agonist with various ratios. Accordingly, a combined therapy capable of using various activity ratios by adjusting the contents of GLP-1 and glucagon may be more effective. However, for the combined therapy, it is required to improve the physical characteristics of glucagon, which aggregates at a neutral pH and precipitates with time, thus showing poor solubility.

Under these circumstances, the present inventors have developed glucagon derivatives with partial modifications of the amino acid sequence of glucagon for improving the therapeutic effects of glucagon on hypoglycemia and obesity by improving the physical properties of glucagon, and have discovered that these glucagon derivatives, due to the altered pI values which are different from that of native glucagon, have improved solubility and higher stability at a neutral pH and have confirmed that the developed glucagon derivative activates its receptors in *in vitro* assay, thereby completing the present invention.

【SUMMARY】

【Technical Problem】

An object of the present invention is to provide a pharmaceutical composition for treating or preventing metabolic syndrome, containing a glucagon derivative and at least one compound or material having a therapeutic activity for metabolic syndrome.

Another object of the present invention is to provide a novel glucagon derivative.

Still another object of the present invention is to provide an isolated polynucleotide encoding the glucagon derivative, a vector including the polynucleotide, and an isolated cell including the polynucleotide or the vector.

Still another object of the present invention is to provide an isolated conjugate in which a glucagon derivative and a biocompatible material which is capable of increasing *in vivo* half-life are linked.

Still another object of the present invention is to provide a composition containing the glucagon derivative and the isolated conjugate.

Still another object of the present invention is to provide a pharmaceutical composition for treating or preventing hypoglycemia or metabolic syndrome, containing the glucagon derivative or the isolated conjugate.

Still another object of the present invention is to provide a method for preventing or treating hypoglycemia or metabolic syndrome including administering the above composition to the subject in need thereof.

Still another object of the present invention is to provide use of the glucagon derivative or the isolated conjugate or the composition in the preparation of a

medicament (or a pharmaceutical composition) for preventing or treating hypoglycemia or metabolic syndrome.

【Technical Solution】

In order to achieve the above objects, an aspect of the present invention provides a pharmaceutical composition for treating or preventing metabolic syndrome containing a glucagon derivative and at least one compound or material which has a therapeutic activity for metabolic syndrome.

More specifically, an aspect of the present invention refers to a pharmaceutical composition for treating or preventing metabolic syndromes comprising i) a peptide comprising the amino acid sequence of the following General Formula 1 and ii) at least one compound or material having a therapeutic activity for metabolic syndrome:

X1-X2-QGTF-X7-SD-X10-S-X12-X13-X14-X15-X16-X17-X18-X19-X20-X21-F-X23-X24-W-L-X27-X28-X29-X30 (General Formula 1, SEQ ID NO: 45)

wherein, in General Formula 1,

X1 is histidine, desamino-histidyl, N-dimethyl-histidyl, β-hydroxy imidazopropionyl, 4-imidazoacetyl, β-carboxy imidazopropionyl, tryptophan, or tyrosine, or is absent;

X2 is α-methyl-glutamic acid, aminoisobutyric acid (Aib), D-alanine, glycine, Sar (N-methylglycine), serine, or D-serine;

X7 is threonine, valine, or cysteine;

X10 is tyrosine or cysteine;

X12 is lysine or cysteine;

X13 is tyrosine or cysteine;

X14 is leucine or cysteine;

X15 is aspartic acid, glutamic acid, or cysteine;

X16 is glutamic acid, aspartic acid, serine, α-methyl-glutamic acid, or cysteine, or is absent;

X17 is aspartic acid, glutamine, glutamic acid, lysine, arginine, serine, cysteine, or valine, or is absent;

X18 is alanine, aspartic acid, glutamic acid, arginine, valine, or cysteine, or is absent;

X19 is alanine, arginine, serine, valine, or cysteine, or is absent;

X20 is lysine, histidine, glutamine, aspartic acid, lysine, arginine, α-methyl-glutamic acid, or cysteine, or is absent;

X21 is aspartic acid, glutamic acid, leucine, valine, or cysteine, or is absent;

X23 is isoleucine, valine, or arginine, or is absent;

X24 is valine, arginine, alanine, cysteine, glutamic acid, lysine, glutamine, α-methyl-glutamic acid, or leucine, or is absent;

X27 is isoleucine, valine, alanine, lysine, methionine, glutamine, or arginine, or is absent;

X28 is glutamine, lysine, asparagine, or arginine, or is absent;

X29 is lysine, alanine, glycine, or threonine, or is absent; and

X30 is cysteine, or is absent;

with the proviso that when the amino acid sequence of General Formula 1 is identical to SEQ ID NO: 1, it is excluded, and the peptide satisfies at least one condition selected from the following (1) to (4):

(1) in General Formula 1,

X1 is tyrosine;

X2 is aminoisobutyric acid (Aib);

X7 is threonine;

X10 is tyrosine;

X12 is lysine;

X13 is tyrosine;

X14 is leucine;

X15 is aspartic acid or cysteine;

X16 is glutamic acid, serine, or cysteine;

X17 is lysine or arginine;

X18 is arginine;

X19 is alanine;

X20 is glutamine, cysteine, or lysine;

X21 is aspartic acid, cysteine, valine, or glutamic acid;

X23 is valine;

X24 is valine or arginine;

X27 is methionine;

X28 is asparagine or arginine;

X29 is threonine; and

X30 is absent,

(2) the peptide comprises the amino acid sequence of the following General Formula 2:

Y-Aib-QGTF-X7-SD-X10-S-X12-Y-L-X15-X16-X17-R-A-X20-X21-F-V-X24-W-L-M-N-T-X30 (General Formula 2, SEQ ID NO: 46)

wherein, in General Formula 2,

X7 is threonine, valine, or cysteine;

X10 is tyrosine or cysteine;

X12 is lysine or cysteine;

X15 is aspartic acid or cysteine;

X16 is glutamic acid or serine;

X17 is lysine or arginine;

X20 is glutamine or lysine;

X21 is aspartic acid or glutamic acid;

X24 is valine or glutamine; and

X30 is cysteine or is absent,

(3) the peptide comprising the amino acid sequence of General Formula 1 has an isoelectric point (pI) value different to that of native glucagon (6.8), or

(4) the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 2 to 44.

In another aspect, the present invention refers to an isolated peptide comprising the amino acid sequence of the following General Formula 2:

Y-Aib-QGTF-X7-SD-X10-S-X12-Y-L-X15-X16-X17-R-A-X20-X21-F-V-X24-W-L-M-N-T-X30 (General Formula 2, SEQ ID NO: 46)

wherein, in General Formula 2,

X7 is threonine, valine, or cysteine;

X10 is tyrosine or cysteine;

X12 is lysine or cysteine;

X15 is aspartic acid or cysteine;

X16 is glutamic acid or serine;

X17 is lysine or arginine;

X20 is glutamine or lysine;

X21 is aspartic acid or glutamic acid;

X24 is valine or glutamine; and

X30 is cysteine or is absent;

with the proviso that when the amino acid sequence of General Formula 2 is identical to any one of SEQ ID NOS: 14, 19, 20, 25, 27, 31, and 33, it is excluded.

In another aspect, the present invention refers to an isolated polynucleotide encoding the isolated peptide of any of the present invention.

In yet another aspect, the present invention refers to a vector comprising the isolated polynucleotide of the present invention.

In yet another aspect, the present invention refers to an isolated conjugate, wherein the isolated peptide of the present invention and a biocompatible material capable of increasing *in vivo* half-life are linked.

In another aspect, the present invention refers to a composition comprising the isolated

peptide of the present invention or the isolated conjugate of the present invention.

Also described herein is:

An example of a pharmaceutical composition for treating or preventing metabolic syndrome, which contains: i) a peptide including the amino acid sequence of the following General Formula 1, and ii) at least one compound or material having a therapeutic activity for metabolic syndrome:

X1-X2-QGTF-X7-SD-X10-S-X12-X13-X14-X15-X16-X17-X18-X19-X20-X21-F-X23-X24-W-L-X27-X28-X29-X30 (General Formula 1, SEQ ID NO: 45)

In General Formula 1,

X1 is histidine, desamino-histidyl, N-dimethyl-histidyl, β-hydroxy imidazopropionyl, 4-imidazoacetyl, β-carboxy imidazopropionyl, tryptophan, or tyrosine, or is absent;

X2 is α-methyl-glutamic acid, aminoisobutyric acid (Aib), D-alanine, glycine, Sar(N-methylglycine), serine, or D-serine;

X7 is threonine, valine, or cysteine;

X10 is tyrosine or cysteine;

X12 is lysine or cysteine;

X13 is tyrosine or cysteine;

X14 is leucine or cysteine;

X15 is aspartic acid, glutamic acid, or cysteine;

X16 is glutamic acid, aspartic acid, serine, α-methyl-glutamic acid, or cysteine, or is absent;

X17 is aspartic acid, glutamine, glutamic acid, lysine, arginine, serine, cysteine, or valine, or is absent;

X18 is alanine, aspartic acid, glutamic acid, arginine, valine, or cysteine, or is absent;

X19 is alanine, arginine, serine, valine, or cysteine, or is absent;

X20 is lysine, histidine, glutamine, aspartic acid, lysine, arginine, α-methyl-glutamic acid, or cysteine, or is absent;

X21 is aspartic acid, glutamic acid, leucine, valine, or cysteine, or is absent;

X23 is isoleucine, valine, or arginine, or is absent;

X24 is valine, arginine, alanine, cysteine, glutamic acid, lysine, glutamine, α-methyl-glutamic acid, or leucine, or is absent;

X27 is isoleucine, valine, alanine, lysine, methionine, glutamine, or arginine, or is absent;

X28 is glutamine, lysine, asparagine, or arginine, or is absent;

X29 is lysine, alanine, glycine, or threonine, or is absent; and

X30 is cysteine, or is absent;

with the proviso that when the amino acid sequence of General Formula 1 is identical to SEQ ID NO: 1, it is excluded.

In another specific example,

in General Formula 1,

X1 is histidine, tryptophan, or tyrosine, or is absent;

X2 is serine or aminoisobutyric acid (Aib);

X7 is threonine, valine, or cysteine;

X10 is tyrosine or cysteine;

X12 is lysine or cysteine;

X13 is tyrosine or cysteine;

X14 is leucine or cysteine;

X15 is aspartic acid or cysteine;

X16 is glutamic acid, serine, or cysteine;

X17 is aspartic acid, glutamic acid, lysine, arginine, serine, cysteine, or valine;

X18 is aspartic acid, glutamic acid, arginine, or cysteine;

X19 is alanine or cysteine;

X20 is glutamine, aspartic acid, lysine, or cysteine;

X21 is aspartic acid, glutamic acid, leucine, valine, or cysteine;

X23 is isoleucine, valine, or arginine;

X24 is valine, arginine, alanine, glutamic acid, lysine, glutamine, or leucine;

X27 is isoleucine, valine, alanine, methionine, glutamine, or arginine;

X28 is glutamine, lysine, asparagine, or arginine;

X29 is threonine; and

X30 is cysteine or is absent.

In still another specific example, in General Formula 1,

X1 is histidine, tryptophan, or tyrosine, or is absent;
X2 is serine or aminoisobutyric acid (Aib);
X7 is threonine, valine, or cysteine;
X10 is tyrosine or cysteine;

X12 is lysine or cysteine;

X13 is tyrosine or cysteine;

X14 is leucine or cysteine;

X15 is aspartic acid or cysteine;

X16 is glutamic acid, serine, or cysteine;

X17 is aspartic acid, glutamic acid, lysine, arginine, serine, cysteine, or valine;

X18 is aspartic acid, glutamic acid, arginine, or cysteine;

X19 is alanine or cysteine;

X20 is glutamine, aspartic acid, or lysine;

X21 is aspartic acid or glutamic acid;

X23 is valine;

X24 is valine or glutamine;

X27 is isoleucine or methionine;

X28 is asparagine or arginine;

X29 is threonine; and

X30 is cysteine or is absent.

In still another specific example, in General Formula 1,

X1 is tyrosine, X2 is aminoisobutyric acid (Aib);

X7 is threonine;

X10 is tyrosine;

X12 is lysine;

X13 is tyrosine;

X14 is leucine;

X15 is aspartic acid or cysteine;

X16 is glutamic acid, serine, or cysteine;

X17 is lysine or arginine;

X18 is arginine;

X19 is alanine;

X20 is glutamine, cysteine, or lysine;

X21 is aspartic acid, cysteine, valine, or glutamic acid;

X23 is valine;

X24 is valine or arginine;

X27 is methionine;
X28 is asparagine or arginine;
X29 is threonine; and
X30 is absent.

In still another specific example, the above peptide is characterized in that it is a peptide including the amino acid sequence of the following General Formula 2:

Y-Aib-QGTF-X7-SD-X10-S-X12-Y-L-X15-X16-X17-R-A-X20-X21-F-V-X24-W-L-M-N-T-X30 (General Formula 2, SEQ ID NO: 46)

In General Formula 2,

X7 is threonine, valine, or cysteine;

X10 is tyrosine or cysteine;

X12 is lysine or cysteine;

X15 is aspartic acid or cysteine;

X16 is glutamic acid or serine;

X17 is lysine or arginine;

X20 is glutamine or lysine;

X21 is aspartic acid or glutamic acid;

X24 is valine or glutamine; and

X30 is cysteine or is absent,

wherein, among the peptides including the amino acid sequence of General Formula 2, the peptides corresponding to SEQ ID NOS: 14, 19, 20, 25, 27, 31, and 33 may be excluded.

In still another specific example, the peptide including the amino acid sequence of General Formula 1 is characterized in that it has a pI value different to that of native glucagon, e.g., a pI of 6.5 or less, or a pI of 7.0 or higher.

In still another specific example, the peptide including the amino acid sequence of General Formula 1 is characterized in that at least one amino acid pair among the amino acid pairs of X10 and X14, X12 and X16, X16 and X20, X17 and X21, X20 and X24, and X24 and X28 in General Formula 1 is substituted with glutamic acid or lysine, which is capable of forming a ring, respectively.

In still another specific example, the peptide including the amino acid sequence of General Formula 1 is characterized in that the amino acid pair of X12 and X16 or the amino acid pair of X16 and X20 is respectively substituted with glutamic acid or lysine, which is capable of forming a ring.

In still another specific example, the peptide including the amino acid sequence of General Formula 1 is characterized in that at least one amino acid pair among the amino acid pairs of X10 and X14, X12 and X16, X16 and X20, X17 and X21, X20 and X24, and X24 and X28 in General Formula 1 forms a ring (e.g., a lactam ring).

In still another specific example, the peptide including the amino acid sequence

of General Formula 1 is characterized in that the C-terminus of the peptide is amidated.

In still another specific example, the peptide including the amino acid sequence of the following General Formula 1 is characterized in that it is a glucagon derivative capable of activating a glucagon receptor.

In still another specific example, the peptide is characterized in that it includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 2 to 44.

In still another specific example, the peptide is characterized in that it includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 2 to 13, 15, 17, 20 to 24, 26 to 30, and 32 to 44.

In still another specific example, the peptide is characterized in that it includes an amino acid sequence of SEQ ID NO: 12 or SEQ ID NO: 20.

In still another specific example, the compound or material having a therapeutic activity for metabolic syndrome is characterized in that it is selected from the group consisting of an insulinotropic peptide, a glucagon-like peptide-1 (GLP-1) receptor agonist, a leptin receptor agonist, a dipeptidyl peptidase-IV (DPP-IV) inhibitor, a Y5 receptor antagonist, a melanin-concentrating hormone (MCH) receptor antagonist, a Y2/4 receptor agonist, a melanocortin 3/4 (MC 3/4) receptor agonist, a gastric/pancreatic lipase inhibitor, an agonist of 5-hydroxytryptamine receptor 2C (5HT2C), a β3A receptor agonist, an amylin receptor agonist, a ghrelin antagonist, a ghrelin receptor antagonist, a peroxisome proliferator-activated receptor alpha (PPARα) agonist, a peroxisome proliferator-activated receptor delta (PPARδ) agonist, a Farnesoid X receptor (FXR) agonist, an acetyl-CoA carboxylase inhibitor, a peptide YY, cholecystokinin (CCK), xenin, glicentin, obestatin, secretin, nesfatin, insulin, and a glucose-dependent insulinotropic peptide (GIP).

In still another specific example, the insulinotropic peptide is characterized in that it is selected from the group consisting of GLP-1, exendin-3, exendin-4, an agonist thereof, a derivative thereof, a fragment thereof, a variant thereof, and a combination thereof.

In still another specific example, the insulinotropic peptide is characterized in that it is an insulinotropic peptide derivative in which the N-terminal histidine residue of the insulinotropic peptide is substituted with one selected from the group consisting of desamino-histidyl, N-dimethyl-histidyl, β-hydroxy imidazopropionyl, 4-imidazoacetyl, and β-carboxy imidazopropionyl.

In still another specific example, the insulinotropic peptide is characterized in that it is selected from the group consisting of a native exendin-4; an exendin-4 derivative in which the N-terminal amine group of exendin-4 is deleted; an exendin-4 derivative in which the N-terminal amine group of exendin-4 is substituted with a hydroxyl group; an exendin-4 derivative in which the N-terminal amine group of

exendin-4 is modified with a dimethyl group; an exendin-4 derivative in which the α-carbon of the 1st amino acid of exendin-4, histidine, is deleted; an exendin-4 derivative in which the 12th amino acid of exendin-4, lysine, is substituted with serine, and an exendin-4 derivative in which the 12th amino acid of exendin-4, lysine, is substituted with arginine.

In still another specific example, the peptide including the amino acid sequence of General Formula 1 is characterized in that the peptide including the amino acid sequence of General Formula 1 is in the form of a long-acting conjugate to which a biocompatible material capable of increasing *in vivo* half-life of the peptide is linked; and the insulinotropic peptide is in the form of a long-acting conjugate to which a biocompatible material capable of increasing *in vivo* half-life of the insulinotropic peptide is linked.

In still another specific example, the biocompatible material is characterized in that it is selected from the group consisting of polyethylene glycol, fatty acid, cholesterol, albumin and a fragment thereof, an albumin-binding material, a polymer of repeating units of a particular amino acid sequence, an antibody, an antibody fragment, an FcRn-binding material, *in vivo* connective tissue or a derivative thereof, a nucleotide, fibronectin, transferrin, a saccharide, and a polymer.

In still another specific example, the peptide including the amino acid sequence of General Formula 1 and the insulinotropic peptide are characterized in that they are respectively linked to a biocompatible material by a linker selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, a polysaccharide, dextran, polyvinyl ethyl ether, a biodegradable polymer such as polylactic acid (PLA) and polylactic-glycolic acid (PLGA), lipid polymer, chitin, hyaluronic acid, fatty acid, a polymer, a low molecular weight compound, a nucleotide, and a combination thereof.

In still another specific example, the biocompatible material is characterized in that it is an FcRn-binding material, and the peptide including the amino acid sequence of General Formula 1 and the insulinotropic peptide are respectively linked to a biocompatible material by a peptide linker or a non-peptide linker selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, a polysaccharide, polyvinyl ethyl ether, dextran, a biodegradable polymer such as polylactic acid (PLA) and polylactic-glycolic acid (PLGA), lipid polymer, chitin, hyaluronic acid, and a combination thereof.

In still another specific example, the FcRn-binding material is characterized in that it is a polypeptide including an immunoglobulin Fc region.

In still another specific example, the immunoglobulin Fc region is characterized

in that it is aglycosylated.

In still another specific example, the immunoglobulin Fc region is characterized in that it is selected from the group consisting of:

(a) a CH1 domain, a CH2 domain, a CH3 domain, and a CH4 domain;

(b) a CH1 domain and a CH2 domain;

(c) a CH1 domain and a CH3 domain;

(d) a CH2 domain and a CH3 domain;

(e) a combination between one or two or more domains among a CH1 domain, a CH2 domain, a CH3 domain, and a CH4 domain and an immunoglobulin hinge region or a part of the hinge region; and

(f) a dimer between each domain of the heavy chain constant region and the light chain constant region.

In still another specific example, the polypeptide including the immunoglobulin Fc region is in the form of a dimer.

In still another specific example, the immunoglobulin Fc region is characterized in that it is a native Fc derivative in which the region capable of forming a disulfide bond is deleted, a native Fc derivative in which a part of the amino acid(s) in the N-terminus is deleted, a native Fc derivative in which a methionine is added to the N-terminus, a native Fc derivative in which a complement-binding site is deleted, or a native Fc derivative in which an antibody dependent cell mediated cytotoxicity (ADCC) site is deleted.

In still another specific example, the immunoglobulin Fc region is characterized in that it is an Fc region derived from an immunoglobulin selected from the group consisting of IgG, IgA, IgD, IgE, and IgM.

In still another specific example, the immunoglobulin Fc region is characterized in that it is an IgG4 Fc region.

In still another specific example, the immunoglobulin Fc region is characterized in that it is an aglycosylated Fc region derived from human IgG4.

In still another specific example, the non-peptide linker is characterized in that it is linked to the cysteine residue of a peptide including the amino acid sequence of General Formula 1.

In still another specific example, the non-peptide linker is characterized in that both ends of the non-peptide linker are respectively linked to an amine group or a thiol group of a peptide, which includes the amino acid sequence of General Formula 1, or an insulinotropic peptide, and a biocompatible material.

In still another specific example, the metabolic syndrome is characterized in that it is selected from the group consisting of impaired glucose tolerance, hypercholesterolemia, dyslipidemia, obesity, diabetes, hypertension, nonalcoholic steatohepatitis

(NASH), atherosclerosis caused by dyslipidemia, atherosclerosis, arteriosclerosis, coronary heart disease, and stroke.

In another example, the present disclosure provides a novel glucagon derivative.

In a specific example, the glucagon derivative is characterized in that it is an isolated peptide including the amino acid sequence of the following General Formula 2:

Y-Aib-QGTF-X7-SD-X10-S-X12-Y-L-X15-X16-X17-R-A-X20-X21-F-V-X24-W-L-M-N-T-X30 (General Formula 2, SEQ ID NO: 46)

In General Formula 2,

X7 is threonine, valine, or cysteine;

X10 is tyrosine or cysteine;

X12 is lysine or cysteine;

X15 is aspartic acid or cysteine;

X16 is glutamic acid or serine;

X17 is lysine or arginine;

X20 is glutamine or lysine;

X21 is aspartic acid or glutamic acid;

X24 is valine or glutamine; and

X30 is cysteine or is absent,

wherein, among the peptides including the amino acid sequence of General Formula 2, the peptides corresponding to SEQ ID NOS: 14, 19, 20, 25, 27, 31, and 33 may be excluded.

In still another specific example, the peptide including the amino acid sequence of General Formula 2 is characterized in that the amino acid pair of X16 and X20 is substituted with glutamic acid or lysine, which is capable of forming a ring.

In still another specific example, the peptide including the amino acid sequence of General Formula 2 is characterized in that the C-terminus of the peptide is amidated.

In still another specific example, the peptide is characterized in that it is a glucagon derivative capable of activating a glucagon receptor.

In still another specific example, the peptide is characterized in that it includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 12, 13, 15, and 36 to 44.

In still another specific example, the peptide is characterized in that it includes

the amino acid sequence of SEQ ID NO: 12.

In one example, the present disclosure provides an isolated polynucleotide encoding the glucagon derivative, a vector including the polynucleotide, and an isolated cell including the polynucleotide or the vector.

In one example, the present disclosure provides an isolated conjugate in which a glucagon derivative and a biocompatible material capable of increasing *in vivo* half-life are linked.

In a specific example, the biocompatible material is characterized in that the biocompatible material is selected from the group consisting of polyethylene glycol, fatty acid, cholesterol, albumin and a fragment thereof, an albumin-binding material, a polymer of repeating units of a particular amino acid sequence, an antibody, an antibody fragment, an FcRn-binding material, *in vivo* connective tissue or a derivative thereof, a nucleotide, fibronectin, transferrin, a saccharide, and a polymer.

In a specific example, the isolated peptide is characterized in that it is linked to a biocompatible material by a linker selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, a polysaccharide, dextran, polyvinyl ethyl ether, a biodegradable polymer such as polylactic acid (PLA) and polylactic-glycolic acid (PLGA), lipid polymer, chitin, hyaluronic acid, fatty acid, a polymer, a low molecular weight compound, a nucleotide, and a combination thereof.

In a specific example, the biocompatible material is characterized in that it is an FcRn-binding material, and

the isolated peptide and the insulinotropic peptide are respectively linked to a biocompatible material by a peptide linker or a non-peptide linker selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, a polysaccharide, polyvinyl ethyl ether, dextran, a biodegradable polymer such as polylactic acid (PLA) or polylactic-glycolic acid (PLGA), lipid polymer, chitin, hyaluronic acid, and a combination thereof.

In a specific example,

the FcRn-binding material is characterized in that it is a polypeptide including an immunoglobulin Fc region.

In one example, the present disclosure provides a composition including the glucagon derivative or the isolated conjugate.

In a specific example, the composition is characterized in that it is a pharma-

ceutical composition for treating or preventing hypoglycemia or metabolic syndrome.

In another example, the present disclosure provides a method for preventing or treating hypoglycemia or metabolic syndrome including administering the composition to the subject in need thereof.

In still another example, the present disclosure provides use of the glucagon derivative or the isolated conjugate or the composition in the preparation of a medicament (or a pharmaceutical composition) for preventing or treating hypoglycemia or metabolic syndrome.

【Advantageous Effects】

The glucagon derivatives described herein have improved physical properties compared to that of native glucagon and thus can be effectively used as a therapeutic agent for treating hypoglycemia by improving the compliance of patients. Additionally, the glucagon derivatives of the described herein can be effectively used for the prevention and treatment of hypoglycemia and metabolic syndrome such as obesity, diabetes, and nonalcoholic steatohepatitis (NASH).

【Description of Drawings】

FIG. 1 shows a graph illustrating the changes in body weight of obesity animal models (rats), which were prepared by high-fat diet, during a single or combined administration of a long-acting insulinotropic peptide conjugate (named as a long-acting exendin-4 derivative) and a long-acting glucagon derivative conjugate (named as a long-acting derivative of SEQ ID NO: 12) with an adjusted dose to the rats, at 3-day intervals for 15 days.

FIG. 2 shows a result illustrating the amount of mesenteric fat of obesity animal models (rats), which were prepared by high-fat diet, measured after a single or combined administration of a long-acting insulinotropic peptide conjugate (named as a long-acting exendin-4 derivative) and a long-acting glucagon derivative conjugate (named as a long-acting derivative of SEQ ID NO: 12) with an adjusted dose to the rats for 15 days (*$p<0.05$, **$p<0.01$ vs. vehicle by ANOVA test).

FIG. 3 shows a result illustrating the difference in liver weight of obesity animal models (rats), which were prepared by high-fat diet, measured after a single or combined administration of a long-acting insulinotropic peptide conjugate (named as a long-acting exendin-4 derivative) and a long-acting glucagon derivative conjugate (named as a long-acting derivative of SEQ ID NO: 12) with an adjusted dose to the rats

X21 is aspartic acid, cysteine, valine, or glutamic acid;

X23 is valine;

X24 is valine or arginine;

X27 is methionine;

X28 is asparagine or arginine;

X29 is threonine; and

X30 is absent.

For example, the peptide may be one which includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 14, 16, 18, 19, 25 and 31, and specifically, one which (essentially) consists of an amino acid sequence selected from the group consisting of SEQ ID NOS: 14, 16, 18, 19, 25 and 31, but is not limited thereto.

More specifically, the peptide may be a peptide which includes the amino acid sequence of the following General Formula 2:

Y-Aib-QGTF-X7-SD-X10-S-X12-Y-L-X15-X16-X17-R-A-X20-X21-F-V-X24-W-L-M-N-T-X30 (General Formula 2, SEQ ID NO: 46)

In General Formula 2,

X7 is threonine, valine, or cysteine;

X10 is tyrosine or cysteine;

X12 is lysine or cysteine;

X15 is aspartic acid or cysteine;

X16 is glutamic acid or serine;

X17 is lysine or arginine;

X20 is glutamine or lysine;

X21 is aspartic acid or glutamic acid;

X24 is valine or glutamine; and

X30 is cysteine or is absent,

with the proviso that when the amino acid sequence of General Formula 2 is identical to any one of SEQ ID NOS: 14, 19, 20, 25, 27, 31, and 33, it may be excluded, but it is not limited thereto.

For example, the peptide may be one which includes an amino acid sequence selected from the group consisting of SEQ ID NOS: 12, 13, 15, and 36 to 44, and specifically, one which (essentially) consists of an amino acid sequence selected from

X16 is glutamic acid or serine;

X17 is lysine or arginine;

X20 is glutamine or lysine;

X21 is aspartic acid or glutamic acid;

X24 is valine or glutamine; and

X30 is cysteine or is absent,

with the proviso that when the amino acid sequence of General Formula 2 is identical to any one of SEQ ID NOS: 14, 19, 20, 25, 27, 31, and 33, it may be excluded.

In still another aspect, the present invention provides a method for preventing or treating hypoglycemia or metabolic syndrome, including administering the above composition to a subject.

The composition, hypoglycemia, metabolic syndrome, prevention, and treatment are the same as explained above.

In the present invention, the term "subject" refers to those suspected of having hypoglycemia or metabolic syndrome, which means mammals including humans, mice, and livestock having hypoglycemia or metabolic syndrome or having the risk of hypoglycemia or metabolic syndrome. However, any subject to be treated with the glucagon derivative of the present invention or the composition containing the same is included without limitation. Further, the subject suspected of having hypoglycemia or obesity can be effectively treated by administering with the pharmaceutical composition containing the glucagon derivative of the present invention. The hypoglycemia and obesity are the same as explained above.

The method of the present invention may include administering the pharmaceutical composition containing the peptide at a pharmaceutically effective amount. The total daily dose should be determined within appropriate medical judgment by a physician, and administered once or several times in divided doses. Regarding the objects of the present invention, the specific therapeutically effective dose for any particular patient may be preferably applied differently, depending on various factors well known in the medical art, including the kind and degree of the response to be achieved, specific compositions including whether other agents are occasionally used therewith or not, the patient's age, body weight, general health conditions, sex and diet, the time and route of administration, secretion rate of the composition, duration of treatment, other drugs used in combination or concurrently with the composition of the present invention, and like factors well known in the medical arts.

【CLAIMS】

【Claim 1】

A pharmaceutical composition for treating or preventing metabolic syndromes comprising i) a peptide comprising the amino acid sequence of the following General Formula 1 and ii) at least one compound or material having a therapeutic activity for metabolic syndrome:

X1-X2-QGTF-X7-SD-X10-S-X12-X13-X14-X15-X16-X17-X18-X19-X20-X21-F-X23-X24-W-L-X27-X28-X29-X30 (General Formula 1, SEQ ID NO: 45)

wherein, in General Formula 1,

X1 is histidine, desamino-histidyl, N-dimethyl-histidyl, β-hydroxy imidazopropionyl, 4-imidazoacetyl, β-carboxy imidazopropionyl, tryptophan, or tyrosine, or is absent;

X2 is α-methyl-glutamic acid, aminoisobutyric acid (Aib), D-alanine, glycine, Sar (N-methylglycine), serine, or D-serine;

X7 is threonine, valine, or cysteine;

X10 is tyrosine or cysteine;

X12 is lysine or cysteine;

X13 is tyrosine or cysteine;

X14 is leucine or cysteine;

X15 is aspartic acid, glutamic acid, or cysteine;

X16 is glutamic acid, aspartic acid, serine, α-methyl-glutamic acid, or cysteine, or is absent;

X17 is aspartic acid, glutamine, glutamic acid, lysine, arginine, serine, cysteine, or valine, or is absent;

X18 is alanine, aspartic acid, glutamic acid, arginine, valine, or cysteine, or is absent;

X19 is alanine, arginine, serine, valine, or cysteine, or is absent;

X20 is lysine, histidine, glutamine, aspartic acid, lysine, arginine, α-methyl-glutamic acid, or cysteine, or is absent;

X21 is aspartic acid, glutamic acid, leucine, valine, or cysteine, or is absent;

X23 is isoleucine, valine, or arginine, or is absent;

X24 is valine, arginine, alanine, cysteine, glutamic acid, lysine, glutamine,

α-methyl-glutamic acid, or leucine, or is absent;

X27 is isoleucine, valine, alanine, lysine, methionine, glutamine, or arginine, or is absent;

X28 is glutamine, lysine, asparagine, or arginine, or is absent;

X29 is lysine, alanine, glycine, or threonine, or is absent; and

X30 is cysteine, or is absent;

with the proviso that when the amino acid sequence of General Formula 1 is identical to SEQ ID NO: 1, it is excluded, and the peptide satisfies at least one condition selected from the following (1) to (4):

(1) in General Formula 1,

X1 is tyrosine;

X2 is aminoisobutyric acid (Aib);

X7 is threonine;

X10 is tyrosine;

X12 is lysine;

X13 is tyrosine;

X14 is leucine;

X15 is aspartic acid or cysteine;

X16 is glutamic acid, serine, or cysteine;

X17 is lysine or arginine;

X18 is arginine;

X19 is alanine;

X20 is glutamine, cysteine, or lysine;

X21 is aspartic acid, cysteine, valine, or glutamic acid;

X23 is valine;

X24 is valine or arginine;

X27 is methionine;

X28 is asparagine or arginine;

X29 is threonine; and

X30 is absent,

(2) the peptide comprises the amino acid sequence of the following General Formula

2:

Y-Aib-QGTF-X7-SD-X10-S-X12-Y-L-X15-X16-X17-R-A-X20-X21-F-V-X24-W-L-M-N-T-X30 (General Formula 2, SEQ ID NO: 46)

wherein, in General Formula 2,
X7 is threonine, valine, or cysteine;
X10 is tyrosine or cysteine;
X12 is lysine or cysteine;
X15 is aspartic acid or cysteine;
X16 is glutamic acid or serine;
X17 is lysine or arginine;
X20 is glutamine or lysine;
X21 is aspartic acid or glutamic acid;
X24 is valine or glutamine; and
X30 is cysteine or is absent,

(3) the peptide comprising the amino acid sequence of General Formula 1 has an isoelectric point (pI) value different to that of native glucagon (6.8), or

(4) the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 2 to 44.

【Claim 2】

The pharmaceutical composition of claim 1, wherein, in the peptide comprising the amino acid sequence of General Formula 1, at least one amino acid pair among the amino acid pairs of X10 and X14, X12 and X16, X16 and X20, X17 and X21, X20 and X24, and X24 and X28 in General Formula 1 is substituted with glutamic acid or lysine, which is capable of forming a ring, respectively.

【Claim 3】

The pharmaceutical composition of claim 2, wherein, in the peptide comprising the

amino acid sequence of General Formula 1, the amino acid pair of X12 and X16 or the amino acid pair of X16 and X20 is respectively substituted with glutamic acid or lysine, which is capable of forming a ring.

【Claim 4】

The pharmaceutical composition of claim 1, wherein the peptide comprising the amino acid sequence of General Formula 1 is a glucagon derivative capable of activating a glucagon receptor.

【Claim 5】

The pharmaceutical composition of claim 1, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 12, 13, 15, and 36 to 44.

【Claim 6】

The pharmaceutical composition of claim 1, wherein the compound or material having a therapeutic activity for metabolic syndrome is selected from the group consisting of an insulinotropic peptide, a glucagon-like peptide-1 (GLP-1) receptor agonist, a leptin receptor agonist, a dipeptidyl peptidase-IV (DPP-IV) inhibitor, a Y5 receptor antagonist, a melanin-concentrating hormone (MCH) receptor antagonist, a Y2/4 receptor agonist, a melanocortin 3/4 (MC 3/4) receptor agonist, a gastric/pancreatic lipase inhibitor, an agonist of 5-hydroxytryptamine receptor 2C (5HT2C), a β3A receptor agonist, an amylin receptor agonist, a ghrelin antagonist, a ghrelin receptor antagonist, a peroxisome proliferator-activated receptor alpha (PPARα) agonist, a peroxisome proliferator-activated receptor delta (PPARδ) agonist, a Farnesoid X receptor (FXR) agonist, an acetyl-CoA carboxylase inhibitor, a peptide YY, cholecystokinin (CCK), xenin, glicentin, obestatin, secretin, nesfatin, insulin, and a glucose-dependent insulinotropic peptide (GIP).

【Claim 7】

The pharmaceutical composition of claim 6, wherein the insulinotropic peptide is selected from the group consisting of GLP-1, exendin-3, exendin-4, an agonist thereof, a derivative thereof, a fragment thereof, a variant thereof, and a combination thereof.

【Claim 8】

The pharmaceutical composition of claim 7, wherein the insulinotropic peptide is an insulinotropic peptide derivative, in which the N-terminal histidine residue is substituted with one selected from the group consisting of desamino-histidyl, N-dimethyl-histidyl, β-hydroxy imidazopropionyl, 4-imidazoacetyl, and β-carboxy imidazopropionyl.

【Claim 9】

The pharmaceutical composition of claim 7, wherein the insulinotropic peptide is selected from the group consisting of a native exendin-4; an exendin-4 derivative in which the N-terminal amine group of exendin-4 is deleted; an exendin-4 derivative in which the N-terminal amine group of exendin-4 is substituted with a hydroxyl group; an exendin-4 derivative in which the N-terminal amine group of exendin-4 is modified with a dimethyl group; an exendin-4 derivative in which the α-carbon of the 1^{st} amino acid of exendin-4, histidine, is deleted; an exendin-4 derivative in which the 12^{th} amino acid of exendin-4, lysine, is substituted with serine, and an exendin-4 derivative in which the 12^{th} amino acid of exendin-4, lysine, is substituted with arginine.

【Claim 10】

The pharmaceutical composition of claim 6, wherein:

the peptide comprising the amino acid sequence of General Formula 1 is in the form of a long-acting conjugate, to which a biocompatible material capable of increasing *in vivo* half-life of the peptide is linked; and

the insulinotropic peptide is in the form of a long-acting conjugate, to which a biocompatible material capable of increasing *in vivo* half-life of the insulinotropic peptide is linked.

【Claim 11】

The pharmaceutical composition of claim 10, wherein the biocompatible material is selected from the group consisting of polyethylene glycol, fatty acid, cholesterol, albumin and a fragment thereof, an albumin-binding material, a polymer of repeating units of a particular amino acid sequence, an antibody, an antibody fragment, an FcRn-binding material, *in vivo* connective tissue or a derivative thereof, a nucleotide, fibronectin, transferrin, a

saccharide, and a polymer.

【Claim 12】

The pharmaceutical composition of claim 10, wherein the peptide comprising the amino acid sequence of General Formula 1 and the insulinotropic peptide are respectively linked to a biocompatible material by a linker selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, a polysaccharide, dextran, polyvinyl ethyl ether, a biodegradable polymer including polylactic acid (PLA) or polylactic-glycolic acid (PLGA), lipid polymer, chitin, hyaluronic acid, fatty acid, a polymer, a low molecular weight compound, a nucleotide, and a combination thereof.

【Claim 13】

The pharmaceutical composition of claim 10,

wherein the biocompatible material is an FcRn-binding material, and

the peptide comprising the amino acid sequence of General Formula 1 and the insulinotropic peptide are respectively linked to a biocompatible material by a peptide linker or a non-peptide linker selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, a polysaccharide, dextran, polyvinyl ethyl ether, a biodegradable polymer including polylactic acid (PLA) or polylactic-glycolic acid (PLGA), lipid polymer, chitin, hyaluronic acid, and a combination thereof.

【Claim 14】

The pharmaceutical composition of claim 13, wherein the FcRn-binding material is a polypeptide comprising an immunoglobulin Fc region.

【Claim 15】

The pharmaceutical composition of claim 14, wherein the immunoglobulin Fc region is aglycosylated.

【Claim 16】

The pharmaceutical composition of claim 14, wherein the immunoglobulin Fc region is selected from the group consisting of:

(a) a CH1 domain, a CH2 domain, a CH3 domain, and a CH4 domain;

(b) a CH1 domain and a CH2 domain;

(c) a CH1 domain and a CH3 domain;

(d) a CH2 domain and a CH3 domain;

(e) a combination between one or at least two domains among a CH1 domain, a CH2 domain, a CH3 domain, and a CH4 domain, and an immunoglobulin hinge region or a part of the hinge region; and

(f) a dimer between each domain of the heavy chain constant region and the light chain constant region.

【Claim 17】

The pharmaceutical composition of claim 14, wherein the polypeptide comprising the immunoglobulin Fc region is in the form of a dimer.

【Claim 18】

The pharmaceutical composition of claim 14, wherein the immunoglobulin Fc region is a native Fc derivative in which the region capable of forming a disulfide bond is deleted, a native Fc derivative in which a part of the amino acid(s) in the N-terminus is removed, a native Fc derivative in which a methionine is added to the N-terminus, a native Fc derivative in which a complement-binding site is deleted, or a native Fc derivative in which an antibody dependent cell mediated cytotoxicity (ADCC) site is deleted.

【Claim 19】

The pharmaceutical composition of claim 14, wherein the immunoglobulin Fc region is derived from an immunoglobulin selected from the group consisting of IgG, IgA, IgD, IgE, and IgM.

【Claim 20】

The pharmaceutical composition of claim 19, wherein the immunoglobulin Fc region

is an IgG4 Fc region.

【Claim 21】

The pharmaceutical composition of claim 14, wherein the immunoglobulin Fc region is an aglycosylated Fc region derived from human IgG4.

【Claim 22】

The pharmaceutical composition of claim 13, wherein the non-peptide linker is linked to the cysteine residue of a peptide comprising the amino acid sequence of General Formula 1.

【Claim 23】

The pharmaceutical composition of claim 13, wherein both ends of the non-peptide linker are respectively linked to an amine group or a thiol group of a peptide, which comprises the amino acid sequence of General Formula 1, or an insulinotropic peptide, and a biocompatible material.

【Claim 24】

The pharmaceutical composition of claim 1, wherein the metabolic syndrome is selected from the group consisting of impaired glucose tolerance, hypercholesterolemia, dyslipidemia, obesity, diabetes, hypertension, nonalcoholic steatohepatitis (NASH), atherosclerosis caused by dyslipidemia, atherosclerosis, arteriosclerosis, coronary heart disease, and stroke.

【Claim 25】

An isolated peptide comprising the amino acid sequence of the following General Formula 2:

Y-Aib-QGTF-X7-SD-X10-S-X12-Y-L-X15-X16-X17-R-A-X20-X21-F-V-X24-W-L-M-N-T-X30 (General Formula 2, SEQ ID NO: 46)

wherein, in General Formula 2,

X7 is threonine, valine, or cysteine;

X10 is tyrosine or cysteine;

X12 is lysine or cysteine;

X15 is aspartic acid or cysteine;

X16 is glutamic acid or serine;

X17 is lysine or arginine;

X20 is glutamine or lysine;

X21 is aspartic acid or glutamic acid;

X24 is valine or glutamine; and

X30 is cysteine or is absent;

with the proviso that when the amino acid sequence of General Formula 2 is identical to any one of SEQ ID NOS: 14, 19, 20, 25, 27, 31, and 33, it is excluded.

【Claim 26】

The peptide of claim 25, wherein, in the peptide comprising the amino acid sequence of General Formula 2, the amino acid pair of X16 and X20 is respectively substituted with glutamic acid or lysine, which is capable of forming a ring.

【Claim 27】

The peptide of claim 25, wherein the C-terminus of the peptide is amidated.

【Claim 28】

The peptide of claim 25, wherein the peptide is a glucagon derivative capable of activating a glucagon receptor.

【Claim 29】

The peptide of claim 25, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 12, 13, 15, and 36 to 44.

【Claim 30】

An isolated polynucleotide encoding the isolated peptide of any of claims 25 to 29.

【Claim 31】

A vector comprising the isolated polynucleotide of claim 30.

【Claim 32】

An isolated conjugate, wherein the isolated peptide of claim 25 and a biocompatible material capable of increasing *in vivo* half-life are linked.

【Claim 33】

The isolated conjugate of claim 32, wherein the biocompatible material is selected from the group consisting of polyethylene glycol, fatty acid, cholesterol, albumin and a fragment thereof, an albumin-binding material, a polymer of repeating units of a particular amino acid sequence, an antibody, an antibody fragment, an FcRn-binding material, *in vivo* connective tissue or a derivative thereof, a nucleotide, fibronectin, transferrin, a saccharide, and a polymer.

【Claim 34】

The isolated conjugate of claim 32, wherein the peptide is linked to a biocompatible material by a linker selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, a polysaccharide, dextran, polyvinyl ethyl ether, a biodegradable polymer including polylactic acid (PLA) or polylactic-glycolic acid (PLGA), lipid polymer, chitin, hyaluronic acid, fatty acid, a polymer, a low molecular weight compound, a nucleotide, and a combination thereof.

【Claim 35】

The isolated conjugate of claim 32,

wherein the biocompatible material is an FcRn-binding material, and

the isolated peptide is linked to a biocompatible material by a peptide linker or a non-peptide linker selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, a polysaccharide, dextran, polyvinyl ethyl ether, a biodegradable polymer including polylactic acid (PLA) or polylactic-glycolic acid (PLGA), lipid polymer, chitin, hyaluronic

acid, and a combination thereof.

【Claim 36】

The isolated conjugate of claim 35, wherein the FcRn-binding material is a polypeptide comprising an immunoglobulin Fc region.

【Claim 37】

A composition comprising the isolated peptide of claim 25 or the isolated conjugate of claim 32.

【Claim 38】

The composition of claim 37, which is a pharmaceutical composition for treating or preventing hypoglycemia or metabolic syndrome.