to indicate that certain "Exendin agonist"/"Gastrin compound" combinations described therein may have a beneficial effect with respect to normalizing blood glucose and Insulin levels in acutely diabetic NOD mice compared to the effect seen when employing the "Exendin agonist" (or the "Gastrin compound") in question alone, and that certain "GLP-1 receptor agonist" /Gastrin combinations described therein may have a beneficial effect with respect to inducing islet cell regeneration compared to the effect seen when employing the "GLP-1 receptor agonist" alone.

WO 2005/072045 and WO 2007/095737 also disclose the possibility of forming conjugates comprising a "GLP-1 agonist" or "Exendin agonist", respectively, and a "Gastrin compound" covalently coupled or linked (i.e. conjugated) to one another, optionally via an intermediate linker or spacer. As a suitable spacer is mentioned a mono- or disaccharide, an amino acid, a sulfate, a succinate, an acetate, or an oligomeric polymeric spacer or linker comprising one or more of such moieties. Contemplated methods by which conjugates of the types in question might be prepared are also described. However, no preparative or other data are provided in either of the latter documents in question to substantiate that any conjugate of the type in question had in fact been prepared and characterized - or tested with respect to its biological/physiological properties or activity – at the time of filing of the respective international application.

It may further be noted that neither WO 2005/072045 nor WO 2007/095737 provide any *in vivo*, *in vitro* or other data to substantiate that the "GLP-1 agonist"/"Gastrin compound" or "Exendin agonist"/"Gastrin compound" combinations, respectively, described and utilized therein might be beneficial in the treatment, for example, of type 2 diabetes.

SUMMARY ~~OF THE INVENTION~~

It has now been found that certain conjugates comprising two covalently coupled or linked peptide moieties may exhibit unexpectedly high therapeutic activity in the treatment, for example, of diabetes (type 1 and/or type 2 diabetes), or of various other diabetes-related diseases or disorders, by comparison with the therapeutic activity of a combination of the two individual peptides in question.

In a broad ~~aspect~~embodiment, the ~~invention~~disclosure provides a peptide conjugate of a GLP-1 receptor agonist and Gastrin, in particular Gastrin with a substitution at position 15 selected from Leu, Nle, Phe and Thr. More particularly, the ~~invention~~disclosure provides peptide conjugates of Exendin-4 and Gastrin as well as peptide conjugates of GLP-1 and Gastrin.

In one aspect, there is provided a peptide conjugate having the formula I

H-HGEGTFTSDLSKQMEEEAVRLFIEWLKN-Peg3-Peg3-YGWLDF-NH_2,

H-HGEGTFTSDLSKQMEEEAVRLFIEWLKN-Peg3-Peg3-GWLDF-NH_2;

H-HGEGTFTSDLSKQMEEEAVRLFIEWLKN-Peg3-Peg3-AGWLDF-NH_2; or

H-HGEGTFTSDLSKQMEEEAVRLFIEWLKNYGWTDF-NH_2;

or a pharmaceutically acceptable salt or solvate thereof.

In ~~a first aspect~~one embodiment, the ~~invention~~disclosure thus provides a peptide conjugate having the formula I

R^1-Z-L-Y-R^2 (I)

wherein

R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

Z comprises the sequence of Exendin-4(1-39) having the sequence

His - Gly - Glu - Gly - Thr - Phe - Thr - Ser - Asp - Leu - Ser - Lys - Gln - Met - Glu - Glu - Glu - Ala - Val - Arg - Leu - Phe - Ile - Glu - Trp - Leu - Lys - Asn - Gly - Gly - Pro - Ser - Ser - Gly - Ala - Pro - Pro - Pro – Ser (SEQ ID NO: 115)

or an analogue thereof Z_a;

L is an optional linker moiety; and

Y comprises the sequence of Gastrin17 having the sequence

Gln-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Y15-Asp-Phe (SEQ ID NO: 116)

wherein Y15 is selected from Leu, Nle, Phe and Thr

or an analogue thereof Y_a.

Further, the ~~invention~~disclosure provides a peptide conjugate wherein:

(i) Z_a has substitutions in up to 10 positions, and/or comprises a C-terminal truncation of 1 to 12 amino acids, with respect to the sequence of Exendin-4; and/or

(ii) Y_a has substitutions in up to 5 positions with respect to the sequence of Gastrin17, and/or comprises an N-terminal truncation from 1 to 13 amino acids, with respect to the sequence of Gastrin17.

In one embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein

Z_a is a peptide sequence having the formula IIa

His - Z2 – Z3 - Gly - Thr - Phe - Thr - Ser - Z9 – Z10 – Z11 – Z12 – Z13 – Z14 – Glu – Z16 – Z17– Z18 – Z19 – Z20 – Z21 - Z22 – Z23 – Z24 – Z25 – Z26 – Z27 – Z28 – Z29 – Z30 – Z31 – Z32 – Z33 – Z34 – Z35 – Z36 – Z37 – Z38 – Z39 (IIa)

Y10 is Glu or is absent;

Y11 is Ala or is absent;

Y12 is selected from Ala and Tyr or is absent;

Y13 is selected from Gly and Ala or is absent;

Y14 is selected from Trp, Phe, 1Nal and Met;

Y15 is selected from Leu, Nle, Phe and Thr; and

Y17 is selected from Phe and 3-(3-Pyridyl)-alanine.

In another embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein

Z_a is a peptide sequence having the formula IIIa

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Z9-Leu-Ser-Z12-Z13-Z14-Glu-Z16-Glu-Ala-Val-Z20-Leu-Phe-Ile-Z24-Z25-Leu-Z27-Z28 (IIIa)

wherein

Z9 is selected from Asp and Glu;

Z12 is selected from Lys, Arg and Orn;

Z13 is selected from Gln and Tyr;

Z14 is selected from Met and Leu;

Z16 is selected from Glu, Cys and Lys;

Z20 is selected from Arg, Lys and Orn;

Z24 is selected from Lys and Glu;

Z25 is selected from Trp, Lys, Cys and Phe;

Z27 is selected from Lys, Arg and Orn;

Z28 is selected from Asn and Asp or is absent;

L is a peptide sequence having the formula IIIb

L1-L2-L3-L4 (IIIb)

wherein

L1 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L2 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L3 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L4 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

Y_a is a peptide sequence having the formula IIIc

Y12-Y13-Y14-Y15-Asp-Y17 (IIIc)

wherein

Y12 is selected from Tyr and Ala or is absent;

Y13 is selected from Gly and Ala or is absent;

Y14 is selected from Trp, 1Nal and Phe;

Y15 is selected from Leu, Nle, Thr and Phe.; and

Y17 is selected from Phe and 3-(3-pyridyl)-alanine.

In a further embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein

Z_a is a peptide sequence having the formula IVa

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Z9-Leu-Ser-Z12-Gln-Met-Glu-Z16-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Z27-Z28 (IVa)

wherein

Z9 is selected from Glu and Asp;

Z12 is selected from Lys and Orn;

Z16 is selected from Glu and Lys;

Z27 is selected from Lys and Orn;

Z28 is selected from Asn and Asp or is absent;

L is a peptide sequence having the formula IVb

L1-L2-L3-L4 (IVb)

wherein

L1 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L2 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L3 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L4 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

Y_a is a peptide sequence having the formula IVc

Y12-Y13-Trp-Leu-Asp-Phe (IVc)

wherein

Y12 is Tyr or is absent; and

Y13 is Gly or is absent.

In a still further embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein

Z_a is a peptide sequence having the formula Va

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Z9-Leu-Ser-Z12-Tyr-Leu-Glu-Z16-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Phe-Leu-Z27-Z28 (Va)

wherein

Z9 is selected from Glu and Asp;

Z12 is selected from Lys and Orn;

Z16 is selected from Glu and Lys;

Z27 is selected from Lys and Orn;

Z28 is selected from Asn and Asp or is absent;

L is a peptide sequence having the formula Vb

L1-L2-L3-L4 (Vb)

wherein

L1 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L2 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L3 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L4 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

Y_a is a peptide sequence having the formula Vc

Y12-Y13-Trp-Leu-Asp-Phe (Vc)

wherein

Y12 is Tyr or is absent; and

Y13 is Gly or is absent.

Further, the ~~invention~~disclosure provides a peptide conjugate wherein the peptide sequence of formula I comprises one or more intramolecular bridges.

Still further, the ~~invention~~disclosure provides a peptide conjugate wherein said intramolecular bridge is formed between the side chains of two amino acid residues which are separated by three amino acids in the linear amino acid sequence of formula I.

In a specific embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein the intramolecular bridge is formed between the side chains of residue pairs x and x+3, x+4, x+5 etc.

In another specific embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein the intramolecular bridge is a lactam ring.

In a further specific embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein the intramolecular bridge involves a pair of residues wherein:

Z12 is Lys and Z16 is Glu; Z12 is Glu and Z16 is Lys;

Z16 is Glu and Z20 is Lys; Z16 is Lys and Z20 is Glu;

Z20 is Glu and Z24 is Lys; Z20 is Lys and Z24 is Glu;

In another ~~aspect~~embodiment of the ~~invention~~disclosure is provided a peptide conjugate having the formula VI:

R^1-X-L-Y-R^2 (VI)

wherein

R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

X comprises the sequence of GLP-1(7-36) having the sequence

His – Ala - Glu - Gly - Thr - Phe - Thr - Ser - Asp - Val - Ser - Ser - Tyr –Leu - Glu - Gly - Gln - Ala - Ala – Lys - Glu –Phe - Ile - Ala - Trp - Leu - Val – Lys - Gly – Arg (SEQ ID NO: 114)

or an analogue thereof Xa;

L is a linker containing up to 4 naturally or non-naturally amino acids or combinations thereof or is absent;

Y comprises the sequence of Gastrin-17 having the sequence

Gln-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Y15- Asp-Phe

wherein Y15 is selected from Leu, Nle, Phe and Thr

or an analogue thereof Y_a.

In one embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein

(i) X_a has substitutions in up to 5 positions and/or comprises a C-terminally truncation of 1-2 amino acids, with respect to the sequence of GLP-1; and/or

(ii) Y_a has substitutions in up to 5 positions with respect to the sequence of Gastrin17, and/or comprises an N-terminal truncation of 1 to 13 amino acids, with respect to the sequence of Gastrin17.

In another embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein

X_a is a peptide sequence having the formula VIIa

His – X8 - Glu - Gly - Thr - Phe - Thr - Ser - Asp - Val - Ser - Ser - Tyr –Leu - Glu - Gly - Gln - Ala - Ala – X26 - Glu –Phe - Ile - Ala - Trp - Leu - Val – X34 - Gly – X36 (VIIa)

Y10 is Glu or is absent;

Y11 is Ala or is absent;

Y12 is selected from Ala, Tyr or is absent;

Y13 is selected from Gly, Ala or is absent;

Y14 is selected from Trp, Phe, 1Nal and Met;

Y15 is selected from Leu, Nle, Phe and Thr: and

Y17 is selected from Phe and 3-(3-pyridyl)-alanine.

In still another embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein

X_a is a peptide sequence having the formula VIIIa

His – X8 - Glu - Gly - Thr - Phe - Thr - Ser - Asp - Val - Ser - Ser - Tyr –Leu - Glu - Gly - Gln - Ala - Ala – X26 - Glu –Phe - Ile - Ala - Trp - Leu - Val – X34 - Gly – X36 (VIIIa)

wherein

X8 is selected from Ala, Aib and Gly;

X26 is selected from Arg and Lys;

X34 is selected from Arg and Lys;

X36 is selected from Arg and Lys;

L is a peptide sequence having the formula VIIIb

L1-L2-L3-L4 (VIIIb)

wherein

L1 is selected from Peg3, Orn, Cys, Lys, Gln or is absent;

L2 is selected from Ser, Ala, Orn, Cys, Lys, Gln or is absent;

L3 is selected from Lys, Ala, Cys, Orn, Gln or is absent;

L4 is selected from Lys, Orn, Ala, Peg3, Cys, Lys, Gln or is absent;

Y_a is a peptide sequence having the formula VIIIc

Y12-Y13-Y14-Y15-Asp-Phe (VIIIc)

wherein

Y12 is selected from Tyr, Ala or is absent;

Y13 is selected from Gly, Ala or is absent;

Y14 is selected from Trp and Phe; and

Y15 is selected from Leu, Thr and Phe.

In a specific embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein

X_a is a peptide sequence having the formula IXa

His – X8 - Glu - Gly - Thr - Phe - Thr - Ser - Asp - Val - Ser - Ser - Tyr –Leu - Glu - Gly - Gln - Ala - Ala – X26 - Glu –Phe - Ile - Ala - Trp - Leu - Val – X34 - Gly – X36 (IXa)

wherein

X8 is selected from Ala, Aib and Gly;

X26 is selected from Arg and Lys;

X34 is selected from Arg and Lys;

X36 is selected from Arg and Lys;

L is a peptide sequence having the formula IXb

L1-L2-L3-L4 (IXb)

wherein

L1 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L2 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L3 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

L4 is selected from Orn, Peg3, Cys, Lys and Gln or is absent;

Y_a is a peptide sequence having the formula IXc

Y12-Y13-Trp-Leu-Asp-Phe (IXc)

wherein

Y12 is Tyr or is absent; and

Y13 is Gly or is absent.

In a further specific embodiment, the ~~invention~~disclosure provides a peptide conjugate wherein at least one of Lys or Cys is further conjugated to a lipophilic substituent.

A a further ~~aspect~~embodiment of the ~~invention~~disclosure relates to a peptide conjugate having the formula:

Exendin-4(1-39)-Peg3-Peg3-[Gln1,Leu15]Gastrin17, (1)

Exendin-4(1-39)-[Gln1,Leu15]Gastrin17, (2)

Exendin-4(1-39)-K-[Gln1,Leu15]Gastrin17, (3)

Exendin-4(1-39)-AAA-[Gln1,Leu15]Gastrin17, (4)

Exendin-4(1-39)-SKK-[Gln1,Leu15]Gastrin17, (5)

Exendin-4(1-39)-Peg3-SKK-[Gln1,Leu15]Gastrin17, (6)

Exendin-4(1-39)-8Aoc-SKK-[Gln1,Leu15]Gastrin17, (7)

Exendin-4(1-39)-DBF-SKK-[Gln1,Leu15]Gastrin17, (8)

Exendin-4(1-39)-8Aoc-8Aoc-[Gln1,Leu15]Gastrin17, (9)

Exendin-4(1-39)-[Leu4]Gastrin6, (10)

Exendin-4(1-39)-K-[Leu4]Gastrin6, (11)

Exendin-4(1-39)-AAA-[Leu4]Gastrin6, (12)

Exendin-4(1-39)-SKK-[Leu4]Gastrin6, (13)

Exendin-4(1-39)-Peg3-SKK-[Leu4]Gastrin6, (14)

Exendin-4(1-39)-Peg3-Peg3-[Leu4]Gastrin6, (15)

Exendin-4(1-39)-8Aoc-SKK-[Leu4]Gastrin6, (16)

In one aspect, there is provided a method of manufacturing a peptide conjugate as defined herein synthetically.

In one aspect, there is provided a method of manufacturing a peptide conjugate as defined herein recombinantly.

In one aspect, there is provided a process for manufacturing a peptide conjugate, or pharmaceutically acceptable salt or solvate thereof as defined herein.

In a specific ~~aspect~~embodiment, the peptide conjugates of the ~~invention~~disclosure may be manufactured by standard synthetic methods, by use of recombinant expression systems, or by any other suitable method. Thus, the conjugates may be synthesized in a number of ways, including, e.g., methods which comprise:

(a) synthesizing the peptide conjugate by means of standard solid-phase or liquid-phase methodology, either stepwise or by fragment assembly, and isolating and purifying the final peptide conjugate product;

(b) expressing a nucleic acid construct that encodes the peptide conjugate in a host cell and recovering the expression product from the host cell culture; or

(c) effecting cell-free *in vitro* expression of a nucleic acid construct that encodes the peptide conjugate, and recovering the expression product;

or by any combination of methods of (a), (b) or (c) to obtain fragments of the peptide conjugate, subsequently ligating the fragments to obtain the peptide conjugate, and recovering the peptide conjugate.

In one aspect, there is provided a pharmaceutical composition comprising a peptide conjugate, or pharmaceutically acceptable salt or solvate thereof, as defined herein, together with a pharmaceutically acceptable carrier, excipient or vehicle.

In one aspect, there is provided a peptide conjugate or pharmaceutically acceptable salt or solvate thereof as defined herein for use as a medicament.

Among further ~~aspects~~embodiments of the ~~invention~~disclosure are methods of treatment of a variety of conditions, diseases or disorders [including diabetes (type 1 and type 2) and various diabetes-related conditions, diseases or disorders] comprising administration of a peptide conjugate of the ~~invention~~disclosure (in free form or in the form of a pharmaceutically acceptable salt or solvate thereof), as well as pharmaceutical compositions comprising a peptide conjugate or pharmaceutically acceptable salt or solvate thereof of the ~~invention~~disclosure.

In one aspect, there is provided the use of a peptide conjugate or pharmaceutically acceptable salt or solvate thereof as defined herein, in the manufacture of a medicament for the treatment, in a subject in need thereof, of a disease or disorder selected from the group consisting of:

type 1 diabetes, type 2 diabetes, pre-diabetes, Insulin resistance syndrome, impaired glucose tolerance (IGT), disease states associated with elevated blood glucose levels, hyperglycemia, hypertension, atherogenic dyslipidemia, arteriosclerosis (e.g. atherosclerosis), coronary heart disease, peripheral artery disease, stroke, microvascular disease, gastric disease, metabolic syndrome, cancer (e.g. colon cancer), inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).

In one aspect, there is provided the use of a peptide conjugate or pharmaceutically acceptable salt or solvate thereof as defined herein, in the manufacture of a medicament for inducing, in a subject in need thereof, pancreatic islet neogenesis.

In one aspect, there is provided the use of a peptide conjugate or pharmaceutically acceptable salt or solvate thereof as defined herein in the manufacture of a medicament for preventing, in a subject in need thereof, β-cell apoptosis in the pancreatic islets.

In one aspect, there is provided the use of a peptide conjugate, or pharmaceutically acceptable salt or solvate thereof, as defined herein, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for preventing weight gain or promoting weight loss.

In one aspect, there is provided the use of a peptide conjugate, or pharmaceutically acceptable salt or solvate thereof, as defined herein in the manufacture of a medicament for the treatment of a condition caused or characterized by excess body weight, e.g. the treatment and/or prevention of obesity, morbid obesity, obesity linked inflammation, obesity linked gallbladder disease, obesity induced sleep apnea, metabolic syndrome, or pre-diabetes.

In one aspect, there is provided the use of a peptide conjugate, or pharmaceutically acceptable salt or solvate thereof, as defined herein, in the manufacture of a medicament for improving circulating glucose levels, glucose tolerance and/or circulating cholesterol levels, lowering circulating LDL levels, and/or increasing HDL/LDL ratio.

In one aspect, there is provided the use of a peptide conjugate, or pharmaceutically acceptable salt or solvate thereof as defined herein in the manufacture of a medicament for improving circulating glucose levels, glucose tolerance and/or circulating cholesterol levels, lowering circulating LDL levels, and/or increasing HDL/LDL ratio in an individual in need thereof wherein a Drug holiday dosage regimen is used.

In a specific ~~aspect~~embodiment, the peptide conjugates of the present ~~invention~~disclosure may also be useful as pharmaceutical agents for treatment of Insulin resistance, glucose intolerance, pre-diabetes, elevated fasting glucose levels, type 1 and/or type 2 diabetes, hypertension and/or dyslipidemia (or a combination of these metabolic risk factors), atherosclerosis, arteriosclerosis, coronary heart disease, peripheral artery disease and stroke. They may also be useful in preventing weight gain, promoting weight loss, reducing excess body weight and/or treating obesity (e.g. by control of appetite, feeding, food intake, calorie intake, and/or energy expenditure), including morbid obesity, as well as associated diseases, disorders and health conditions, including, but not limited to, obesity-linked inflammation, obesity-linked gallbladder disease and obesity-induced sleep apnea. Effects of the peptide conjugates of the ~~invention~~disclosure on these conditions may be mediated in whole or in part via an effect on body weight, or may be independent thereof.

In one aspect, there is provided a device comprising at least one peptide conjugate, or pharmaceutically acceptable salt or solvate thereof, as defined herein, for delivery of the peptide conjugate to a subject.

In one aspect, there is provided a kit comprising at least one peptide conjugate, or pharmaceutically acceptable salt or solvate thereof, as defined herein, and further comprising packaging or instructions for use.

Further ~~aspects~~embodiments of the ~~invention~~disclosure will become apparent from the disclosure below.