. The agent according to any one of the preceding items, wherein the agent comprises at least 2 additional amino acids, such as at least 3, such as at least 4, such as at least 5, such as at least 6, such as at least 7, such as at least 8, such as

at least 9, such as at least 10, such as at least 15 or such as at least 20 amino acids conjugated to the N- or C-terminus of the peptide.

36. The agent according to any of the preceding items, wherein the agent further comprises a detectable moiety.

37. The agent according to any of the preceding items, wherein the detectable moiety comprises or consists of a radioisotope.

38. The agent according to any of the preceding items, wherein the radioisotope is selected from the group consisting of ^{99m}Tc, ^{111}In, ^{67}Ga, ^{68}Ga, ^{72}As, ^{89}Zr, ^{123}I and ^{201}Tl.

39. The agent according to any of the preceding items, wherein the detectable moiety is detectable by an imaging technique such as SPECT, PET, MRI, optical or ultrasound imaging.

40. Use of the agent of any of the preceding items, for the preparation of a diagnostic composition for the diagnosis of a disease, disorder or damage of the pancreas in an individual.

41. A composition comprising the agent according to any of the preceding items.

42. The composition according to any one of the preceding items, wherein the composition is a pharmaceutical composition.

43. The agent or the composition according to any one of the preceding items, for use as a medicament.

44. An agent selected from the group consisting of:

a) a peptide selected from the group consisting of

(i) a peptide comprising or consisting of an amino acid sequence of the general formula:

$KX_2LAX_5X_6X_7X_8IX_{10}LX_{12}YGIK$ **(SEQ ID NO: 140)**

wherein:

X_2 is C, P or G;

X_5 is E or G;

X_6 is C, D or I;

X_7 is D, I, S or G;
X_8 is S, D or G;
X_{10} is E or G;
X_{12} is S or T;

with the proviso that if X_{12} is T, the peptide comprises no more than 25 amino acid residues;

or a biologically active fragment and/or variant of SEQ ID NO: 140;

(ii) a peptide comprising or consisting of an amino acid sequence of the general formula:

$VDZ_3Z_4Z_5GZ_7Z_8SZ_{10}Z_{11}YGLR$ **(SEQ ID NO: 68)**

wherein:

Z_3 is T or V;
Z_4 is Y or P;
Z_5 is D or N;
Z_7 is D or G;
Z_8 is I or G;
Z_{10} is V or L;
Z_{11} is V or A; and

b) a polynucleotide encoding upon expression, the peptide of a);
c) a vector comprising the polynucleotide of b); and
d) a cell comprising the polynucleotide of b), or the vector of c);

for use in the treatment of an endocrine disease, a nutritional disease and/or a metabolic disease in a mammal.

45. The agent or the composition for use according to item 44, wherein the peptide comprises or consists of an amino acid sequence selected from the group consisting of KCLAECDSIELSYGIK (SEQ ID NO: 141), CLAEIDSC (SEQ ID NO: 142), CFKPLAEIDSIECSYGIK (SEQ ID NO: 143), KPLAEIELSYGIK (SEQ ID NO: 148), KCLAEIDSCELSYGIK (SEQ ID NO: 155) and CFKPLAEIDSIEC (SEQ ID NO: 156);

46. The agent or the composition for use according to any one of the preceding items, wherein the peptide is selected from the group consisting of SEQ ID NO: 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155 and 156.

47. The agent or the composition for use according to any one of the preceding items, wherein the peptide is selected from the group consisting of SEQ ID NO: 1, 136, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 67, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 135, 137, 138, 139, 157, 158, 159, 160, 161, 167, 168 and 169.

48. The agent or the composition for use according to any one of the preceding items, wherein said agent comprises a second or further active ingredient.

49. The agent or the composition for use according to item 48, wherein the second or further active ingredient is selected from the group consisting of insulin, glucagon-like peptide-1 (GLP-1), sulfonylurea, a dipeptidyl peptidase-4 (DPP4) inhibitor, an alpha-glucosidase inhibitor, a thiazolidinedione, a meglitidine and a sodium-glucose cotransporter-2 (SGLT2) inhibitor.

50. The agent or the composition according to any of the preceding items for use in the treatment of an endocrine disease, a nutritional disease and/or a metabolic disease in a mammal.

51. The agent or the composition for use according to item 50, wherein the mammal is a human.

52. The agent or the composition for use according to any one of the preceding items, wherein the endocrine disease, nutritional disease and/or metabolic disease are selected from the group consisting of diabetes mellitus, type 1 diabetes mellitus, type 2 diabetes mellitus, malnutrition-related diabetes mellitus, disorders of glucose

regulation and pancreatic internal secretion, insulin resistance syndrome, impaired glucose tolerance, hyperglycemia, hyperinsulinemia, and any combinations thereof.

53. The agent or the composition for use according to any one of the preceding items, wherein the endocrine disease, nutritional disease and/or metabolic disease are selected from the group consisting of diabetes mellitus, disorders of the thyroid gland, disorders of glucose regulation and pancreatic internal secretion, disorders of endocrine glands, malnutrition, nutritional deficiencies, obesity, hyperalimentation, and metabolic disorders.

54. The agent or the composition for use according to any one of the preceding items, wherein the diabetes mellitus is selected from the group consisting of type 1 diabetes mellitus, type 2 diabetes mellitus, malnutrition-related diabetes mellitus, specified diabetes mellitus, and unspecified diabetes mellitus.

55. The agent or the composition for use according to any one of the preceding items, wherein the disorder of glucose regulation and pancreatic internal secretion is selected from the group consisting of nondiabetic hypoglycaemic coma and disorders of pancreatic internal secretion.

56. The agent or the composition for use according to any one of the preceding items, wherein the disorder of obesity and hyperalimentation is selected from the group consisting of localized adiposity, hyperalimentation, and sequelae of hyperalimentation.

57. The agent or the composition for use according to any one of the preceding items, wherein the disorder of nutritional deficiencies is selected from the group consisting of disorders of aromatic amino-acid metabolism, disorders of branched-chain amino-acid metabolism and fatty-acid metabolism, disorders of amino-acid metabolism, lactose intolerance, disorders of carbohydrate metabolism, disorders of sphingolipid metabolism, disorders of lipid storage disorders, disorders of glycosaminoglycan metabolism, disorders of glycoprotein metabolism, disorders of lipoprotein metabolism, lipiemias, disorders of purine and pyrimidine metabolism, disorders of porphyrin and bilirubin metabolism, disorders of mineral metabolism , cystic fibrosis, amyloidosis, volume depletion, disorders of fluid, electrolyte and acid-base balance, and postprocedural endocrine and metabolic disorders.

58. A method of treating an endocrine disease, a nutritional disease and/or a metabolic disease, the method comprising administering an agent according to any one of the preceding items to a subject in need thereof.

59. Use of an agent according to any one of the preceding items for the manufacture of a medicament for use in treatment of an endocrine disease, a nutritional disease and/or a metabolic disease in a mammal.

60. A method for delaying onset of diabetes and diabetes associated disorders and diseases, the method comprising administering a therapeutically effective amount of the agent as defined in any one of the preceding items, to an individual in need thereof.

61. A method for decreasing blood glucose levels, the method comprising administering a therapeutically effective amount of an agent of any one of the preceding items, to an individual in need thereof.

62. The method according to item 61, wherein insulin secretion is increased.

63. The method according to item 61, wherein cellular uptake of glucose is increased.

64. The method according to item 61, wherein the insulin production is increased.

65. The method according to item 61, wherein the glucagon production is decreased.

66. A method for improving beta cell viability, the method comprising administering a therapeutically effective amount of an agent of any one of the preceding items, to an individual in need thereof.

67. A method for improving beta cell morphology, the method comprising administering a therapeutically effective amount of an agent of any one of the preceding items, to an individual in need thereof.

68. A method for stabilising or improving viability and/or morphology of pancreatic islets, the method comprising administering a therapeutically effective amount of an agent of any one of the preceding items, to an individual in need thereof.

Examples

The disclosure is further illustrated by the following examples, which however should not be construed as being limiting for the disclosure. These examples demonstrate that exemplary peptides of the present disclosure stimulate β–cell proliferation, and have the ability to protect and rescue β–cells from apoptosis induced by glucotoxic conditions. It is also demonstrated that the exemplary peptides have the ability to stimulate insulin secretion from rat β-cells as well as isolated mouse pancreatic islets, where the peptides also are demonstrated to reduce glucagon levels. Furthermore, the examples demonstrate that the peptides reduce plasma glucose levels *in vivo* in a glucose tolerance test and that the peptides delay onset of type 1 diabetes in BB *lyp/lyp* rats

Example 1: Peptide design

The novel peptides were designed following rational structure activity investigations. For FOL-005 (SEQ ID NO: 1) the peptides were designed around the RGD site but mutated in order to generate different structures that potentially could interact with different integrins. A sequence similar to FOL-005 was identified in the third fibronectin type III repeat domain (TNfn3) in tenascin-C and found to be reasonably similar to the mutated RGD site of FOL-005. A peptide was designed from this sequence denoted FOL-014. The X-ray crystal structure of the tenascin-3 TNfn3 domain (PDB code 1TEN, Leahy et al. (1992) Science 258(5084):987-91) was analyzed. The FOL-014 (SEQ ID NO: 136) sequence span the beta-turn before and the entire 3rd beta sheet. FOL-014 variants were designed to allow for structural modification and stabilization of the 3-dimensional molecular structure. Specifically, the peptides variants covered the beta-turn region with exposed side chains and some cyclized variants to maintain geometry.

All peptides were synthesized by solid phase peptide synthesis using several peptide manufacturers. Mainly, the peptide variants have been provided by Biopeptide Inc., California.

Example 2: FOL-005 and FOL-014 induced proliferation of INS-1 Cells

To investigate if FOL-005 and FOL-014 could induce proliferation of β-cells we used INS-1 cells. Rat INS-1 cells were seeded in 96-well plates in RPMI medium with supplement and after 2 hours the medium was changed to RPMI without supplement.

During the proliferation experiment the cells were incubated at different test conditions (FOL-005, FOL-014, coated or in solution, 48h incubation) and during the last 20 hours of culture period the cells were pulsed with 1µ Ci/well of [methyl-3H] thymidine. The cells were then harvested onto glass fiber filters using a FilterMate harvester. The filters were air dried, and the bound radioactivity was measured using a liquid scintillation counter. To study whether FOL-005 influenced β-cell proliferation, INS-1 cells were treated with increasing amounts of soluble FOL-005 (0.06-6 µM) during 48 hours and proliferation was measured with radiolabeled thymidine incorporation into newly synthesized DNA. FOL-005 stimulated INS-1 cell proliferation (Fig. 1A). Wells coated with either FOL-005 or FOL-014 and later blocked with bovine serum albumin (BSA) before addition of INS-1 cells also stimulated proliferation compared to control (ctrl) coated wells (Fig. 1B-C).

This demonstrated that FOL-005 and FOL-014 interacted with β–cells and induced proliferation.

Example 3: FOL-005 protected β-cells from glucotoxicity

Since glucotoxicity in pancreatic β-cells is a well-established process in type 2 diabetes we next investigated the protective effects of FOL-005 on β-cells during glucotoxic conditions. First we confirmed that 20 mM glucose induced cell apoptosis in INS cells after 48h of exposure. High glucose (20 mM) containing RPMI medium induced more Annexin V positive cells and more caspase-3 activity in INS cells compared to cells incubated with medium containing 5 mM glucose (Fig. 2A-B). Exposure of INS-1 cells to 20 mM of glucose at the same time as FOL-005 decreased cell apoptosis as detected both by Annexin V staining and by caspase-3 activity (Fig.2 A-B). The rate of apoptosis in INS-1 cells was measured with either Caspase-3 Assay Kit or stained with Annexin V Apoptosis Detection Kit with 7-AAD. Caspase-3 activity was measured with fluorescence at an excitation wavelength of 380 nm and an emission wavelength of 440 nm. Caspase-3 activity was then normalized to protein concentration in each well. Measurements of Annexin V stained cells were performed using a CyAn ADP flow cytometer and analyzed with Summit V4.3 software.

In conclusion, it is well known that glucotoxicity induces β-cell apoptosis, however in the presence of FOL-005 glucotoxicity-induced apoptosis was diminished.

Example 4: FOL-005 induced insulin secretion from INS-1 cells

To investigate the stimulatory effect of FOL-005 on insulin secretion, INS-1 β-cells were used in the following experiments. Cells were seeded overnight in cRPMI and then washed with PBS before pre-incubation for 60 min at 37° C in Krebs-Ringer bicarbonate buffer (KRB), pH 7.4, supplemented with 10 mM HEPES, 0.1 % bovine serum albumin. After pre-incubation, the buffer was changed and the INS-1 cells were incubated at different test conditions (0 mM, 5 mM or 20 mM glucose) and stimulated with peptide FOL-005 or FOL-015 (SEQ ID NO: 158) or left untreated during 60 min at 37° C. Immediately after incubation, an aliquot of the buffer was removed and frozen for subsequent assay of insulin with an insulin radioimmunoassay kit.

The results demonstrated that β-cells stimulated with FOL-005 peptide secreted more insulin compared to unstimulated control cells or to cells stimulated with the FOL-015 control peptide (Fig. 3A) under conditions without glucose. INS-1 β-cells subjected to glucose (5 mM or 20 mM) responded with insulin secretion after FOL-005 peptide (6 µM) stimulation (Fig. 3B). INS-1 cells stimulated with 6 µM FOL-005 peptide in the presence of 20 mM glucose responded with more insulin secretion compared to FOL-005 stimulated cells incubated with 5 mM glucose (Fig. 3B).

Example 5: FOL-005 induced insulin secretion from mouse pancreatic islets

Mouse pancreatic islets were isolated from 8-week old C57BL/6J male mice (Taconic). Mice were sacrificed by an overdose of isoflurane and cervical dislocation. 3 ml of 0.9 U/ml collagenase P was injected into the pancreatic duct to inflate the pancreas. The pancreas was then removed and collagen digested for 19 min at 37 °C. The samples were vigorously shaken to disrupt the tissue. The digest was transferred into ice cold Hank's Balanced Salt Solution (HBSS) with Ca^{2+} and Mg^{2+}. The suspension was allowed to sit for 10 min to allow the islet to sink, and the islets were washed in fresh HBSS four times. The islets were then hand-picked and sorted according to size. Islets (n=3 per well in a 96 well plate) were pre-incubated in KRB buffer during 10 min 37º C, pH 7.4, supplemented with 10 mM HEPES, 0.1 % bovine serum albumin. After pre-incubation, the buffer was changed and islets were incubated at different test conditions in new KRB buffer with 0.1 % bovine serum albumin (non-treated ctrl, FOL-005 peptide, or GLP-1) for 60 min at 37° C. Immediately after incubation, an aliquot of the buffer was removed and frozen for subsequent assay of insulin.

The results demonstrated that isolated mouse pancreatic islets stimulated with GLP-1 (100 nM) or FOL-005 (6 µM) secreted more insulin compared to unstimulated control islets (Fig. 3C).

Example 6: FOL-014 induced insulin secretion from INS-1 cells

INS-1 β-cells were used to investigate the stimulatory effect of FOL-014 on insulin secretion. Cells were seeded overnight and then washed with PBS before pre-incubation for 60 min at 37° C in Krebs-Ringer bicarbonate buffer (KRB), pH 7.4, supplemented with 10 mM HEPES, 0.1 % bovine serum albumin. After pre-incubation, the buffer was changed and the INS-1 cells were incubated in new KRB buffer supplemented with 10 mM HEPES, 0.1 % bovine serum albumin and stimulated with peptide FOL-014 or left untreated during 60 min at 37° C. Immediately after incubation, an aliquot of the buffer was removed and frozen for subsequent assay of insulin.

The results demonstrated that β-cells stimulated with FOL-014 peptide secreted more insulin compared to unstimulated control cells (Fig. 4A).

Example 7: FOL-014 induced insulin secretion from mouse pancreatic islets

Mouse pancreatic islets were isolated from 8-week old C57BL/6J male mice as described under example 5. The islets were then hand-picked and sorted according to size. Islets (n=5 per well in a 96 well plate) were pre-incubated in 200 µl KRB buffer during 10 min 37º C, pH 7.4, supplemented with 10 mM HEPES, 0.1 % bovine serum albumin. Following pre-incubation, the buffer was changed and islets were incubated in different test conditions in new KRB buffer with 0.1 % bovine serum albumin (non-treated ctrl, FOL-014 peptide, and GLP-1) for 60 min at 37° C. Immediately after incubation, an aliquot of the buffer was removed and frozen for subsequent assay of insulin.

The result show that mouse pancreatic islets stimulated with FOL-014 (6 µM) secreted more insulin compared to unstimulated control islets (Fig. 4B). GLP-1 (100 nM) or FOL-014 (0.6 µM) did not affect insulin secretion (Fig. 4B).

Example 8-11: Stimulation of insulin secretion from INS-1 cell lines by FOL-014, FOL-005 and related peptides

Materials and methods: Rat INS-1 β-cells (passages 60–70) were cultured at 37 ℃ and 5% CO_2 in cRPMI media (RPMI 1640 supplemented with 10% fetal bovine serum,

50 IU/mL penicillin, 50 mg/L streptomycin, 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, and 50 µM beta-mercaptoethanol) unless otherwise stated. INS-1 cells were seeded in 96-well plates (2×10^3 cells/well) in cRPMI medium and following overnight incubation, the cells were washed in PBS before pre-incubation for 120 min at 37° C in Krebs-Ringer bicarbonate buffer, pH 7.4, supplemented with 10 mM HEPES, 0.1 % bovine serum albumin and 2.8 mM glucose. Following pre-incubation, the buffer was exchanged with fresh Krebs-Ringer buffer as described above and supplemented with specific glucose concentrations and peptides for the individual experiments as described below. Immediately after 60 minutes incubation at 37°C, an aliquot of the buffer was removed and frozen for subsequent insulin ELIZA assay.

Example 8. FOL-014 induced insulin secretion is dose-dependent in a non-linear manner

Insulin release from INS-1 cells were measured following exposure to increasing concentrations of FOL-014 and compared with the stimulatory effect of GLP-1 and untreated control during high glucose concentration (16.7 mM). All concentrations of FOL-014 tested elicited significantly higher insulin release as compared with the untreated control. At 6 nM or higher, FOL-014 triggered insulin release within the same range as 100 mM GLP-1. At concentrations ranging from 0.6–60 nM, insulin secretion increased in a linear fashion in relation to increasing FOL-014 concentrations. Exposure to FOL-014 concentrations ≥ 600 nM did not increase the insulin secretion (Figure 5).

The results demonstrated that FOL-014 significantly increased insulin secretion from INS-1 β-cells *in vitro* in a non-linear dose dependent fashion.

Example 9. The capacity of FOL-014 to induce insulin secretion is glucose dependent

Insulin release from INS-1 cells was measured following exposure to 60 nM FOL-014 at increasing concentrations of glucose. In untreated control samples, elevated glucose concentrations increased the insulin secretion at 11.1 mM glucose or higher. In the presence of FOL-014, insulin secretion increased significantly in a glucose dependent fashion already from 5.5 mM glucose. (Figure 6).

The results demonstrated that the presence of FOL-014 significantly increased insulin secretion from INS-1 β-cells *in vitro* in a glucose concentration dependent fashion and that FOL-014 was effective also at marginally elevated glucose levels.

Example 10. FOL-014 or FOL-005 in combination with GLP-1 increased insulin secretion as compared with either peptide alone.

Insulin secretion from INS-1 cells was measured following exposure to FOL-005, FOL-014, GLP-1 or combinations of those, expressed as percentage of untreated control. The combined effect of GLP-1 and FOL-014 resulted in a significantly higher insulin release than GLP-1 or FOL-014 alone. The additive effect of the combination of FOL-005 and GLP-1 was less pronounced, but did however increase the insulin secretion as compared with GLP-1 alone. The experiments were performed in the presence of 16.7 mM glucose (Figure 7).

The results demonstrated that the combination of GLP-1 and FOL-014 could further potentiate the insulin secretion from INS-1 cells *in vitro* as compared with each peptide alone. Furthermore, the combination of FOL-005 and GLP-1 tendentially increased insulin secretion.

Example 11. The ability of novel peptide analogues to induce insulin secretion in pancreatic β-cell-lines was investigated

Novel peptide analogues, derived from either FOL-005 or FOL-014 were tested concerning their ability to induce insulin secretion in two separate INS-1 cell lines in the presence of 16.7 mM glucose. FOL-005, FOL-014 and GLP-1 as well as a high glucose (16.7 mM) and a low glucose (2.8 mM) control (not shown) was included in each experiment and the peptide concentration was 100 nM. In order to correct for the variance between experiments, all values were normalized to, and expressed as percentage of the average value of the high glucose control in the individual experiments. The analogues were subsequently ranked according to performance (Figure 11A and 11B). Peptide analogues eliciting an insulin response below the high glucose control average value were considered non-functional and were hence excluded (not shown).

The results demonstrated the capacity of several novel peptide analogues to enhance insulin secretion from INS-1 β-cells *in vitro*.

Example 12. FOL-014 increase insulin secretion from mouse-derived pancreatic islets

Twelve-week-old male C57/bl6 mice were euthanized with isoflurane and cervical dislocation. After clamping the hepatic ducts, 3 ml of 0.9 U/ml collagenase P was injected into the bile duct to inflate the pancreas. The pancreas was then removed and digested for 19 min at 37 °C. The samples were vigorously shaken to disrupt the tissue. The digest was quickly transferred into ice cold Hank's Balanced Salts Solution with Ca^{2+} and Mg^{2+}. The suspension was allowed to sit for 8 min to allow the islet to sink, and the islets were washed in the same manner four times. The islets were then handpicked and sorted according to size.

Freshly isolated islets were seeded in groups of 5 in a 96-well plate and preincubated for 1h at 37°C in a Krebs-Ringer bicarbonate buffer (pH 7.4). The islets were incubated for 1h at 37°C in Krebs-Ringer buffered solution supplemented with 0.6 or 6 µM FOL-014 or 100 nM GLP-1 or left unsupplemented for control. Immediately after incubation, the medium was removed for assays of insulin and glucagon using Mercodia's ELISA kits. The effect of FOL-014 on insulin (Figure 8A and B) and glucagon (Figure 8C and D) secretion from isolated mouse islets was measured in the presence of low glucose (2.8 mM; Figure 8A and C) or high glucose (16.7 mM; Figure 8B and D) concentrations. A significant effect of FOL-014 was observed in the presence of high glucose for insulin and in the presence of both high and low glucose for glucagon. The effect of FOL-014 differed from that of GLP-1, which enhanced insulin secretion also in low glucose samples but failed to inhibit glucagon secretion in low glucose conditions.

The results demonstrated that FOL-014 enhanced insulin secretion and inhibited glucagon secretion in pancreatic islets.

Example 13. FOL-014 reduced plasma glucose levels in an Intraperitoneal Glucose Tolerance Test (IPGTT) in mice

Whole blood was collected for glucose and insulin measurements from 10-week-old wild type maleC57bl/6 mice. After a 4 hour fast, the mice were divided into three groups and given an intraperitoneal injection (ip) of either saline, 30 nmol/kg peptide (Figure 9A) or 200 nmol/kg peptide (Figure 9B). 15 min after the FOL-014 or saline (control) injections, the mice were administered 2 g of glucose/kg ip. Blood glucose concentrations were measured at 5, 15, 30, 45 and 60 minutes after the glucose

injection. Statistical calculations were performed using student's t-test. FOL-014 dosed at 200 nmol/kg significantly lowered the plasma glucose levels as compared to the control when measured as area under the curve. In addition, the difference was significant at 15, 30 and 45 minutes. At the 30 nmol/kg dose, FOL-014 lowered the plasma glucose levels with a significant effect at 45 minutes after the glucose injection.

The results demonstrated that FOL-014 could lower plasma glucose levels in a glucose tolerance test performed on healthy wild type mice.

Example 14. FOL-014 delayed onset of Type 1 Diabetes in BB *lyp/lyp* rats

BB *lyp/lyp* rats were randomized for placebo (sodium chloride, 9 mg/ml) or FOL-014 treatment 3 times/week from day 40 until onset of type 1 diabetes, defined as plasma glucose levels ≥ 11.1 mM. The dose of 100 nmol/kg FOL-014 peptide in saline or placebo (saline) was administered subcutaneously and the animals were terminated immediately upon exceeding critical plasma glucose levels. The difference between FOL-014 treated animals and animals receiving placebo treatment was significant both when expressed as average age for onset of type 1 diabetes (Figure 10A) and when described as percentage of animals developing type 1 diabetes per day (Figure 10B).

The results demonstrated that FOL-014 treatment significantly delayed the onset of type-1 diabetes in BB *lyp/lyp* rats.

Example 15. FOL-005 and FOL-014 displayed organ specific distribution patterns in mice.

C57Bl/6 mice were injected subcutaneously with H^3 labelled FOL-005 and euthanized at 1h (Figure 12A) or 2h (Figure 12B) after injection. Following whole body sectioning the distribution of the labelled peptide was visualised. Strong binding was evident in pancreas and at the site of injection. Using Pearl Trilogy Small Animal Imaging System, *in vivo* bio-distribution and tissue localization of two Cy7.5 labelled peptides, FOL-005 (Figure 12C) and FOL-014 (Figure 12D) in NMRI nude mice via subcutaneous injection was investigated. High accumulation of the peptide was evident in the pancreatic tissue area. The same distribution pattern was found after i.v. administrations (not shown). The dose of each peptide was 10 nmol per mouse. The mice were imaged before injection, at 5min, 20min, 50min, 60min, 2hrs, 4hrs, 6hrs, 24hrs and 48 hrs post administration of labelled peptide.

Example 16. Tissue specific imaging for diagnostic use

Agents prepared as defined herein above are labelled by conjugation to suitable imaging probe or moiety, using methods known by those of skill in the art. The conjugated peptide-probe agents are subsequently administered to a subject and biodistribution is subsequently monitored e.g. up to 48h after administration. The conjugated agent is thus used as a diagnostic or prognostic tool for investigation of pancreatic status. As such, the conjugated agents are suitable for detecting, diagnosing, or monitoring disease, disease processes and progression, susceptibility, as well as to determine efficacy of a treatment. The agents are particularly suited for monitoring the diabetic status of a subject. The conjugated agents are also used for monitoring and/or predicting risk of developing a disease, specifically diabetes. The test is used alone or in combination with other tests known by those of skill in the art, such as blood tests, genetic testing, urine test, and biopsies.

Example 17: Sequence overview

SEQ ID NO	Sequence	Notes
1	VDTYDGDISVVYGLR	FOL-005
2	VDTYDGDISVVYGLS	
3	VDTYDGDISVVYGL	FOL-025
4	DTYDGDISVVYGLR	
5	TYDGDISVVYGLRS	
6	VDTYDGDISVVYG	FOL-024
7	DTYDGDISVVYGL	
8	TYDGDISVVYGLR	
9	YDGDISVVYGLRS	
10	VDTYDGDISVVY	
11	DTYDGDISVVYG	
12	TYDGDISVVYGL	
13	YDGDISVVYGLR	
14	DGDISVVYGLRS	
15	VDTYDGDISVV	
16	DTYDGDISVVY	
17	TYDGDISVVYG	

18	YDGDISVVYGL	
19	DGDISVVYGLR	
20	GDISVVYGLRS	
21	VDTYDGDISV	
22	DTYDGDISVV	
23	TYDGDISVVY	
24	YDGDISVVYG	
25	DGDISVVYGL	
26	GDISVVYGLR	FOL-009h
27	DISVVYGLRS	
28	VDTYDGDIS	FOL-019h
29	DTYDGDISV	
30	TYDGDISVV	
31	YDGDISVVY	
32	DGDISVVYG	
33	GDISVVYGL	
34	DISVVYGLR	
35	ISVVYGLRS	
36	VDTYDGDI	
37	DTYDGDIS	
38	TYDGDISV	
39	YDGDISVV	
40	DGDISVVY	
41	GDISVVYG	
42	DISVVYGL	
43	ISVVYGLR	
44	VDTYDGD	
45	DTYDGDI	
46	TYDGDIS	
47	YDGDISV	
48	DGDISVV	
49	GDISVVY	
50	DISVVYG	
51	ISVVYGL	

52	DTYDGD	
53	TYDGDI	
54	YDGDIS	
55	DGDISV	
56	GDISVV	
57	DISVVY	
58	ISVVYG	
59	TYDGD	
60	YDGDI	
61	DGDIS	
62	GDISV	
63	DISVV	
64	ISVVY	
65	SVVYG	
66	MRIAVICFCLLGITCAIPVKQADSGSSEEKQLY NKYPDAVATWLNPDPSQKQNLLAPQTLPSK SNESHDHMDDMDDEDDDDHVDSQDSIDSN DSDDVDDTDDSHQSDESHHSDESDELVTDF PTDLPATEVFTPVVPT*VDTYDGRGDSVVYGL R*SKSKKFRRPDIQYPDATDEDITSHMESEEL NGAYKAIPVAQDLNAPSDWDSRGKDSYETS QLDDQSAETHSHKQSRLYKRKANDESNEHS DVIDSQELSKVSREFHSHEFHSHEDMLVVDP KSKEEDKHLKFRISHELDSASSEVN	Wildtype human osteopontin, i.e. GenBank: AAA59974.1
67	VDTYDGRGDSVVYGLR	FOL-002
68	$VDZ_3Z_4Z_5GZ_7Z_8SZ_{10}Z_{11}YGLR$	Z_3 is T or V; Z_4 is Y or P; Z_5 is D or N; Z_7 is D or G; Z_8 is I or G; Z_{10} is V or L; Z_{11} is V or A
69	VDVPNGDISLAYGLR	FOL-004
70	DVPNGDISLAYGLRS	

71	VDVPNGDISLAYGL	FOL-016
72	DVPNGDISLAYGLR	FOL-007
73	VPNGDISLAYGLRS	
74	VDVPNGDISLAYG	FOL-017
75	DVPNGDISLAYGL	
76	VPNGDISLAYGLR	
77	PNGDISLAYGLRS	
78	VDVPNGDISLAY	
79	DVPNGDISLAYG	
80	VPNGDISLAYGL	
81	PNGDISLAYGLR	FOL-008
82	NGDISLAYGLRS	
83	VDVPNGDISLA	FOL-018
84	DVPNGDISLAY	
85	VPNGDISLAYG	
86	PNGDISLAYGL	
87	NGDISLAYGLR	
88	GDISLAYGLRS	
89	VDVPNGDISL	
90	DVPNGDISLA	
91	VPNGDISLAY	
92	PNGDISLAYG	
93	NGDISLAYGL	
94	GDISLAYGLR	FOL-009
95	DISLAYGLRS	
96	VDVPNGDIS	FOL-019
97	DVPNGDISL	
98	VPNGDISLA	
99	PNGDISLAY	
100	NGDISLAYG	
101	GDISLAYGL	
102	DISLAYGLR	
103	ISLAYGLRS	
104	VDVPNGDI	

105	DVPNGDIS	
106	VPNGDISL	
107	PNGDISLA	
108	NGDISLAY	
109	GDISLAYG	
110	DISLAYGL	
111	ISLAYGLR	
112	VDVPNGD	
113	DVPNGDI	
114	VPNGDIS	
115	PNGDISL	
116	NGDISLA	
117	GDISLAY	
118	DISLAYG	
119	ISLAYGL	
120	DVPNGD	
121	VPNGDI	
122	PNGDIS	
123	NGDISL	
124	GDISLA	
125	DISLAY	
126	ISLAYG	
127	VPNGD	
128	PNGDI	
129	NGDIS	
130	GDISL	
131	DISLA	
132	ISLAY	
133	SLAYG	
134	MRLAVICFCLFGIASSLPVKVTDSGSSEEKLY SLHPDPIATWLVPDPSQKQNLLAPQNAVSSE EKDDFKQETLPSNSNESHDHMDDDDDDDD DDGDHAESEDSVDSDESDESHHSDESDETV TASTQADTFTPIVPT*VDVPNGRGDSLAYGLR*	Wildtype murine osteopontin, *i.e.* NCBI Reference Sequence: NP_001191162.1

	SKSRSFQVSDEQYPDATDEDLTSHMKSGES KESLDVIPVAQLLSMPSDQDNNGKGSHESS QLDEPSLETHRLEHSKESQESADQSDVIDSQ ASSKASLEHQSHKFHSHKDKLVLDPKSKEDD RYLKFRISHELESSSSEVN	
135	VDVPNGRGDSLAYGLR	FOL-001
136	KPLAEIDSIELSYGIK	FOL-014
137	GDPNDGRGDSVVYGLR	FOL-003
138	VDTYDGGISVVYGLR	FOL-026
139	VDTYDGDGSVVYGLR	FOL-027
140	$KX_2LAX_5X_6X_7X_8IX_{10}LX_{12}YGIK$	X_2 is C, P or G; X_5 is E or G; X_6 is C, D or I; X_7 is D, I, S or G; X_8 is S, D or G; X_{10} is E or G; X_{12} is S or T;
141	KCLAECDSIELSYGIK (Cyclic)	FOL-032
142	CLAEIDSC (Cyclic)	FOL-033
143	CFKPLAEIDSIECSYGIK (Cyclic)	FOL-036
144	KPLAEDISIELSYGIK	FOL-037
145	KPLAEISDIELSYGIK	FOL-038
146	KPLAEIGDIELSYGIK	FOL-039
147	KPLAEGDIELSYGIK	FOL-040
148	KPLAEIELSYGIK	FOL-041
149	KPLAEIDSIELTYGIK	FOL-042
150	KPLAEIDGIELSYGIK	FOL-043
151	KPLAEIDGIELTYGIK	FOL-044
152	KPLAEIGSIELSYGIK	FOL-045
153	KGLAEIDSIELSYGIK	FOL-046
154	KPLAGIDSIGLSYGIK	FOL-047
155	Cyclic K**C**LAEIDS**C**ELSYGIK	FOL-034
156	Cyclic **C**FKPLAEIDSIE**C**	FOL-035
157	VDVPEGDISLAYGLR	FOL-010

158	LDGLVRAYDNISPVG	FOL-015
159	GDPNGDISVVYGLR	FOL-006
160	VDVPNGDISLAYRLR	FOL-011
161	VDVPEGDISLAYRLR	FOL-012
162	$KX_2LAX_5X_6X_7X_8IX_{10}LSYGIK$	X_2 is C, P or G; X_5 is E or G; X_6 is C, I or absent; X_7 is D, G or absent; X_8 is S, G or absent; X_{10} is E or G;
163	$KX_2LAX_5IX_{10}LSYGIK$	X_2 is C, P or G; X_5 is E or G; X_{10} is E or G.
164	$VDVPZ_5GDISLAYZ_{13}LR$	Z_5 is E or N; Z_{13} is R or G.
165	$VDTYDGZ_7Z_8SVVYGLR$	Z_7 is D or G; Z_8 is I or G.
166	$GDPNZ_5Z_6Z_7Z_8Z_9SVVYGLR$	Z_5 is D or G; Z_6 is D or G Z_7 is I or R; Z_8 is G or absent; Z_9 is D or absent.
167	$VZ_2TYDGDISVVYGLR$	Z_2 is beta D FOL-005 (2betaAsp)
168	VDTY Z_5GDISVVYGLR	Z_5 is beta D FOL-005 (5betaAsp)
169	VDTYDG Z_7ISVVYGLR	FOL-005 (7betaAsp) Z_7 is beta D

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Claims

1. An agent comprising:

a) a peptide or peptide analog, wherein the peptide or peptide analog comprises an amino acid sequence of the general formula:

$KX_2LAX_5X_6X_7X_8IX_{10}LX_{12}YGIK$ **(SEQ ID NO: 140)**

wherein:

X_2 is C, P or G;

X_5 is E or G;

X_6 is C, D or I;

X_7 is D, I, S or G;

X_8 is S, D or G;

X_{10} is E or G;

X_{12} is S or T;

with the proviso that if X_{12} is T, the peptide comprises no more than 25 amino acid residues; and

with the proviso that if X_2 is P, X_5 is E, X_6 is I, X_7 is D, X_8 is S, X_{10} is E and X_{12} is S, the peptide comprises no more than 85 amino acid residues;

or a biologically active fragment and/or variant thereof, selected from the group consisting of CLAEIDSC (SEQ ID NO: 142), CFKPLAEIDSIECSYGIK (SEQ ID NO: 143), KPLAEGDIELSYGIK (SEQ ID NO: 147), KPLAEIELSYGIK (SEQ ID NO: 148), KCLAEIDSCELSYGIK (SEQ ID NO: 155), and CFKPLAEIDSIEC (SEQ ID NO: 156);

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c).

2. The agent according to claim 1, wherein the peptide or peptide analog comprises an amino acid sequence of the general formula:

$KX_2LAX_5X_6X_7X_8IX_{10}LSYGIK$ **(SEQ ID NO: 162)**

wherein:

X_2 is C, P or G;
X_5 is E or G;
X_6 is C, I or absent;
X_7 is D, G or absent;
X_8 is S, G or absent;
X_{10} is E or G.

3. The agent according to any of the preceding claims, wherein the agent comprises no more than 85, such as no more than 80, such as no more than 75, such as no more than 70, such as no more than 65, such as no more than 60, such as nor more than 55, such as no more than 50, such as no more than 55, such as no more than 40 amino acids, such as no more than 35, such as no more than 30, such as no more than 28, such as no more than 26, such as no more than 24, such as no more than 22, such as no more than 20, such as no more than 19, such as no more than 18, such as no more than 17, such as no more than 16, such as no more than 15, such as no more than 14, such as no more than 13, such as no more than 12, such as no more than 11, such as no more than 10 amino acids.

4. The agent according to any one of the preceding claims, wherein the agent comprises at least 2 additional amino acids, such as at least 3, such as at least 4, such as at least 5, such as at least 6, such as at least 7, such as at least 8, such as at least 9, such as at least 10, such as at least 15 or such as at least 20 amino acids conjugated to the N- or C-terminus of the peptide.

5. The agent according to any one of the preceding claims, wherein the agent is non-naturally occurring.

6. The agent according to any one of the preceding claims, wherein the agent is conjugated to a moiety.

7. The agent according to claim 6, wherein the moiety is selected from the group consisting of polyethylene glycol (PEG), monosaccharides, fluorophores, chromophores, radioactive compounds, and cell-penetrating peptides.

8. The agent according to any one of the preceding claims, wherein the agent is further modified such as being glycosylated or by PEGylation, amidation, esterification, acylation, acetylation and/or alkylation.

9. The agent according to any one of the preceding claims, wherein the agent comprises or consists of tandem repeats.

10. The agent according to claim 9, wherein the tandem repeats comprise or consist of the amino acid sequence of any one or more of the sequences as described in the preceding claims.

11. The agent according to any of the preceding claims, wherein the agent is fused to another polypeptide.

12. The agent according to claim 11, wherein the said polypeptide is selected from the group consisting of glutathione-S-transferase (GST) and protein A.

13. The agent according to any of the preceding claims, wherein the agent is fused to a tag.

14. The agent according to claim 13, wherein the said tag is an oligo-histidine tag.

15. The agent according to any of the preceding claims, wherein the agent is cyclic.

16. The agent according to any of the preceding claims, wherein the peptide or peptide analog is capable of forming at least one intramolecular cysteine bridge.

17. The agent according to any of the preceding claims, wherein the peptide or peptide analog comprises or consists of an amino acid sequence selected from the group consisting of KCLAECDSIELSYGIK (SEQ ID NO: 141), CLAEIDSC (SEQ ID NO: 142), CFKPLAEIDSIECSYGIK (SEQ ID NO: 143), KPLAEDISIELSYGIK (SEQ ID NO: 145), KPLAEIGDIELSYGIK (SEQ ID NO: 146), KPLAEGDIELSYGIK (SEQ ID NO: 147), KPLAEIELSYGIK (SEQ ID NO: 148), KPLAEIDSIELTYGIK (SEQ ID NO: 149), KPLAEIDGIELSYGIK (SEQ ID NO: 150), KPLAEIDGIELTYGIK (SEQ ID NO:

151), KPLAEIGSIELSYGIK (SEQ ID NO: 152), KGLAEIDSIELSYGIK (SEQ ID NO: 153), KPLAGIDSIGLSYGIK (SEQ ID NO: 154), KCLAEIDSCELSYGIK (SEQ ID NO: 155) and CFKPLAEIDSIEC (SEQ ID NO: 156), or a variant or fragment thereof.

18. The agent according to any of the preceding claims, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KPLAEIDSIELSYGIK (SEQ ID NO: 136), or a variant or fragment thereof.

19. The agent according to any of the preceding claims, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KPLAGIDSIGLSYGIK (SEQ ID NO: 154), or a variant or fragment thereof.

20. The agent according to any of the preceding claims, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KGLAEIDSIELSYGIK (SEQ ID NO: 153), or a variant or fragment thereof.

21. The agent according to any of the preceding claims, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KCLAECDSIELSYGIK (SEQ ID NO: 141), or a variant or fragment thereof.

22. The agent according to any of the preceding claims, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KPLAEIDGIELTYGIK (SEQ ID NO: 151), or a variant or fragment thereof.

23. The agent according to any of the preceding claims, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KPLAEIGSIELSYGIK (SEQ ID NO: 152), or a variant or fragment thereof.

24. The agent according to any of the preceding claims, wherein the peptide or peptide analog comprises or consists of the amino acid sequence KPLAEIELSYGIK (SEQ ID NO: 148), or a variant or fragment thereof.

25. The agent according to any one of the preceding claims, wherein the variant comprises or consists of a sequence wherein any one amino acid has been altered

for another proteinogenic or non-proteinogenic amino acid, with the proviso that no more than five amino acids are so altered.

26. The agent according to any one of the preceding claims, wherein the variant comprises or consists of a sequence wherein no more than five amino acids are altered for another proteinogenic or non-proteinogenic amino acid, such as no more than 4 amino acids, such as no more than 3 amino acids, such as no more than 2 amino acids, such as no more than 1 amino acid is altered.

27. The agent according to any one of the preceding claims, wherein one or more amino acids are conservatively substituted.

28. The agent according to any one of the preceding claims, wherein the peptide or peptide analog comprises or consists of one or more additional amino acids, inserted at the N- and/or C-terminus and/or internally within the sequence.

29. The agent according to any one of the preceding claims, wherein the peptide or peptide analog has 1 additional amino acid.

30. The agent according to any of the preceding claims, wherein the agent further comprises a detectable moiety.

31. The agent according to any of the preceding claims, wherein the detectable moiety comprises or consists of a radioisotope.

32. The agent according to any of the preceding claims, wherein the radioisotope is selected from the group consisting of ^{99m}Tc, ^{111}In, ^{67}Ga, ^{68}Ga, ^{72}As,^{89}Zr, ^{123}I and ^{201}Tl.

33. The agent according to any of the preceding claims, wherein the detectable moiety is detectable by an imaging technique such as SPECT, PET, MRI, optical or ultrasound imaging.

34. Use of the agent according to any of the preceding claims, for the preparation of a diagnostic composition for the diagnosis of a disease, disorder or damage of the pancreas in an individual.

35. A composition comprising the agent according to any of the preceding claims.

36. The composition according to claim 35, wherein the composition is a pharmaceutical composition.

37. An agent comprising:

c) a peptide or peptide analog comprising or consisting of the amino acid sequence GDPNDGRGDSVVYGLR (SEQ ID NO: 137), VDTYDGGISVVYGLR (SEQ ID NO: 138), and VDTYDGDGSVVYGLR (SEQ ID NO: 139). VDVPEGDISLAYGLR (SEQ ID NO: 157), LDGLVRAYDNISPVG (SEQ ID NO: 158), GDPNGDISVVYGLR (SEQ ID NO: 159), VDVPNGDISLAYRLR (SEQ ID NO: 160) VDVPEGDISLAYRLR (SEQ ID NO: 161), V(beta-D)TYDGDISVVYGLR (SEQ ID NO:167), VDTY(beta-D)GDISVVYGLR (SEQ ID NO: 168), VDTYDG(beta-D)ISVVYGLR (SEQ ID NO:169);

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c).

38. The agent or the composition according to any one of the preceding claims, for use as a medicament.

39. An agent comprising:

a) a peptide or a peptide analog selected from the group consisting of:
(i) a peptide comprising or consisting of an amino acid sequence of the general formula:

$KX_2LAX_5X_6X_7X_8IX_{10}LX_{12}YGIK$ **(SEQ ID NO: 140)**

wherein:

X_2 is C, P or G;

X_5 is E or G;

X_6 is C, D or I;

X_7 is D, I, S or G;

X_8 is S, D or G;

X_{10} is E or G;

X_{12} is S or T;

with the proviso that if X_{12} is T, the peptide comprises no more than 25 amino acid residues;

(ii) a peptide comprising or consisting of an amino acid sequence of the general formula:

$VDZ_3Z_4Z_5GZ_7Z_8SZ_{10}Z_{11}YGLR$ **(SEQ ID NO: 68)**

wherein:

Z_3 is T or V;

Z_4 is Y or P;

Z_5 is D or N;

Z_7 is D or G;

Z_8 is I or G;

Z_{10} is V or L;

Z_{11} is V or A; and

(iii) a peptide comprising or consists of an amino acid sequence selected from the group consisting of KCLAECDSIELSYGIK (SEQ ID NO: 141), CLAEIDSC (SEQ ID NO: 142), CFKPLAEIDSIECSYGIK (SEQ ID NO: 143), KPLAEGDIELSYGIK (SEQ ID NO: 147), KPLAEIELSYGIK (SEQ ID NO: 148), KCLAEIDSCELSYGIK (SEQ ID NO: 155) and CFKPLAEIDSIEC (SEQ ID NO: 156);

b) a polynucleotide encoding upon expression, the peptide of a);

c) a vector comprising the polynucleotide of b); and

d) a cell comprising the polynucleotide of b), or the vector of c);

for use in the treatment of an endocrine disease, a nutritional disease and/or a metabolic disease in a mammal.

40. The agent or the composition for use according to any one of the preceding claims, wherein said agent comprises a second or further active ingredient.

41. The agent or the composition for use according to claim 40, wherein the second or further active ingredient is selected from the group consisting of insulin, glucagon-like peptide-1 (GLP-1), sulfonylurea, a dipeptidyl peptidase-4 (DPP4) inhibitor, an alpha-glucosidase inhibitor, a thiazolidinedione, a meglitidine and a sodium-glucose cotransporter-2 (SGLT2) inhibitor.

42. The agent or the composition according to any of the preceding claims for use in the treatment of an endocrine disease, a nutritional disease and/or a metabolic disease in a mammal.

43. The agent or the composition for use according to claim 42, wherein the mammal is a human.

44. The agent or the composition for use according to any of the preceding claims,, wherein the endocrine disease, nutritional disease and/or metabolic disease are selected from the group consisting of diabetes mellitus, type 1 diabetes mellitus, type 2 diabetes mellitus, malnutrition-related diabetes mellitus, disorders of glucose regulation and pancreatic internal secretion, insulin resistance syndrome, impaired glucose tolerance, hyperglycemia, hyperinsulinemia, and any combinations thereof.

45. The agent or the composition for use according to any of the preceding claims,, wherein the endocrine disease, nutritional disease and/or metabolic disease are selected from the group consisting of diabetes mellitus, disorders of the thyroid gland, disorders of glucose regulation and pancreatic internal secretion, disorders of endocrine glands, malnutrition, nutritional deficiencies, obesity, hyperalimentation, and metabolic disorders.

46. The agent or the composition for use according to any of the preceding claims,, wherein the diabetes mellitus is selected from the group consisting of type 1 diabetes mellitus, type 2 diabetes mellitus, malnutrition-related diabetes mellitus, specified diabetes mellitus, and unspecified diabetes mellitus.

47. The agent or the composition for use according to any of the preceding claims,, wherein the disorder of glucose regulation and pancreatic internal secretion is selected from the group consisting of nondiabetic hypoglycaemic coma and disorders of pancreatic internal secretion.

48. The agent or the composition for use according to any of the preceding claims,, wherein the disorder of obesity and hyperalimentation is selected from the group consisting of localized adiposity, hyperalimentation, and sequelae of hyperalimentation.

49. The agent or the composition for use according to any of the preceding claims,, wherein the disorder of nutritional deficiencies is selected from the group consisting of disorders of aromatic amino-acid metabolism, disorders of branched-chain amino-acid metabolism and fatty-acid metabolism, disorders of amino-acid metabolism, lactose intolerance, disorders of carbohydrate metabolism, disorders of sphingolipid metabolism, disorders of lipid storage disorders, disorders of glycosaminoglycan metabolism, disorders of glycoprotein metabolism, disorders of lipoprotein metabolism, lipiemias, disorders of purine and pyrimidine metabolism, disorders of porphyrin and bilirubin metabolism, disorders of mineral metabolism , cystic fibrosis, amyloidosis, volume depletion, disorders of fluid, electrolyte and acid-base balance, and postprocedural endocrine and metabolic disorders.

50. A method of treating an endocrine disease, a nutritional disease and/or a metabolic disease, the method comprising administering an agent according to any one of the preceding claims to a subject in need thereof.

51. Use of an agent according to any one of the preceding claims for the manufacture of a medicament for use in treatment of an endocrine disease, a nutritional disease and/or a metabolic disease in a mammal.

52. A method for delaying onset of diabetes and/or a diabetes associated disorder or disease, the method comprising administering a therapeutically effective amount of an agent of any one of the preceding claims, to an individual in need thereof.

53. A method for decreasing blood glucose levels, the method comprising administering a therapeutically effective amount of an agent of any one of the preceding claims, to an individual in need thereof.

54. The method according to claim 53, wherein insulin secretion is increased.

55. The method according to claim 53, wherein cellular uptake of glucose is increased.

56. The method according to claim 53, wherein the insulin production is increased.

57. The method according to claim 53, wherein the glucagon production is decreased.

58. A method for improving beta cell viability, the method comprising administering a therapeutically effective amount of an agent of any one of the preceding claims, to an individual in need thereof.

59. A method for improving beta cell morphology, the method comprising administering a therapeutically effective amount of an agent of any one of the preceding claims, to an individual in need thereof.

60. A method for stabilising or improving viability and/or morphology of pancreatic islets, the method comprising administering a therapeutically effective amount of an agent of any one of the preceding claims, to an individual in need thereof.

Fig. 1

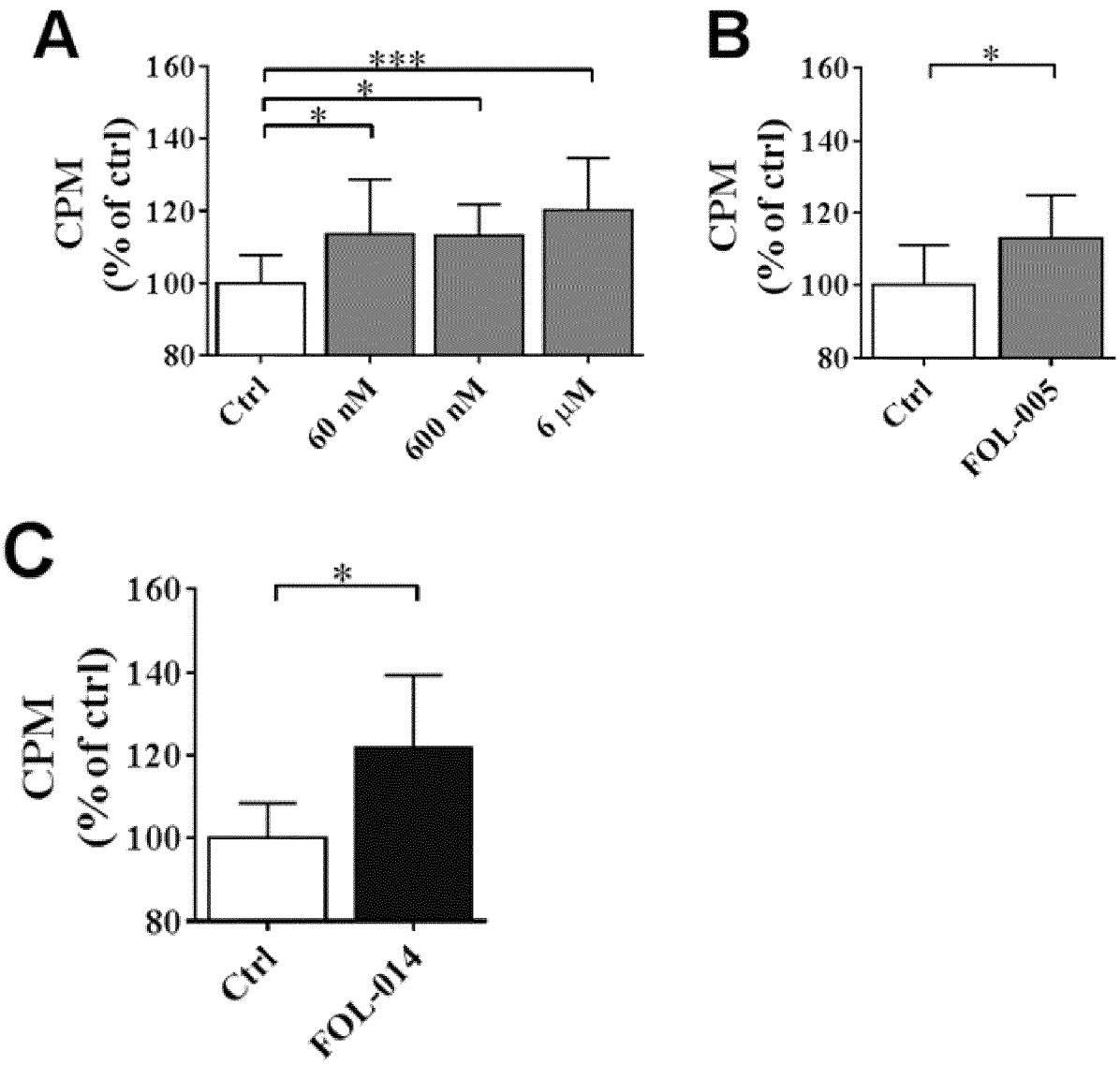

A
CPM (% of ctrl)
160
140
120
100
80
***
*
*
Ctrl
60 nM
600 nM
6 µM
B
CPM (% of ctrl)
160
140
120
100
80
*
Ctrl
FOL-005
C
CPM (% of ctrl)
160
140
120
100
80
*
Ctrl
FOL-014

Fig. 2

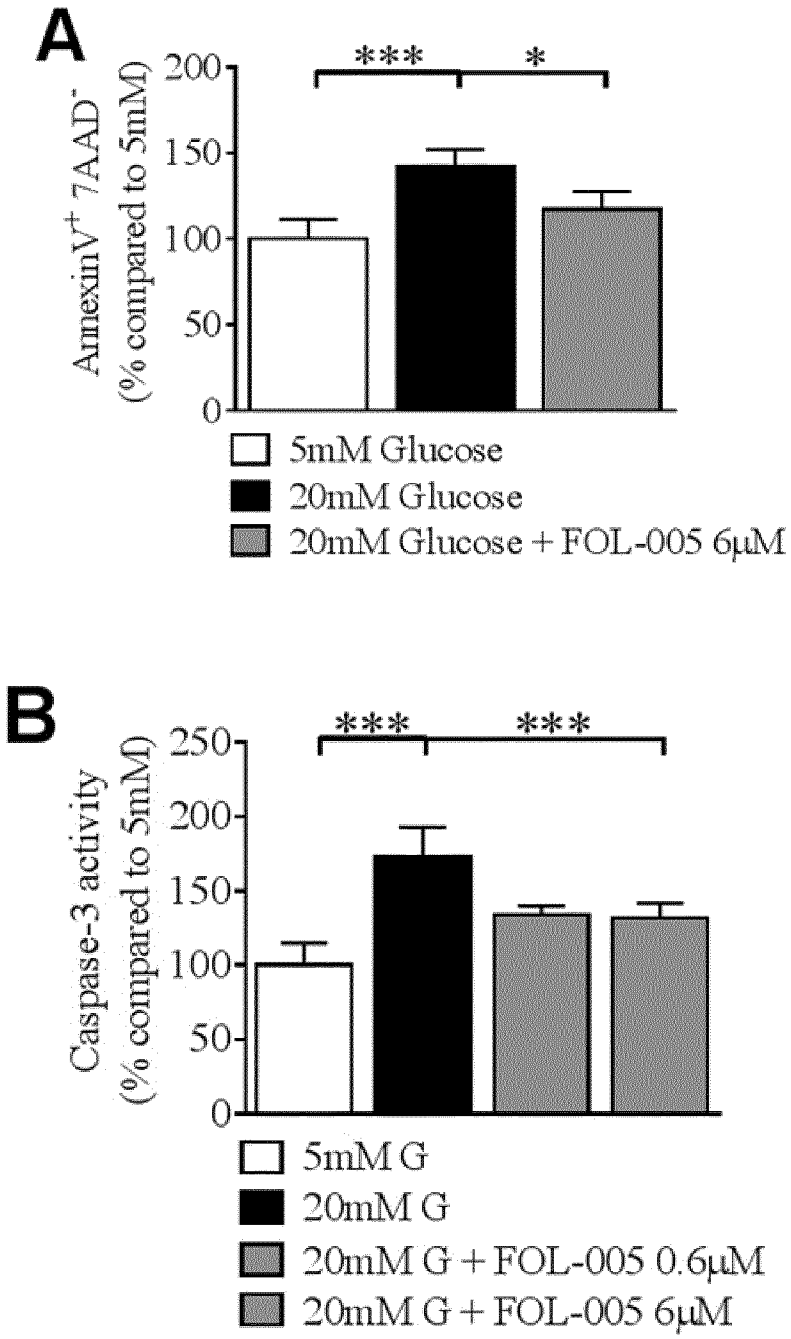

A
AnnexinV+ 7AAD-
(% compared to 5mM)
200
150
100
50
0
***
*
5mM Glucose
20mM Glucose
20mM Glucose + FOL-005 6μM
B
Caspase-3 activity
(% compared to 5mM)
250
200
150
100
50
0
***
***
5mM G
20mM G
20mM G + FOL-005 0.6μM
20mM G + FOL-005 6μM

Fig. 3

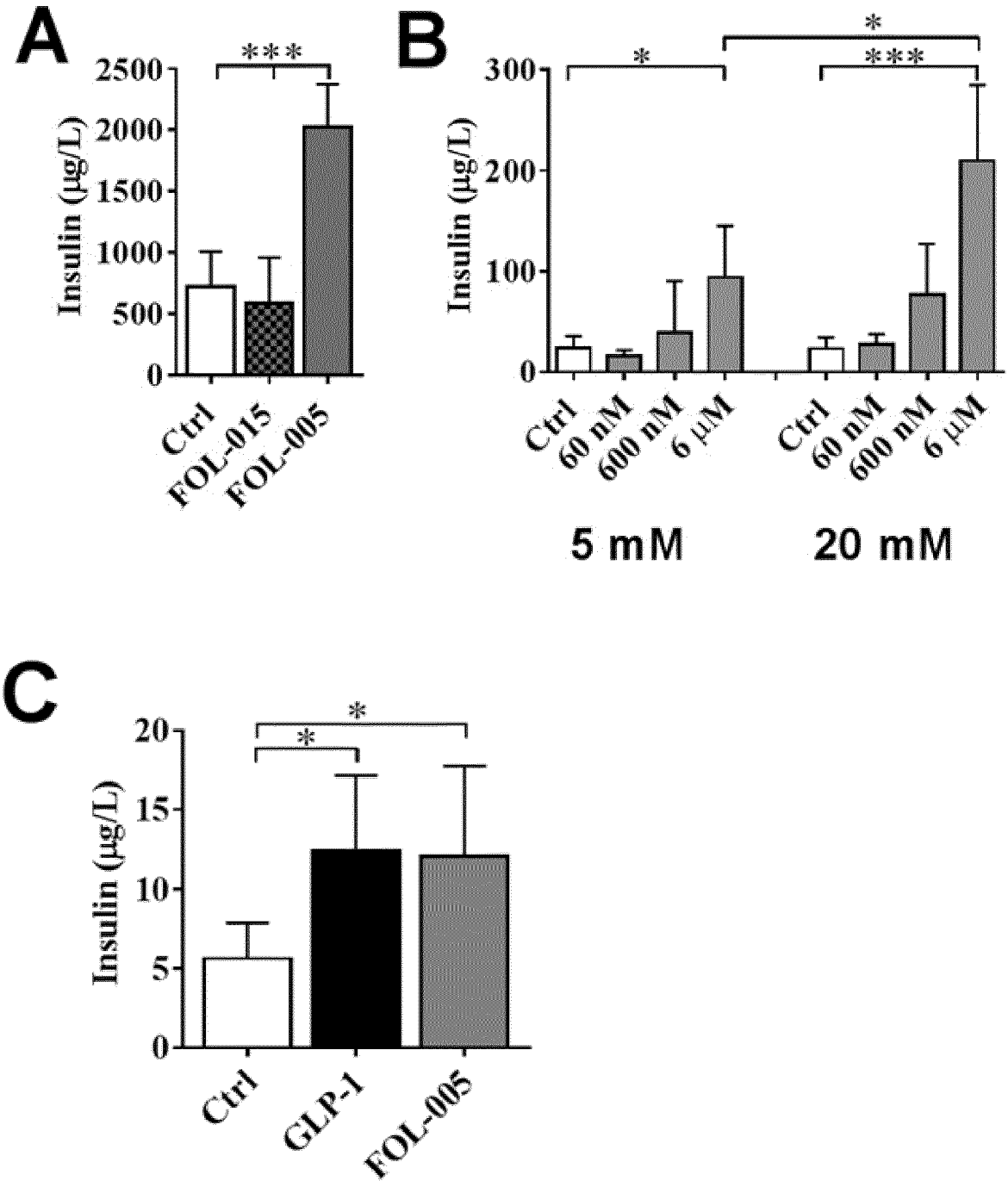

A
***
2500
2000
1500
1000
500
0
Insulin (µg/L)
Ctrl
FOL-015
FOL-005
B
*
*
***
300
200
100
0
Insulin (µg/L)
Ctrl
60 nM
600 nM
6 µM
Ctrl
60 nM
600 nM
6 µM
5 mM
20 mM
C
*
*
20
15
10
5
0
Insulin (µg/L)
Ctrl
GLP-1
FOL-005

Fig. 4

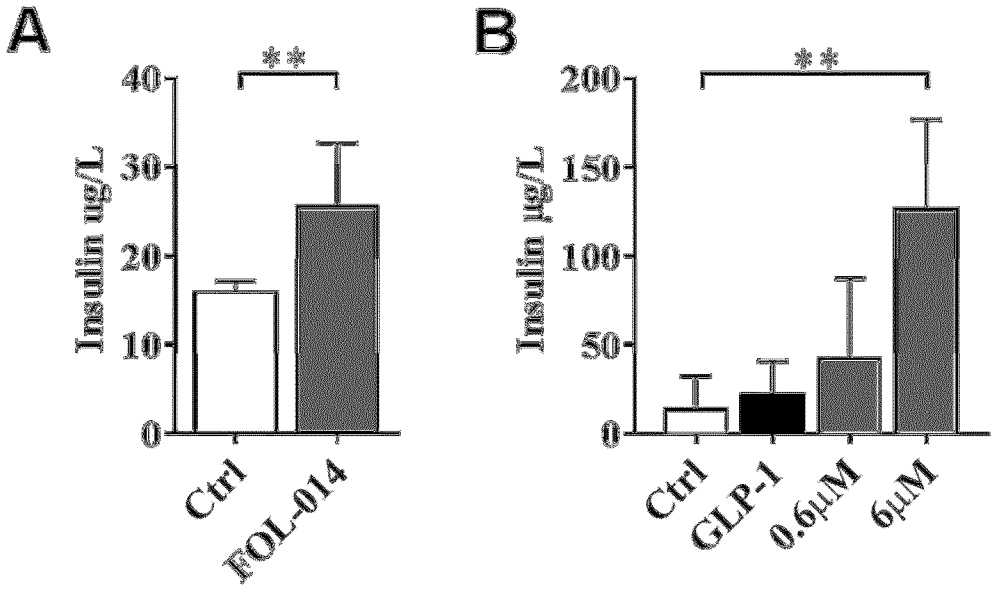

A
**
40
30
20
10
0
Insulin ug/L
Ctrl
FOL-014
B
**
200
150
100
50
0
Insulin µg/L
Ctrl
GLP-1
0.6µM
6µM

Fig. 5

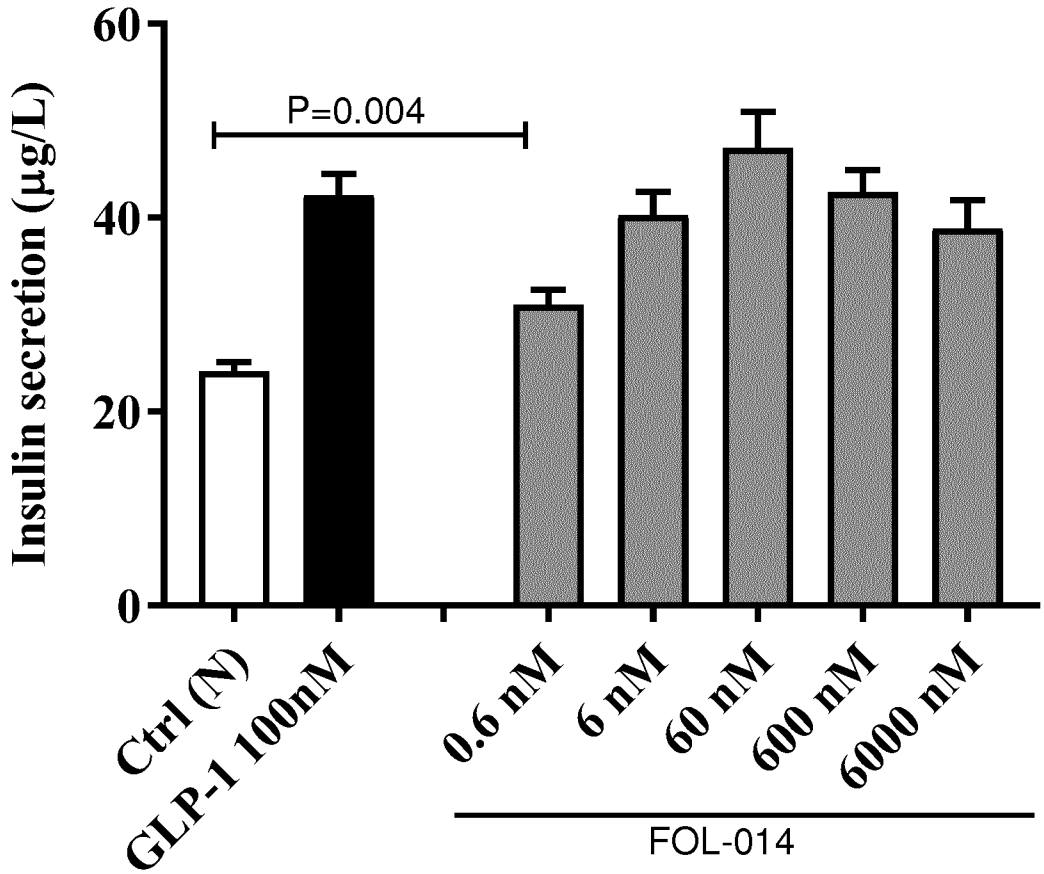

60
40
20
0
Insulin secretion (µg/L)
P=0.004
Ctrl (N)
GLP-1 100nM
0.6 nM
6 nM
60 nM
600 nM
6000 nM
FOL-014

Fig. 6

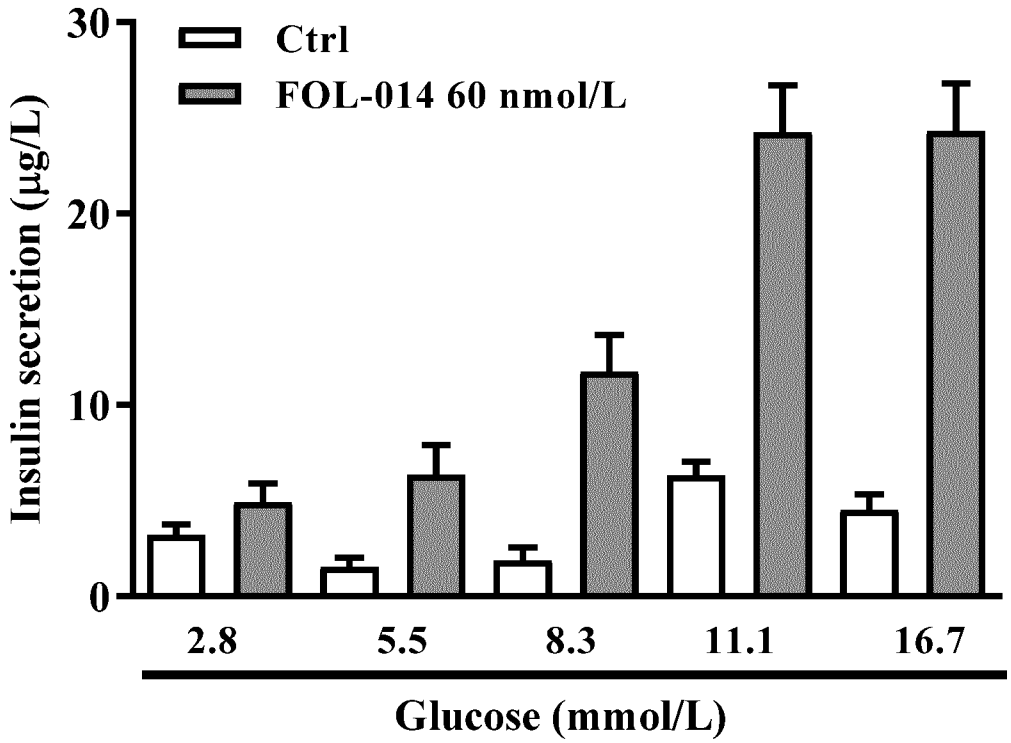

30
20
10
0
Insulin secretion (µg/L)
Ctrl
FOL-014 60 nmol/L
2.8
5.5
8.3
11.1
16.7
Glucose (mmol/L)

Fig. 7

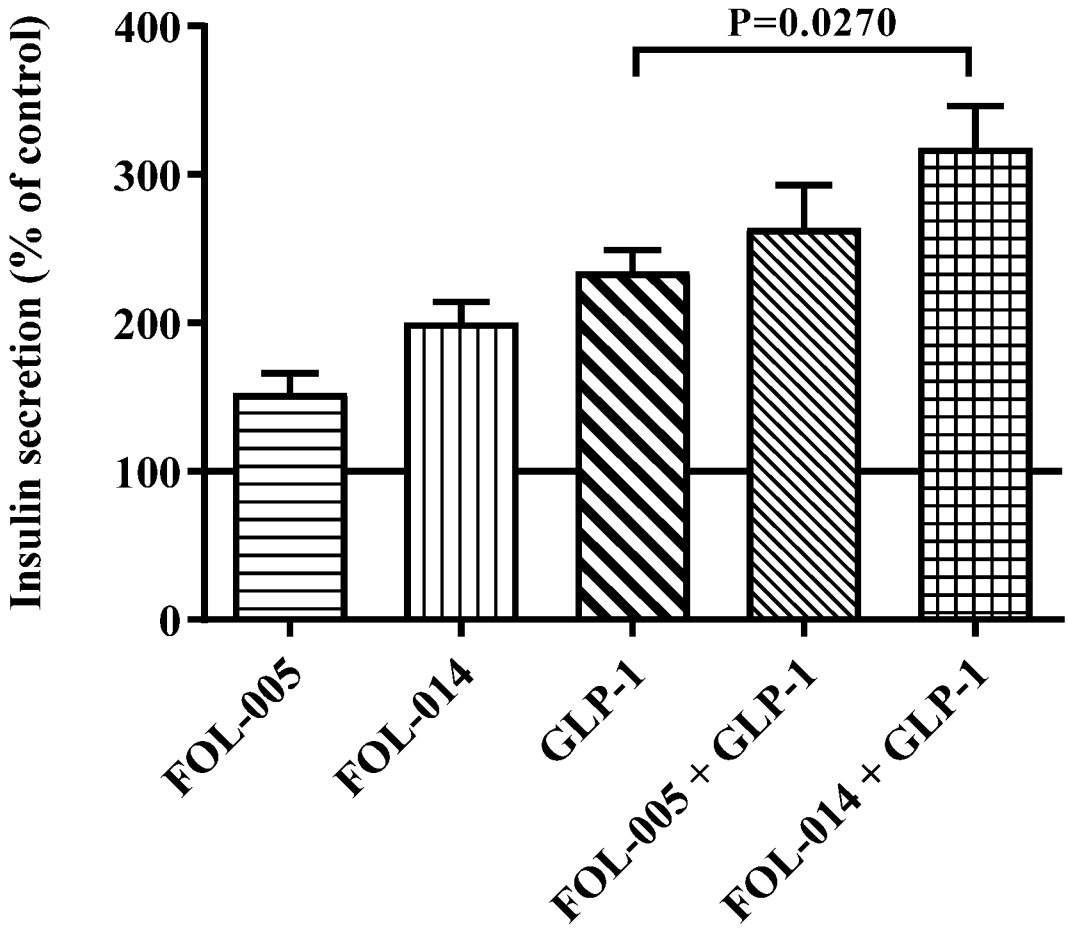

P=0.0270
Insulin secretion (% of control)
400
300
200
100
0
FOL-005
FOL-014
GLP-1
FOL-005 + GLP-1
FOL-014 + GLP-1

Fig. 8

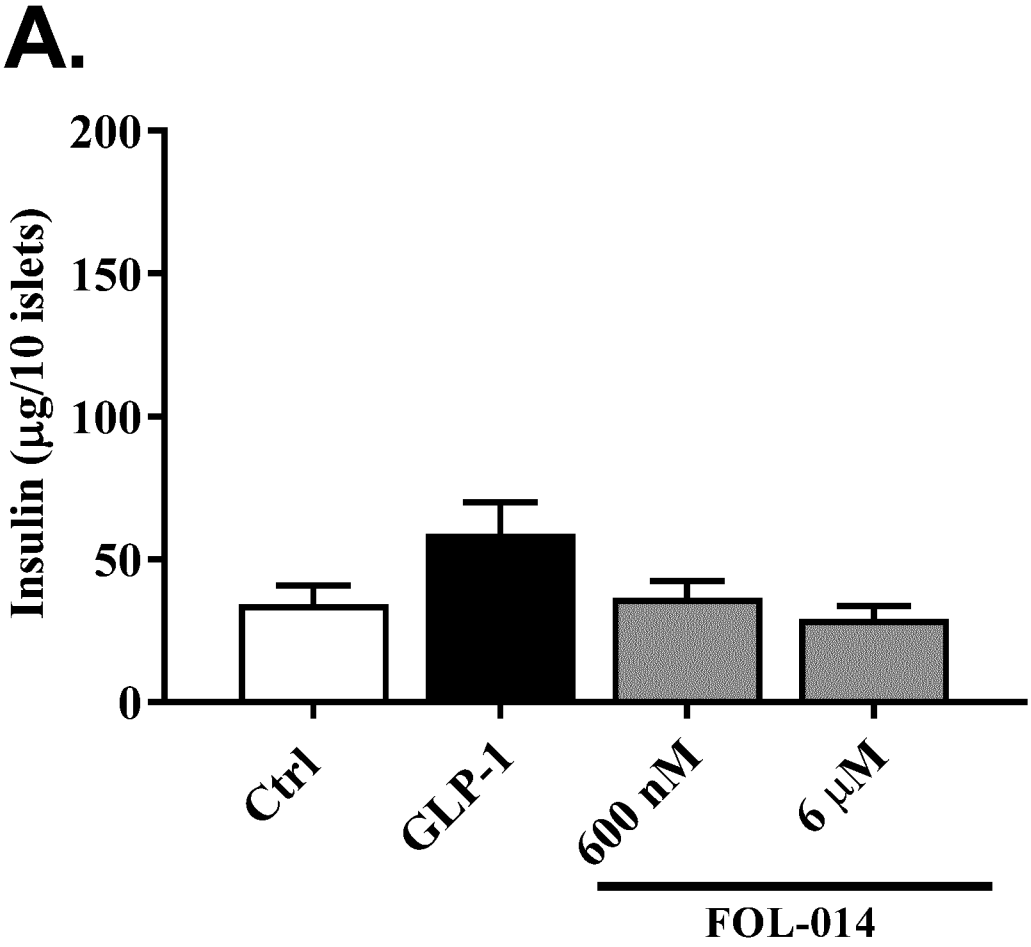

A.
Insulin (µg/10 islets)
200
150
100
50
0
Ctrl
GLP-1
600 nM
6 µM
FOL-014

Fig. 8, cont.

B.

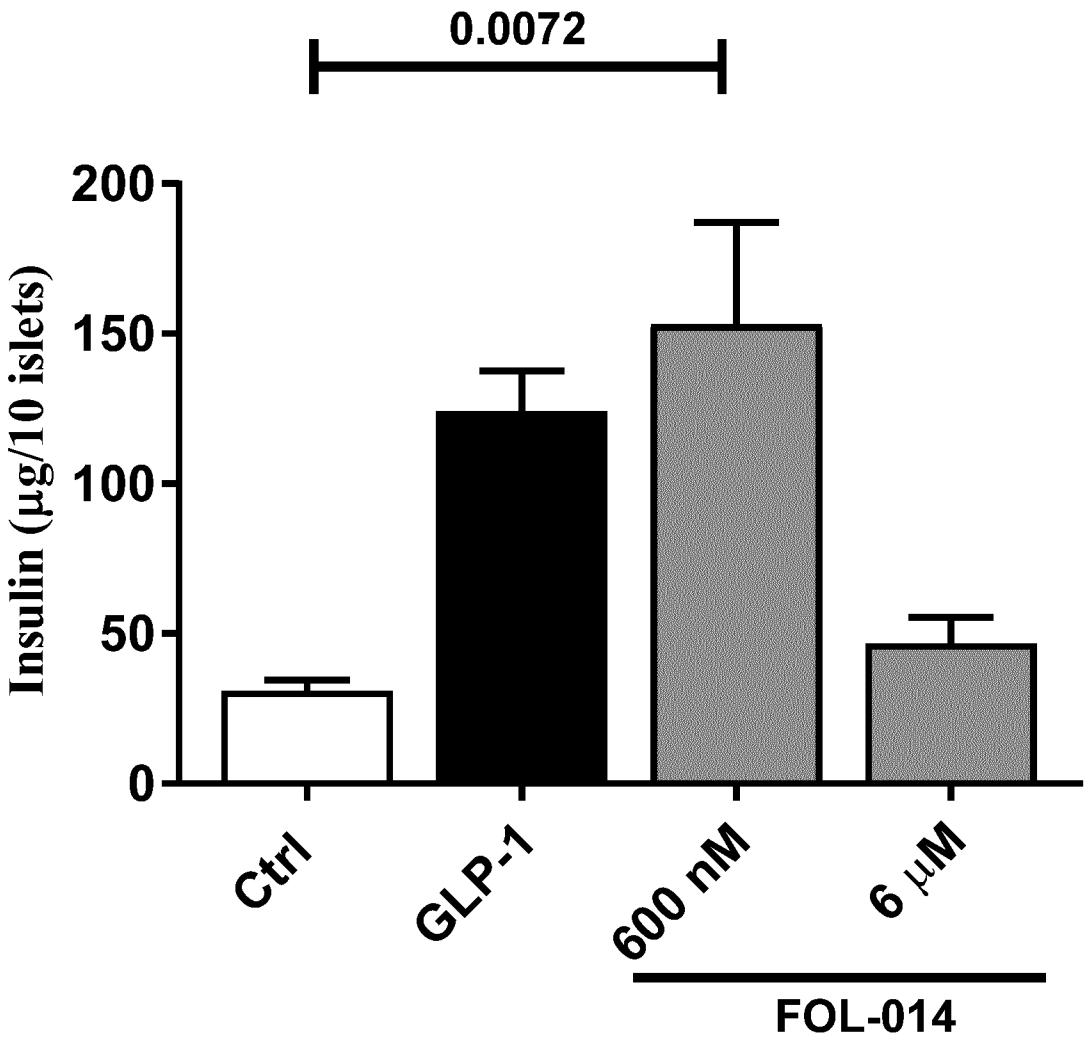

0.0072
200
150
100
50
0
Insulin (µg/10 islets)
Ctrl
GLP-1
600 nM
6 µM
FOL-014

Fig. 8, cont.

C.

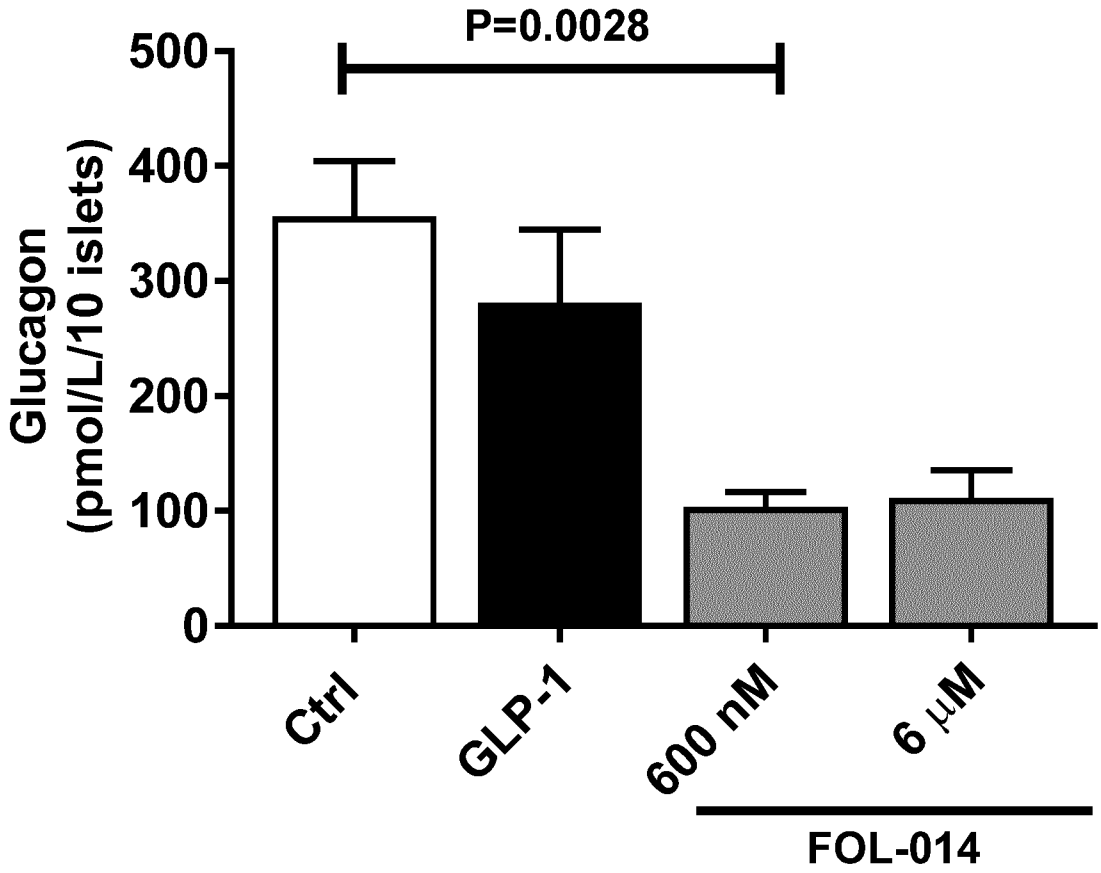

P=0.0028
Glucagon (pmol/L/10 islets)
500
400
300
200
100
0
Ctrl
GLP-1
600 nM
6 μM
FOL-014

Fig. 8, cont.

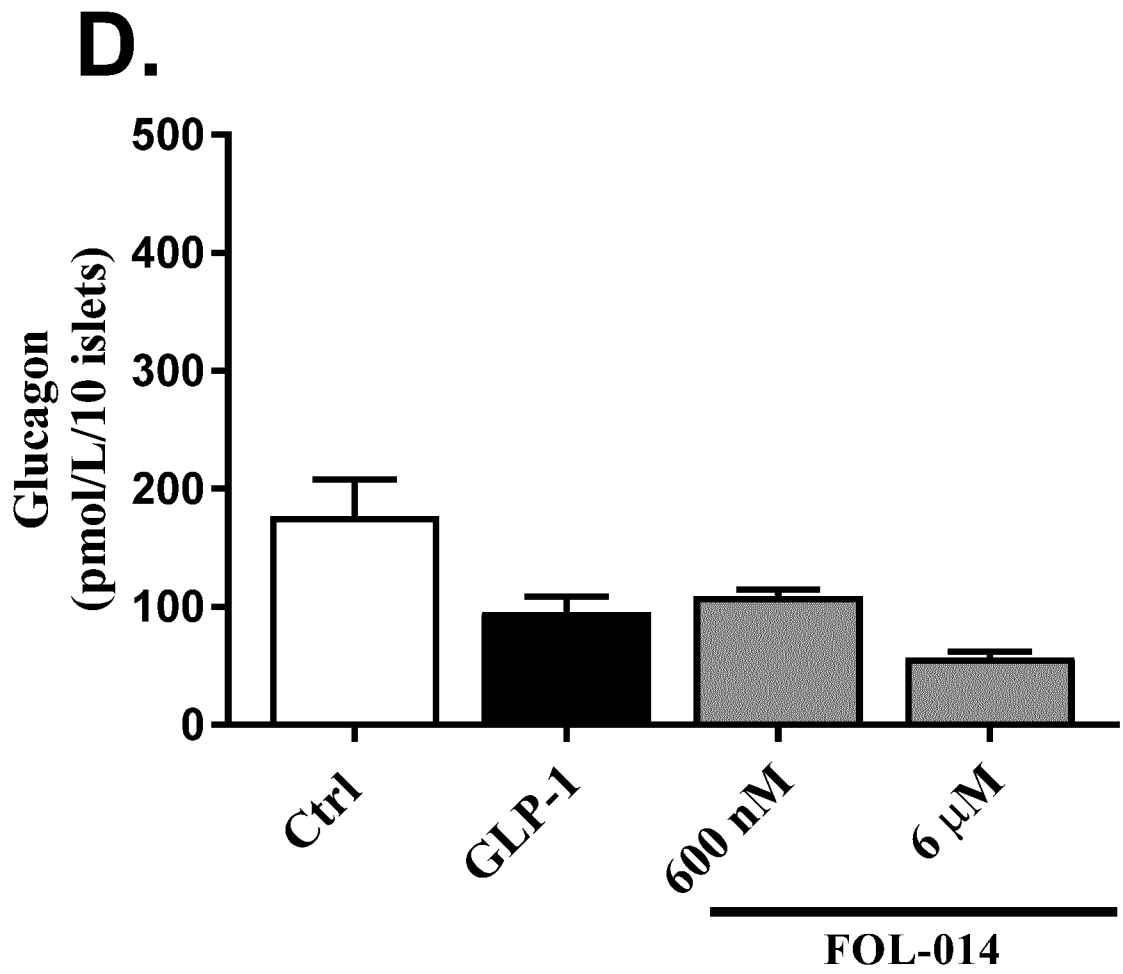

D.
Glucagon (pmol/L/10 islets)
500
400
300
200
100
0
Ctrl
GLP-1
600 nM
6 μM
FOL-014

Fig. 9

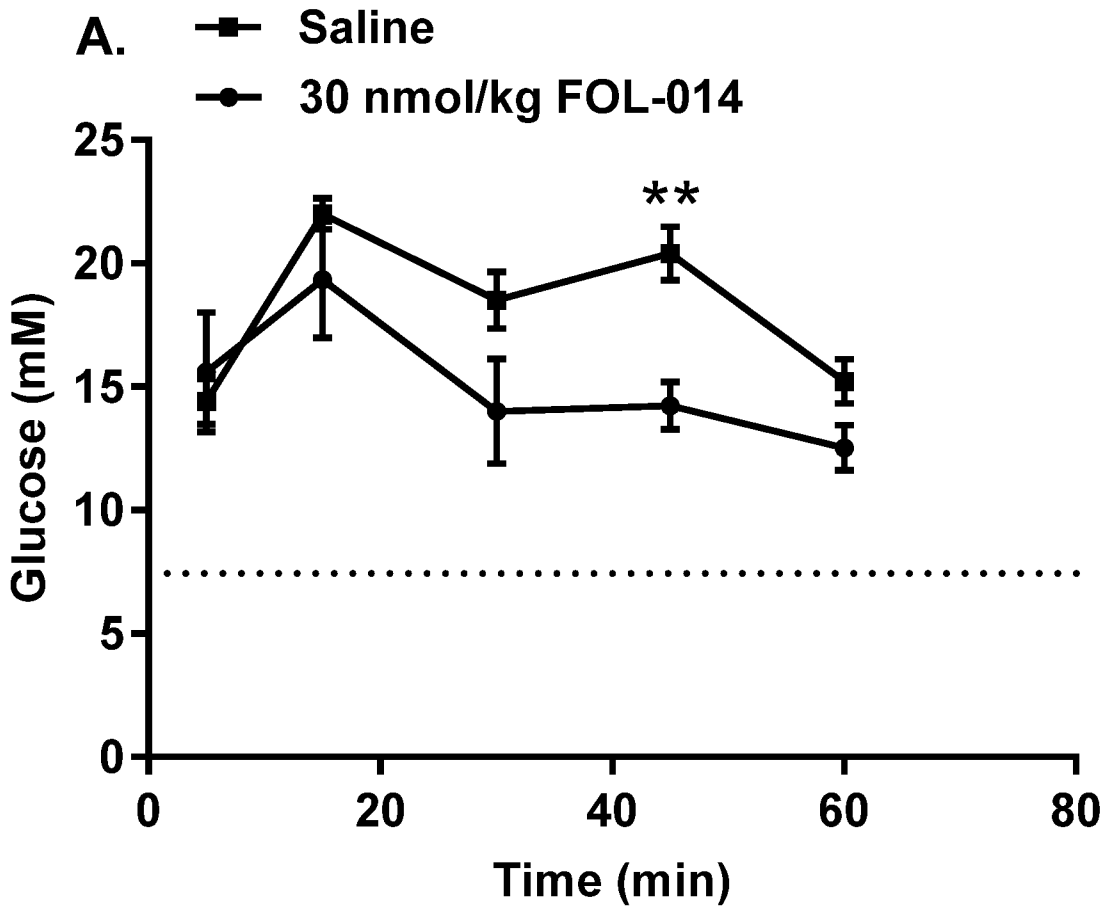

A.
Saline
30 nmol/kg FOL-014
**
Glucose (mM)
25
20
15
10
5
0
0
20
40
60
80
Time (min)

Fig. 9, cont.

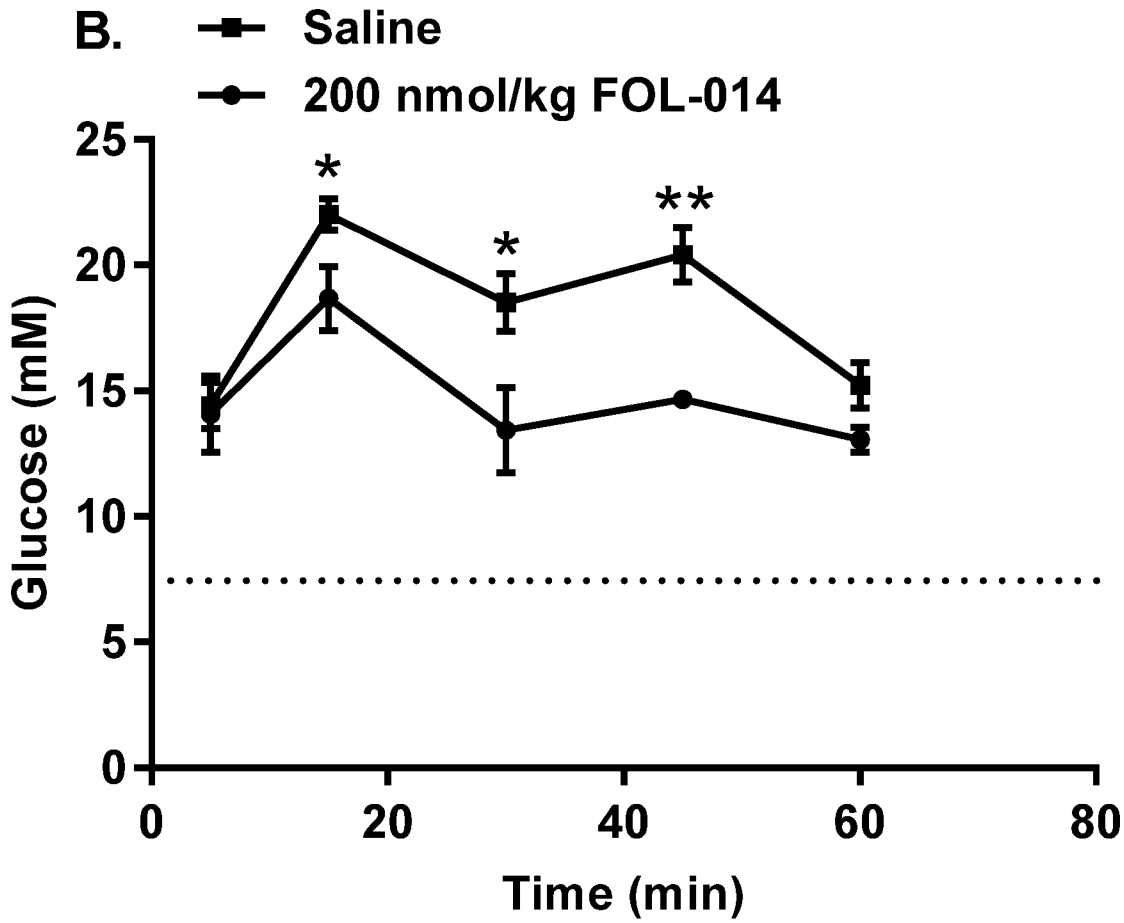

B.
Saline
200 nmol/kg FOL-014
Glucose (mM)
25
20
15
10
5
0
*
*
**
0
20
40
60
80
Time (min)

Fig. 10

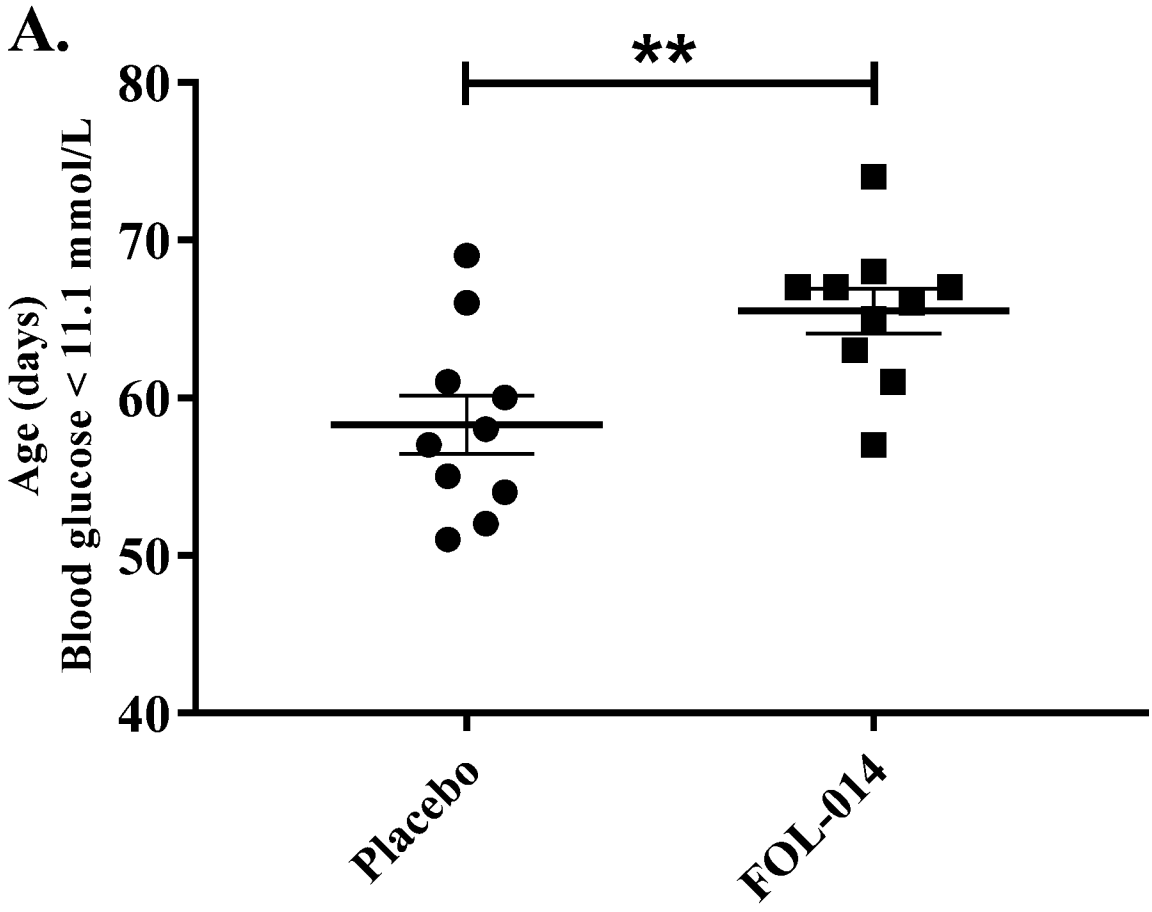
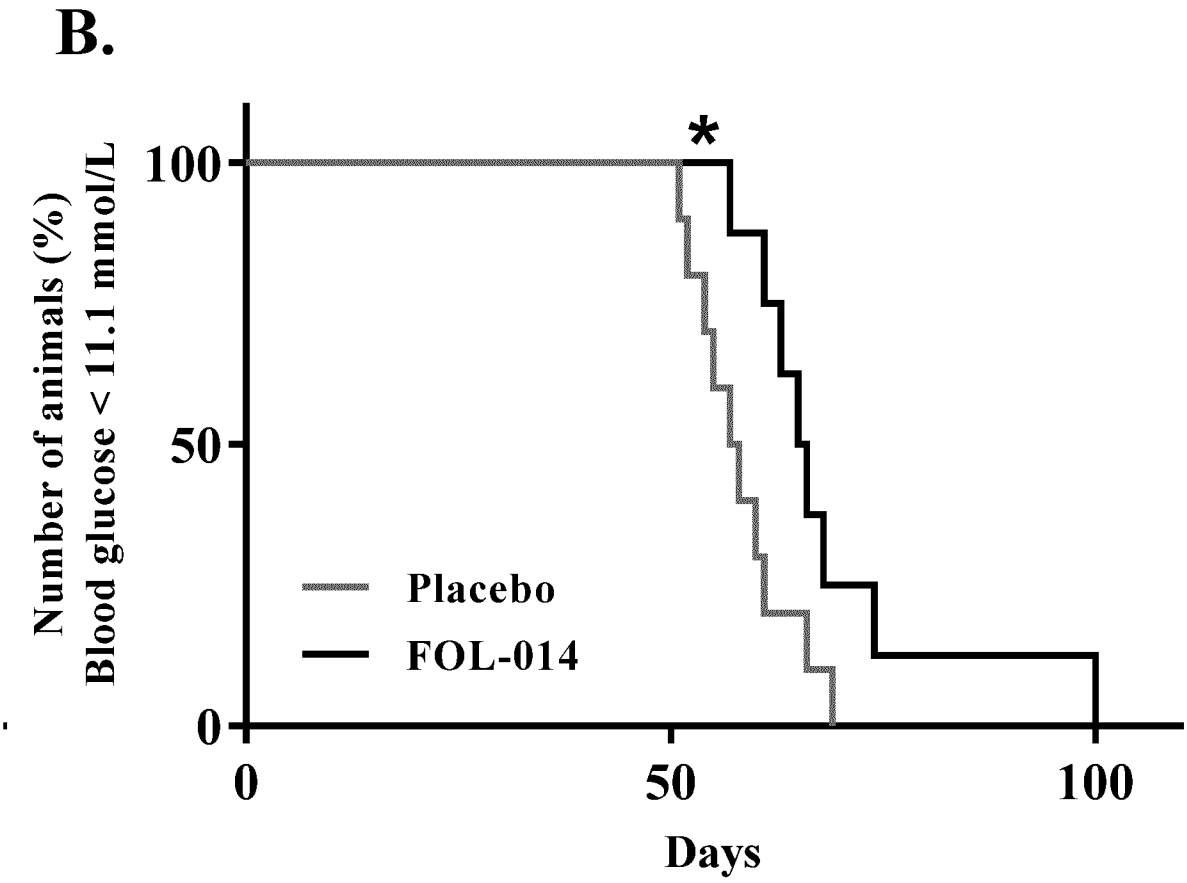

A.
**
80
70
60
50
40
Age (days)
Blood glucose < 11.1 mmol/L
Placebo
FOL-014

Fig. 10, cont.


B.
Number of animals (%)
Blood glucose < 11.1 mmol/L
100
50
0
*
Placebo
FOL-014
0
50
100
Days

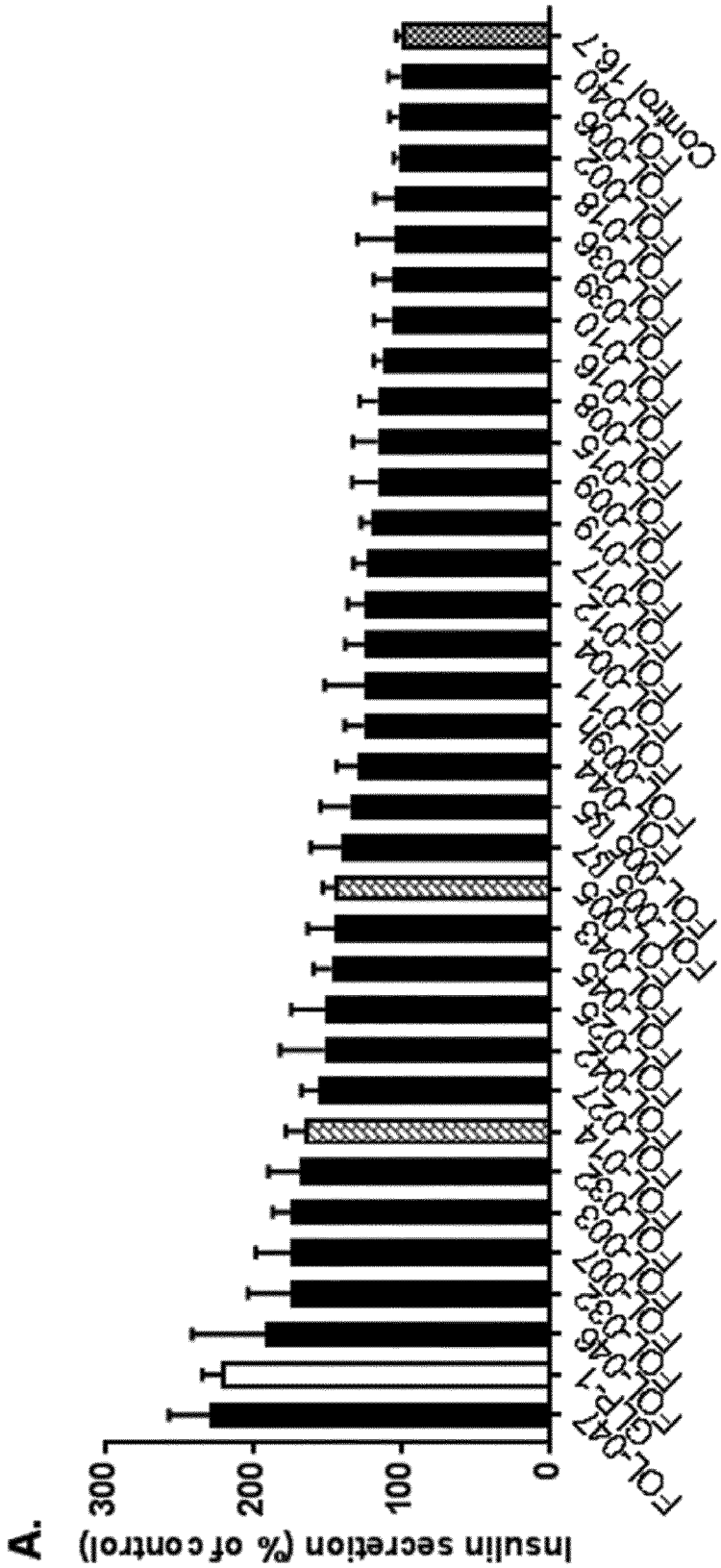


Fig. 11

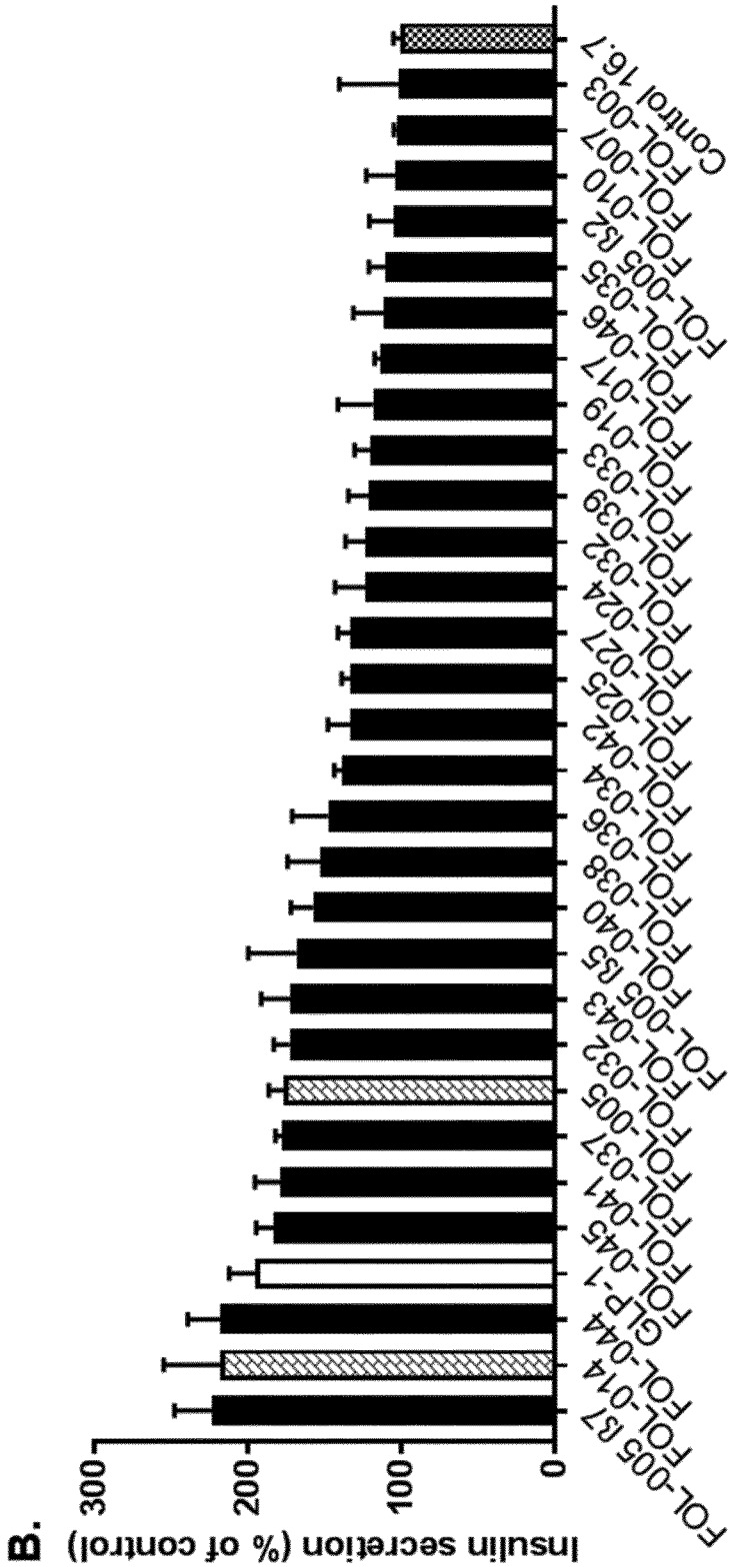
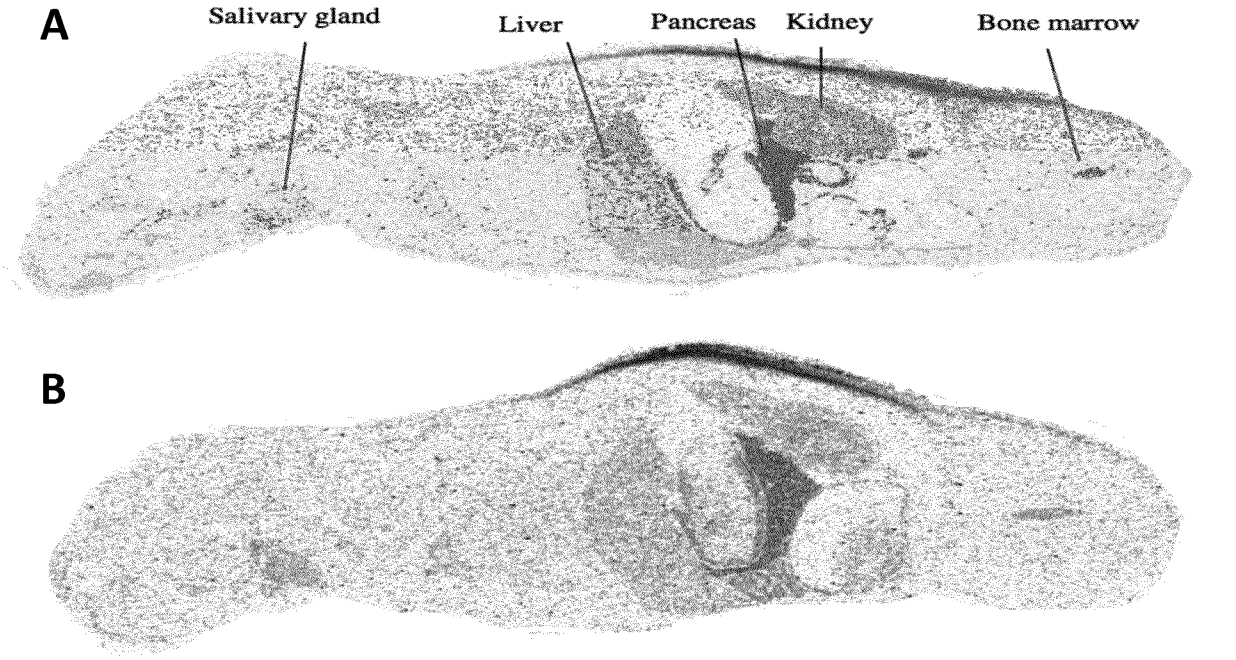

B.
Insulin secretion (% of control)
300
200
100
0
FOL-005 R7
FOL-014
FOL-044
GLP-1
FOL-045
FOL-041
FOL-037
FOL-005
FOL-032
FOL-043
FOL-005 R5
FOL-040
FOL-038
FOL-036
FOL-034
FOL-042
FOL-025
FOL-027
FOL-024
FOL-032
FOL-039
FOL-033
FOL-019
FOL-017
FOL-046
FOL-035
FOL-005 R2
FOL-010
FOL-007
FOL-003
Control 16.7

Fig. 11, cont.

Fig. 12


A
Salivary gland
Liver
Pancreas
Kidney
Bone marrow
B
C
Pre injection
5 min
20 min
50 min
60 min
0000713_01
0000748_01
0000754_01
0000769_01
0000778_01
2 hrs
4 hrs
6 hrs
24 hrs
48 hrs
0000784_01
0000797_02
0000812_01
0000824_01
0000855_01
D
Pre injection
5 min
20 min
50 min
60 min
0000715_01
0000751_01
0000757_01
0000775_01
0000781_01
2 hrs
4 hrs
6 hrs
24 hrs
48 hrs
0000787_01
0000803_01
0000815_01
0000827_01
0000871_01

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/061547

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/00 A61K38/12 A61P3/00 A61P3/06 A61P3/10
C07K14/47
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/058379 A2 (MEDIMMUNE LLC [US]; WU HERREN [US]; BACA MANUEL [US]; SWERS JEFFREY [U) 7 May 2009 (2009-05-07)	1,4-9, 11,17, 22, 25-30, 33-36, 39,40, 42-46, 49-60
Y	the whole document	1-60

X	US 7 662 633 B2 (GLAXOSMITHKLINE LLC [US]) 16 February 2010 (2010-02-16)	37,38, 42-46, 49-60
Y	abstract sequences 4,50	1-60

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
5 July 2018	26/07/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Weisser, Dagmar

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2015/159099 A1 (FOLLICUM AB [SE]; SMITH STEPHEN EDWARD [GB]) 22 October 2015 (2015-10-22) abstract claims 1,13,16,58-60,64-67	1-60
Y	US 2016/317620 A1 (ALENFALL JAN [SE] ET AL) 3 November 2016 (2016-11-03) abstract paragraphs [0015], [0016], [0132] sequence 63	1-60
Y	SAROSIEK KONRAD ET AL: "Osteopontin (OPN) Isoforms, Diabetes, Obesity, and Cancer; What Is One Got to Do with the Other? A New Role for OPN", JOURNAL OF GASTROINTESTINAL SURGERY, QUALITY MEDICAL PUBL., ST. LOUIS, MO, US, vol. 19, no. 4, 13 January 2015 (2015-01-13), pages 639-650, XP035471322, ISSN: 1091-255X, DOI: 10.1007/S11605-014-2735-6 [retrieved on 2015-01-13] the whole document	1-60
Y	US 2010/150877 A1 (O'BRIEN TIMOTHY [IE] ET AL) 17 June 2010 (2010-06-17) paragraphs [0008], [0009] claims 1,2,7	1-60

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Information on patent family members

International application No
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		JP 2017513856 A	01-06-2017
		KR 20160136342 A	29-11-2016
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INTERNATIONAL SEARCH REPORT

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International application No
PCT/EP2018/061547

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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