Description

Title of Invention: COMPOSITION FOR TREATING HYPER-LIPIDEMIA COMPRISING OXYNTOMODULIN DERIVATIVE

Technical Field

[1] The present invention relates to a composition for preventing or treating hyper-lipidemia, fatty liver disease or arteriosclerosis, which comprises an oxyntomodulin derivative as an active ingredient, and to a method for treating hyperlipidemia, fatty liver disease or arteriosclerosis using the composition.

Background Art

[2] In recent years, in Korea, the intake of fats from foods has increased due to economic growth and the westernization of eating habits, and metabolic diseases such as hyper-lipidemia, diabetes, hypertension, arteriosclerosis and fatty liver disease, which are caused by a lack of exercise, have increased.

[3] Hyperlipidemia refers to a condition associated with elevated levels of lipids, such as free cholesterol, cholesterol esters, phospholipids and triglycerides, in blood. Hyper-lipidemia can appear in three forms: (1) hypercholesterolemia, (2) hypertriglyc-eridemia, and (3) combined hyperlipidemia (hypercholesterolemia and hypertriglyc-eridemia). Hyperlipidemia is generally classified into primary hyperlipidemia and secondary hyperlipidemia. Primary hyperlipidemia is generally caused by genetic defects, whereas secondary hyperlipidemia is caused by various disease conditions, drugs and dietary habits. In addition, hyperlipidemia is also caused by a combination of the primary and secondary causes of hyperlipidemia. As criteria for the diagnosis of hyperlipidemia, a total cholesterol level of 220 mg/dl or higher and a triglyceride level of 150 mg/dl or higher are generally used.

[4] There are various forms of cholesterol that naturally occur in mammals. Low-density (LDL) cholesterol is known to be harmful to health, and it is known that an increase in LDL cholesterol increases the risk of heart disease (Assman et al., Am. J. Card, 1996). In addition, high-density (HDL) cholesterol is regarded as good cholesterol and is essential for health, because it prevents atherosclerosis or the like.

[5] Although hyperlipidemia does not show specific symptoms by itself, excessive lipids in blood adhere to the blood vessel walls to reduce the blood vessel size and cause atherosclerosis by inflammatory reactions. For this reason, coronary heart disease, cerebrovascular disease, obstruction of peripheral blood vessels, etc., can occur (E. Falk et al., Circulation, 1995). In addition, excessive blood lipids are accumulated in liver tissue, and thus can cause fatty liver disease. The fatty liver refers to a condition in which the ratio of fats in the weight of the liver is more than 5%. The fatty liver can

be caused not only by excessive intake of fats, but also by intake of alcohol.

[6] Current methods that are used to reduce blood lipid levels include dietary therapy, exercise therapy and drug therapy. However, dietary therapy or excise therapy is difficult to strictly control and perform, and the therapeutic effect thereof is also limited.

[7] Drugs for reducing lipid levels, developed to date, include bile acid binding resin, cholesterol-lowering drugs such as HMG-CoA reductase inhibitors important in cholesterol biosynthesis, triglyceride-lowering drugs such as fibric acid derivatives and nicotinic acid, etc. However, these drugs were reported to have side effects such as hepatic toxicity, gastrointestinal disorder and carcinogenesis. Thus, there is an urgent need for the development of drugs that can be used to treat hyperlipidemia and related diseases (e.g., atherosclerosis and fatty liver disease) while having less side effects.

[8] As a candidate for such drugs, oxyntomodulin has recently received attention. Oxyntomodulin is produced from pre-glucagon and is a peptide that can bind to both glucagon-like peptide-1 (GLP-1) and glucagon receptor to perform dual function. Because of such characteristics, oxyntomodulin has been studied for various purposes, including the treatment of obesity, hyperlipidemia and fatty liver disease. However, oxyntomodulin has a problem in that it should be administered at a high dose, because it has a short half-life *in vivo* and the activity thereof is insufficient for use in the treatment of obesity, hyperlipidemia and fatty liver disease.

[9] Accordingly, the present inventors have developed an oxyntomodulin derivative having increased activity compared to native oxyntomodulin and have found that the oxyntomodulin derivative reduced the content and ratio of lipids in blood in a hyperlipidemia-induced hamster model, indicating that the derivative can be effectively used for the treatment of hyperlipidemia diseases, thereby completing the present invention.

Disclosure of Invention

Technical Problem

[10] It is an object of the present invention to provide a composition for preventing or treating hyperlipidemia, fatty liver disease or atherosclerosis, which contains an oxyntomodulin derivative as an active ingredient.

[11] Another object of the present invention is to provide a method for treating hyperlipidemia, fatty liver disease or atherosclerosis, the method comprising a step of administering an oxyntomodulin derivative to a subject.

[12] Still another object of the present invention is to provide the use of an oxyntomodulin derivative in the preparation of a medicament for preventing or treating hyperlipidemia, fatty liver disease or atherosclerosis.

Solution to Problem

[13] To achieve the above objects, in one aspect, the present invention provides a composition for preventing or treating hyperlipidemia, fatty liver disease or atherosclerosis, which contains an oxyntomodulin derivative as an active ingredient.

[14] As used herein, the term "oxyntomodulin" refers to a peptide produced from preglucagon that a precursor of glucagon. In the present invention, oxyntomodulin is meant include native oxyntomodulin and its precursor, analog (derivative), fragment and variant. Preferably, oxyntomodulin has an amino acid sequence of SEQ ID NO: 1 (HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIA).

[15] As used herein, the term "oxyntomodulin variant" is a peptide that has one or more amino acid residues different from those of the amino acid sequence of native oxyntomodulin and possesses a function of activating GLP-1 and glucagon receptors. The oxyntomodulin variant can be prepared by any one of substitution, addition, deletion, modification, or a combination thereof of some amino acids of native oxyntomodulin.

[16] As used herein, the term "oxyntomodulin derivative" refers to a peptide, peptide derivative or peptide mimic that is prepared by the addition, deletion or substitution of some amino acids of native oxyntomodulin and can activate both GLP-1 receptor and glucagon receptor at a high level compared to the level activated by native oxyntomodulin.

[17] As used herein, the term "oxyntomodulin fragment" refers to a fragment having an addition or deletion of one or more amino acids at the amino or carboxyl terminal end of native oxyntomodulin, in which the added amino acids may also be non-naturally occurring amino acids (e.g., D-type amino acid). Such amino acids have a function of regulating blood glucose levels *in vivo*.

[18] Methods for preparing the oxyntomodulin variant, derivative and fragment may be used alone or in combination. For example, the present invention includes a peptide that has one or more amino acids different from those of native peptide and deamination of the N-terminal amino acid residues and has a function of activating both GLP-1 receptor and glucagon receptor.

[19] Amino acids mentioned herein are abbreviated according to the nomenclature rules of IUPAC-IUB as follows:

[20] Alanine A; Arginine R;

[21] Asparagine N; Aspartic acid D;

[22] Cysteine C; Glutamic acid E;

[23] Glutamine Q; Glycine G;

[24] Histidine H; Isoleucine I;

[25] Leucine L; Lysine K;

[26] Methionine M; Phenylalanine F

[27] Proline P; Serine S;

[28] Threonine T; Tryptophan W;

[29] Tyrosine Y; Valine V.

[30] In the present invention, the oxyntomodulin derivative encompasses any peptide that is prepared by the substitution, addition, deletion or post-translational modification (e.g., methylation, acylation, ubiquitination, or intramolecular covalent bonding) of amino acids in the amino acid sequence of SEQ ID NO: 1 and can activate both the glucagon and GLP-1 receptors. Upon substitution or addition of amino acids, not only 20 amino acids commonly found in human proteins, but also atypical or non-naturally occurring amino acids can be used. Commercial sources of atypical amino acids include Sigma-Aldrich, ChemPep Inc., and Genzyme Pharmaceuticals. The peptides including these amino acids and atypical peptide sequences may be synthesized and purchased from commercial suppliers, for example, American Peptide Company or Bachem (USA) or Anygen (Korea).

[31]

[32] In a specific embodiment of the present invention, the oxyntomodulin derivative of the present invention is a novel peptide including the amino acid sequence of the following formula 1:

[33] [Formula 1]

[34] R1-X1-X2-GTFTSD-X3-X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20-X21-X22-X23-X24-R2

[35] wherein

[36] R1 is histidine, desamino-histidyl, dimethyl-histidyl (N-dimethyl-histidyl), beta-hydroxyimidazopropionyl, 4-imidazoacetyl, beta-carboxy imidazopropionyl or tyrosine;

[37] X1 is Aib (aminosiobutyric acid), d-alanine, glycine, Sar(N-methylglycine), serine, or d-serine;

[38] X2 is glutamic acid or glutamine;

[39] X3 is leucine or tyrosine;

[40] X4 is serine or alanine;

[41] X5 is lysine or arginine;

[42] X6 is glutamine or tyrosine;

[43] X7 is leucine or methionine;

[44] X8 is aspartic acid or glutamic acid;

[45] X9 is glutamic acid, serine, alpha-methyl-glutamic acid or is deleted;

[46] X10 is glutamine, glutamic acid, lysine, arginine or serine or is deleted;

[47] X11 is alanine, arginine or valine or is deleted;

[48] X12 is alanine, arginine, serine or valine or is deleted;

[49] X13 is lysine, glutamine, arginine or alpha-methyl-glutamic acid or is deleted;

[50] X14 is aspartic acid, glutamic acid or leucine or is deleted;

[51] X15 is phenylalanine or is deleted;

[52] X16 is isoleucine or valine or is deleted;

[53] X17 is alanine, cysteine, glutamic acid, lysine, glutamine or alpha-methyl-glutamic acid or is deleted;

[54] X18 is tryptophan or is deleted;

[55] X19 is alanine, isoleucine, leucine, serine or valine or is deleted;

[56] X20 is alanine, lysine, methionine, glutamine or arginine or is deleted;

[57] X21 is asparagine or is deleted;

[58] X22 is alanine, glycine or threonine or is deleted;

[59] X23 is cysteine or lysine or is deleted;

[60] X24 is a peptide having 2 to 10 amino acids consisting of a combination of alanine, glycine and serine or is deleted; and

[61] R2 is KRNRNNIA (SEQ ID NO: 35), GPSSGAPPPS (SEQ ID NO: 36), GPSSGAPPPSK (SEQ ID NO: 37), HSQGTFTSDYSKYLD (SEQ ID NO: 38), HSQGTFTSDYSRYLDK (SEQ ID NO: 39), HGEGTFTSDLSKQMEEEAVK (SEQ ID NO: 40) or is deleted (excluded if the amino acid sequence of formula 1 is identical to that of SEQ ID NO: 1).

[62]

[63] In order to increase the activity of wild-type oxyntomodulin for the glucagon receptor and the GLP-1 receptor, the oxyntomodulin derivative of the present invention may be substituted with 4-imidazoacetyl obtained by deletion of the alpha carbon of histidine at position 1 of the amino acid sequence of SEQ ID NO: 1, desamino-histidyl obtained by deletion of the N-terminal amino group, dimethyl-histidyl (N-dimethyl-histidyl) obtained by modification of the N-terminal amino group with two methyl groups, beta-hydroxy imidazopropionyl obtained by substitution of the N-terminal amino group with a hydroxyl group, or beta-carboxy imidazopropionyl obtained by substitution of the N-terminal amino group with a carboxyl group. In addition, the GLP-1 receptor-binding region may be substituted with amino acids that enhance hydrophobic and ionic bonds or a combination thereof. A portion of the oxyntomodulin sequence may be substituted with the amino acid sequence of GLP-1 or Exendin-4 to increase the activity of the GLP-1 receptor.

[64] Further, a portion of the oxyntomodulin sequence may be substituted with a sequence that enhances alpha helix. Preferably, amino acids at positions 10, 14, 16, 20, 24 and 28 of the amino acid sequence of formula 1 may be substituted with amino acids or amino acid derivatives consisting of Tyr(4-Me), Phe, Phe(4-Me), Phe(4-Cl), Phe(4-CN), Phe(4-NO_2),Phe(4-NH_2), Phg, Pal, Nal, Ala(2-thienyl) and Ala (benzothienyl) that are known to stabilize alpha helix, and the type and number of

alpha helix-stabilizing amino acid or amino acid derivatives to be inserted are not limited.

[65] Preferably, amino acids at positions 10 and 14, 12 and 16, 16 and 20, 20 and 24, and 24 and 28 of the amino acid sequence may be also substituted with glutamic acid or lysine so as to form rings, and the number of rings to be inserted is not limited. Most preferably, the oxyntomodulin derivative may have an amino acid sequence selected from among the following formulas 1 to 6.

[66] In a specific embodiment, the oxyntomodulin derivative of the present invention is a novel peptide including the amino acid sequence of the following formula 2, obtained by substitution of the amino acid sequence of oxyntomodulin with that of exendin or GLP-1:

[67] [Formula 2]

[68] R1-A-R3

[69] In another specific embodiment, the oxyntomodulin derivative of the present invention is a novel peptide including the amino acid sequence of the following formula 3, which is prepared by linking a portion of the amino acid sequence of oxyntomodulin and a portion of the amino acid sequence of exendin or GLP-1 via a proper amino acid linker:

[70] [Formula 3]

[71] R1-B-C-R4

[72] In still another specific embodiment, the oxyntomodulin derivative of the present invention is a novel peptide including the amino acid sequence of the following formula 4, wherein a portion of the amino acid sequence of oxyntomodulin is substituted with an amino acid capable of enhancing the binding affinity to GLP-1 receptor, for example, Leu at position 26 which binds with GLP-1 receptor by hydrophobic interaction is substituted with the hydrophobic residue Ile or Val.

[73] [Formula 4]

[74] R1-SQGTFTSDYSKYLD-D1-D2-D3-D4-D5-LFVQW-D6-D7-N-D8-R3

[75] In still another specific embodiment, the oxyntomodulin derivative of the present invention is a novel peptide including the amino acid sequence of the following formula 5, wherein a portion of the amino acid sequence of native oxyntomodulin is deleted, added, or substituted with other amino acids in order to increase the abilities of native oxyntomodulin to activate GLP-1 receptor and glucagon receptor:

[76] [Formula 5]

[77] R1-E1-QGTFTSDYSKYLD-E2-E3-RA-E4-E5-FV-E6-WLMNT-E7-R5

[78] In formulas 2 to 5, R1 is as described in formula 1;

[79] A is selected from the group consisting of SQGTFTSDYSKYLDSRRAQD-FVQWLMNT (SEQ ID NO: 41), SQGTFTSDYSKYLDEEAVRLFIEWLMNT (SEQ

ID NO: 42), SQGTFTSDYSKYLDERRAQDFVAWLKNT (SEQ ID NO: 43), GQGTFTSDYSRYLEEEAVRLFIEWLKNG (SEQ ID NO: 44), GQGTFTSDYSRQMEEEAVRLFIEWLKNG (SEQ ID NO: 45), GEGTFTSDLSRQMEEEAVRLFIEWAA (SEQ ID NO: 46), and SQGTFTSDYSRQMEEEAVRLFIEWLMNG (SEQ ID NO: 47);

[80] B is selected from the group consisting of SQGTFTSDYSKYLDSRRAQDFVQWLMNT (SEQ ID NO: 41), SQGTFTSDYSKYLDEEAVRLFIEWLMNT (SEQ ID NO: 42), SQGTFTSDYSKYLDERRAQDFVAWLKNT (SEQ ID NO: 43), GQGTFTSDYSRYLEEEAVRLFIEWLKNG (SEQ ID NO: 44), GQGTFTSDYSRQMEEEAVRLFIEWLKNG (SEQ ID NO: 45), GEGTFTSDLSRQMEEEAVRLFIEWAA (SEQ ID NO: 46), SQGTFTSDYSRQMEEEAVRLFIEWLMNG (SEQ ID NO: 47), GEGTFTSDLSRQMEEEAVRLFIEW (SEQ ID NO: 48), and SQGTFTSDYSRYLD (SEQ ID NO: 49);

[81] C is a peptide having 2 to 10 amino acids consisting of a combination of alanine, glycine and serine;

[82] D1 is serine, glutamic acid or arginine;

[83] D2 is arginine, glutamic acid or serine;

[84] D3 is arginine, alanine or valine;

[85] D4 is arginine, valine or serine;

[86] D5 is glutamine, arginine or lysine;

[87] D6 is isoleucine, valine or serine;

[88] D7 is methionine, arginine or glutamine;

[89] D8 is threonine, glycine or alanine;

[90] E1 is serine, Aib, Sar, d-alanine or d-serine;

[91] E2 is serine or glutamic acid;

[92] E3 is arginine or lysine;

[93] E4 is glutamine or lysine;

[94] E5 is aspartic acid or glutamic acid;

[95] E6 is glutamine, cysteine or lysine;

[96] E7 is cysteine or lysine or is deleted;

[97] R3 is KRNRNNIA (SEQ ID NO: 35), GPSSGAPPPS (SEQ ID NO: 36) or GPSSGAPPPSK (SEQ ID NO: 37);

[98] R4 is HSQGTFTSDYSKYLD (SEQ ID NO: 38), HSQGTFTSDYSRYLDK (SEQ ID NO: 39) or HGEGTFTSDLSKQMEEEAVK (SEQ ID NO: 40); and

[99] R5 is KRNRNNIA (SEQ ID NO: 35), GPSSGAPPPS (SEQ ID NO: 36) or GPSSGAPPPSK (SEQ ID NO: 37) or is deleted (excluded if the amino acid sequences of formulas 2 to 5 are identical to that of SEQ ID NO: 1).

[100] Preferably, the oxyntomodulin derivative of the present invention may be a novel

peptide of the following formula 6:

[101] [Formula 6]

[102] R1-X1-X2-GTFTSD-X3-X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19-X20-X21-X22-X23-X24-R2

[103] wherein R1 is histidine, desamino-histidyl, 4-imidazoacetyl or tyrosine;

[104] X1 is Aib(aminosiobutyric acid), glycine, serine or d-serine;

[105] X2 is glutamic acid or glutamine;

[106] X3 is leucine or tyrosine;

[107] X4 is serine or alanine;

[108] X5 is lysine or arginine;

[109] X6 is glutamine or tyrosine;

[110] X7 is leucine or methionine;

[111] X8 is aspartic acid or glutamic acid;

[112] X9 is glutamic acid or alpha-methyl-glutamic acid or is deleted;

[113] X10 is glutamine, glutamic acid, lysine or arginine or is deleted;

[114] X11 is alanine or arginine or is deleted;

[115] X12 is alanine or valine or is deleted;

[116] X13 is lysine, glutamine, arginine or alpha-methyl-glutamic acid or is deleted;

[117] X14 is aspartic acid, glutamic acid or leucine or is deleted;

[118] X15 is phenylalanine or is deleted;

[119] X16 is isoleucine or valine or is deleted;

[120] X17 is alanine, cysteine, glutamic acid, glutamine or alpha-methyl-glutamic acid or is deleted;

[121] X18 is tryptophan or is deleted;

[122] X19 is alanine, isoleucine, leucine or valine or is deleted;

[123] X20 is alanine, lysine, methionine or arginine or is deleted;

[124] X21 is asparagine or is deleted;

[125] X22 is threonine or is deleted;

[126] X23 is cysteine, lysine or is deleted;

[127] X24 is a peptide having 2 to 10 amino acids consisting of glycine or is deleted; and

[128] R2 is KRNRNNIA (SEQ ID NO: 35), GPSSGAPPPS (SEQ ID NO: 36), GPSSGAPPPSK (SEQ ID NO: 37), HSQGTFTSDYSKYLD (SEQ ID NO: 38), HSQGTFTSDYSRYLDK (SEQ ID NO: 39) or HGEGTFTSDLSKQMEEEAVK (SEQ ID NO: 40) or is deleted (excluded if the amino acid sequence of formula 6 is identical to that of SEQ ID NO: 1).

[129] More preferably, the oxyntomodulin derivative of the present invention may be selected from the group consisting of the peptides of SEQ ID NOs: 2 to 34. Even more preferably, the oxyntomodulin derivative of the present invention may be an oxyn-

tomodulin derivative described in Table 1 of Example 2-1.

[130] In an example of the present invention, oxyntomodulin derivatives having the amino acid sequences of SEQ ID NOs: 2 to 34, respectively, were prepared, and it was found that the oxyntomodulin derivatives showed excellent GLP-1 receptor and glucagon receptor activities compared to native oxyntomodulin (Example 2). In other words, it could be seen from the above results that the oxyntomodulin derivative of the present invention exhibited excellent therapeutic effects against hyperlipidemia, fatty liver disease or atherosclerosis by activating the GLP-1 receptor and the glucagon receptor.

[131] The oxyntomodulin derivatives of the present invention are present in the form of conjugates comprising various polymer in order to improve the therapeutic effect and *in vivo* half-life of the derivatives.

[132] The conjugate of the present invention shows an increase in the duration of effects compared to native oxyntomodulin, and the long-acting conjugates include an oxyntomodulin prepared by the modification, substitution, addition or deletion of the amino acids of native oxyntomodulin, an oxyntomodulin conjugated to a biodegradable polymer such as polyethylene glycol (PEG), an oxyntomodulin conjugated to a polysaccharide such as albumin, antibody, elastin, fibronectin or chitin or to a long-acting protein such as an immunoglobulin fragment, an oxyntomodulin conjugated to fatty acid having the ability of binding to albumin *in vivo*, or an oxyntomodulin encapsulated in biodegradable nanoparticles, and the type of long-acting conjugate that is used in the present invention is not limited.

[133] Preferably, the conjugate is a conjugate wherein an oxyntomodulin derivative having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 to 34 is linked to an immunoglobulin Fc region via a non-peptidyl polymer.

[134] The immunoglobulin Fc region is a biodegradable polypeptide that is metabolized *in vivo*,and thus is safe for use as a carrier for a drug. The immunoglobulin Fc region has a low molecular weight compared to the entire immunoglobulin molecule, and thus is advantageous in terms of the preparation, purification and yield of conjugates. In addition, because the amino acid sequence differs between antibodies, a Fab portion showing high non-homogeneity, and thus the homogeneity of the material can be greatly increased and the possibility of inducing blood antigenicity can also be reduced.

[135] As used herein, the term "immunoglobulin Fc region" refers to a protein that contains the heavy-chain constant region 2 (CH2) and heavy-chain constant region 3 (CH3) of an immunoglobulin, excluding the heavy-chain and light-chain variable regions, the heavy-chain constant region 1 (CH1) and the light-chain constant region 1 (CL1) of the immunoglobulin. It may further include a hinge region at the heavy-chain constant region. Also, the immunoglobulin Fc region of the present invention may be an

expanded Fc region including part or all of the heavy-chain constant region 1 (CH1) and/or the light-chain constant region 1 (CL1), except for the heavy-chain and light-chain variable regions, as long as it has an effect that is substantially equal to or better than the native protein. Also, the immunoglobulin Fc region may be a region having a deletion of a portion of a relatively long amino acid sequence corresponding to CH2 and/or CH3. Specifically, the immunoglobulin Fc region of the present invention may comprise 1) a CH1 domain, a CH2 domain, a CH3 domain and a CH4 domain, 2) a CH1 domain and a CH2 domain, 3) a CH1 domain and a CH3 domain, 4) a CH2 domain and a CH3 domain, 5) a combination of one or more domains and an immunoglobulin hinge region (or a portion of the hinge region), or 6) a dimer of each domain of the heavy-chain constant regions and the light-chain constant region.

[136] The immunoglobulin Fc region of the present invention includes a native amino acid sequence, and a sequence derivative (mutant) thereof. As used herein, the term "amino acid sequence derivative" refers to a sequence that is different from the native amino acid sequence due to the deletion, insertion, non-conservative or conservative substitution or a combination thereof of one or more amino acid residues of the native amino acid sequence. For example, in the case of an IgG Fc, amino acid residues at positions 214 to 238, 297 to 299, 318 to 322, or 327 to 331, which are known to be important in binding, may be used as suitable sites for modification.

[137] In addition, other various derivatives are possible, including one that has a deletion of a region capable of forming a disulfide bond, or a deletion of some amino acid residues at the N-terminal end of native Fc or an addition of a methionine residue at the N-terminal end of native Fc. Further, to remove effector functions, a deletion may occur in a complement-binding site, such as a C1q-binding site and an ADCC (antibody dependent cell mediated cytotoxicity) site. Techniques of preparing such sequence derivatives of the immunoglobulin Fc region are disclosed in International Patent Publication Nos. WO 97/34631 and WO 96/32478.

[138] Amino acid exchanges in proteins and peptides, which do not generally alter the activity of the proteins or peptides, are known in the art (H. Neurath, R. L. Hill, The Proteins, Academic Press, New York, 1979). The most commonly occurring exchanges are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Thy/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu and Asp/Gly, in both directions. In addition, the Fc region may, if necessary, be modified by phosphorylation, sulfation, acrylation, glycosylation, methylation, farnesylation, acetylation, amidation, and the like.

[139] The above-described Fc derivatives show biological activity identical to that of the Fc region of the present invention or have increased structural stability against heat, pH, or the like.

[140] In addition, this Fc region may be obtained from native forms isolated from humans and other animals including cows, goats, pigs, mice, rabbits, hamsters, rats and guinea pigs, or may be recombinants or derivatives thereof, obtained from transformed animal cells or microorganisms. Herein, the Fc region may be obtained from a native immunoglobulin by isolating a whole immunoglobulin from a living human or animal body and treating it with proteinase. When the whole immunoglobulin is treated with papain, it is digested into Fab and Fc regions, and when the whole immunoglobulin is treated with pepsin, it is digested into pF'c and $F(ab)_2$ fragments. Fc or pF'c can be isolated using size exclusion chromatography or the like. Preferably, a human-derived Fc region is a recombinant immunoglobulin Fc region obtained from a microorganism.

[141] In addition, the immunoglobulin Fc region of the present invention may be in the form of having native sugar chains or increased or decreased sugar chains compared to a native form, or may be in a deglycosylated form. The increase, decrease or removal of the immunoglobulin Fc sugar chains may be achieved by conventional methods such as a chemical method, an enzymatic method and a genetic engineering method using a microorganism. The Fc region obtained by removal of sugar chains from Fc shows a significant decrease in binding affinity to the C1q part of the first complement component C1 and a decrease or loss in antibody-dependent cell-mediated cytotoxicity or complement-dependent cytotoxicity, and thus does not induce unnecessary immune responses *in vivo*. In this regard, an immunoglobulin Fc region in a deglycosylated or aglycosylated form may be more suitable to the object of the present invention as a drug carrier.

[142] As used herein, the term "deglycosylation" refers to enzymatically removing sugar moieties from an Fc region, and the term "aglycosylation" refers to an unglycosylated Fc region produced in a prokaryote, preferably *E.coli*.

[143] Meanwhile, the immunoglobulin Fc region may be derived from humans or other animals including cows, goats, pigs, mice, rabbits, hamsters, rats and guinea pigs, and preferably from humans.

[144] In addition, the immunoglobulin Fc region may be derived from IgG, IgA, IgD, IgE, IgM, or a combination or hybrid thereof. Preferably, it is derived from IgG or IgM, which are among the most abundant proteins in human blood, and most preferably from IgG, which is known to enhance the half-lives of ligand-binding proteins.

[145] As used herein, the term "combination" means that polypeptides encoding single-chain immunoglobulin Fc regions of the same origin are linked to a single-chain polypeptide of a different origin to form a dimer or multimer. Specifically, a dimer or multimer may be formed from two or more fragments selected from the group consisting of IgG Fc, IgA Fc, IgM Fc, IgD Fc, and IgE Fc fragments.

[146] As used herein, the term "hybrid" means that sequences corresponding to two or

more immunoglobulin Fc fragments of different origins are present in a single-chain immunoglobulin Fc region. In the present invention, various forms of hybrid are possible. In other words, a hybrid composed of 1 to 4 domains selected from the group consisting of the CH1, CH2, CH3 and CH4 of IgG Fc, IgM Fc, IgA Fc, IgE Fc and IgD Fc is possible, and it may include a hinge.

[147] Meanwhile, IgG can also be sub-classified into IgG1, IgG2, IgG3 and IgG4, and in the present invention, a combination or hybrid of these subclasses is also possible. Preferably, IgG is the IgG2 ad IgG4 subclass, and most preferably, it is the Fc region of IgG4 that substantially lacks effector functions such as complement-dependent cytotoxicity (CDC).

[148] In other words, the most preferred immunoglobulin Fc region that is used as a drug carrier in the present invention is an Fc region derived from human IgG4. A human-derived Fc region is more preferable than a non-human-derived Fc region, which may act as an antigen in the human body and cause undesirable immune responses such as the production of a new antibody against the antigen.

[149] As used herein, the term "non-peptidyl polymer" refers to a biocompatible polymer including two or more repeating units linked to each other by any covalent bond in place of a peptide bond. In the present invention, the non-peptidyl polymer may be interchangeably used with the non-peptidyl linker.

[150] The non-peptidyl polymer that can be used in the present invention may be selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol/propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, polysaccharides, dextran, polyvinyl ethyl ether, biodegradable polymers such as PLA (poly(lactic acid)) and PLGA (polylactic-glycolic acid), lipid polymers, chitins, hyaluronic acid, and combinations thereof. Preferably, the non-peptidyl polymer is polyethylene glycol. In addition, derivatives thereof known in the art and derivatives that may be easily prepared by a method known in the art are included in the scope of the present invention.

[151] The peptide linker that is used in a fusion protein obtained by a conventional inframe fusion method has drawbacks in that it is easily cleaved by proteinase *in vivo*,and thus a sufficient effect of increasing the serum half-life of the active drug by a carrier cannot be obtained as expected. However, in the present invention, the polymer having resistance to proteinase can be used to maintain the serum half-life of the peptide, similar to the carrier. Therefore, any non-peptidyl polymer can be used without limitation in the present invention, as long as it is a polymer having the aforementioned function, that is, a polymer having resistance to proteinase *in vivo*. The non-peptidyl polymer has a molecular weight in the range of 1 to 100 kDa, and preferably 1 to 20 kDa. The non-peptidyl polymer of the present invention, which is linked to the im-

munoglobulin Fc region, may be one kind of polymer or a combination of different polymers.

[152] The non-peptidyl polymer that is used in the present invention may have a reactive group capable of binding to the immunoglobulin Fc region and the protein drug. The reactive group at both ends of the non-peptidyl polymer is preferably selected from the group consisting of a reactive aldehyde group, a propionaldehyde group, a butyraldehyde group, a maleimide group and a succinimide derivative.

[153] The succinimide derivative may be succinimidyl propionate, hydroxy succinimidyl, succinimidyl carboxymethyl, or succinimidyl carbonate. In particular, when the non-peptidyl polymer has a reactive aldehyde group at both ends thereof, non-specific reactions can be minimized, and a physiologically active polypeptide and an immunoglobulin can be effectively bound to one and the other end of the non-peptidyl polymer, respectively. A final product generated by reductive alkylation with an aldehyde bond is much more stable than that linked by an amide bond. The aldehyde reactive group selectively binds to an N-terminus at a low pH and can form a covalent bond with a lysine residue at a high pH such as pH 9.0.

[154] The reactive groups at both ends of the non-peptidyl polymer may be the same or different. For example, the non-peptidyl polymer may possess a maleimide group at one end, and an aldehyde group, a propionaldehyde group or a butyraldehyde group at the other end. When a polyethylene glycol having a reactive hydroxy group at both ends thereof is used as the non-peptidyl polymer, the hydroxy group may be activated to various reactive groups by known chemical reactions, or a polyethylene glycol having a commercially available modified reactive group may be used so as to prepare the long acting conjugate of the present invention.

[155] The conjugate of the present invention may be one in which one end of the non-peptidyl polymer and the other are linked to an amine group or a thiol group of the immunoglobulin Fc region and the oxyntomodulin derivative, respectively.

[156] The non-peptidyl polymer of the present invention has a functional group at both ends which can be linked to either an immunoglobulin Fc region or a protein drug. The functional groups can be an aldehyde group, a propionaldehyde group, a butyraldehyde group, a maleimide group and a succinimide derivative (i.e., succinimidyl propionate, hydroxy succinimidyl, succinimidyl carboxymethyl, or succinimidyl carbonate), but are not limited thereto.

[157] The reactive groups at both ends of the linker that is the non-peptidyl polymer may be the same or different. For example, the non-peptidyl polymer may have a maleimide group at one end and an aldehyde group, a propionaldehyde group or a butyraldehyde group at the other end. For example, when the non-peptidyl polymer has a reactive aldehyde group at one end and a reactive maleimide group at the other end, non-

specific reactions can be minimized, and a physiologically active polypeptide and an immunoglobulin can be effectively bound to both ends of the non-peptidyl polymer. According to an embodiment of the present invention, a conjugate was synthesized by linking oxyntomodulin or its derivative to the immunoglobulin Fc region via a covalent bond using the non-peptidyl polymer PEG including a propionaldehyde group alone or both a maleimide group and an aldehyde group.

[158] The pharmaceutical composition of the present invention can be used for the prevention or treatment of hyperlipidemia, fatty liver disease or atherosclerosis.

[159] As used herein, the term "prevention" refers to all actions that inhibit or delay the development of a target disease. As used herein, the term "prevention" means administering the oxyntomodulin derivative of the present invention to inhibit or delay the development of hyperlipidemia, fatty liver disease or atherosclerosis, which shows an increase in blood total cholesterol and low-density cholesterol levels and a decrease in high-density cholesterol levels.

[160] As used herein, the term "treatment" refers to all actions that alleviate, ameliorate or relieve the symptoms of the disease developed. As used herein, the term "treatment" means administering the oxyntomodulin derivative of the present invention to alleviate, ameliorate or relieve hyperlipidemia, fatty liver disease or atherosclerosis, which shows an increase in blood total cholesterol and low-density cholesterol levels and a decrease in high-density cholesterol levels.

[161] As used herein, the term "hyperlipidemia" refers to a condition associated with abnormally elevated levels of lipids, such as free cholesterol, cholesterol esters, phospholipids and triglycerides, in blood. Although hyperlipidemia does not show specific symptoms by itself, excessive lipids in blood adhere to the blood vessel walls to reduce the blood vessel size and cause atherosclerosis by inflammatory reactions. For this reason, coronary heart disease, cerebrovascular disease, obstruction of peripheral blood vessels, etc., can occur.

[162] Thus, the pharmaceutical composition of the present invention can be used for the treatment of not only hyperlipidemia, fatty liver disease or atherosclerosis, but also coronary heart disease, cerebrovascular disease, or obstruction of peripheral blood vessels.

[163] As used herein, the term "fatty liver disease" refers to a condition in which the ratio of fats in the weight of the liver is more than 5%. In the present invention, fatty liver diseases include non-alcoholic fatty liver disease (NAFLD), alcoholic fatty liver disease, nutritional fatty liver disease, starvation fatty liver disease, obesity fatty liver disease, diabetic fatty liver disease or steatohepatitis. The non-alcoholic fatty liver disease is meant to include primary and secondary non-alcoholic fatty liver disease, but may preferably be a non-alcoholic fatty liver disease resulting from primary hyper-

lipidemia, diabetes or obesity.

[164] In addition, in the present invention, non-alcoholic fatty liver disease is meant to include simple steatosis, non-alcoholic steatohepatitis, and liver fibrosis and liver cirrhosis which result from the progression of such diseases.

[165] Atherosclerosis refers to a vascular disease in which atheroma is formed as a result of deposition of cholesterol in the endothelium of blood vessels and proliferation of endothelial cells.

[166] In an example of the present invention, a long-acting oxyntomodulin derivative conjugate was prepared by linking the oxyntomodulin derivative to the immunoglobulin Fc region by a covalent bond using polyethylene glycol, and the prepared conjugate was administered to hamster animal models having hyperlipidemia induced by intake of high-fat diet. As a result, it was shown that the group administered with the long-acting oxyntomodulin derivative conjugate according to the present invention showed a significant decrease in blood triglyceride levels (FIG. 1), a significant decrease in blood total cholesterol levels (FIG. 2), and a significant decrease in blood low-density (LDL) cholesterol levels, compared to the hyperlipidemia-induced animal models. In addition, it was observed that the group administered with the long-acting oxyntomodulin derivative conjugate according to the present invention showed a significant increase in blood high-density (HDL) cholesterol levels (FIG. 4) and a significant increase in the blood HDL-cholesterol/LDL-cholesterol ratio (FIG. 5), compared to the hyperlipidemia-induced animal models.

[167] Further, it could be seen that the long-acting oxyntomodulin derivative conjugate according to the present invention showed a decrease in blood total cholesterol levels (FIG. 6) and a decrease in blood LDL-cholesterol and triglyceride levels (FIG. 7), compared to VICTOZA® that is a commercial long-acting GLP-1 analog. In addition, it could be seen that administration of the long-acting oxyntomodulin derivative conjugate of the present invention showed increases in blood HDL-cholesterol level and the HDL/LDL-cholesterol ratio compared to administration of VICTOZA®(FIGS.8 and 9). Particularly, a long-acting conjugate of the peptide of SEQ ID NO: 25 with Fc showed significant increases in blood HDL levels and HDL/LDL-cholesterol ratio compared to VICTOZA®.

[168] In other words, the oxyntomodulin derivative according to the present invention reduce blood lipid levels, and thus can be used as an agent for treating hyperlipidemia, fatty liver disease or arteriosclerosis. In addition, the conjugate of the present invention has an excellent ability to activate GLP-1 receptor and glucagon receptor compared to native oxyntomodulin and shows an increased blood half-life *in vivo,* and thus the activity thereof can be maintained *in vivo* for an extended period of time.

[169] The oxyntomodulin derivative of the present invention can increase the activity of a factor (Protein kinase C-ζ or PKC-ζ) regulating the activity of enzymes that are involved in the lipolysis of fats, and increase the expression of a factor (Glut2) that is involved in the lipolysis of fats, thereby treating hyperlipidemia, fatty liver disease or arteriosclerosis, but the scope of the present invention is not limited to the above mechanism of action.

[170] The pharmaceutical composition of the present invention may further comprise a pharmaceutical agent exhibiting preventive or therapeutic effects against hyperlipidemia, fatty liver disease or arteriosclerosis. Specifically, the composition of the present invention may further comprise a pharmaceutical agent known as an agent for treating hyperlipidemia, fatty liver disease or arteriosclerosis in order to administer the pharmaceutical agent in combination with the derivative of the present invention.

[171] Thus, the composition of the present invention may be administered alone or in combination with other drugs in order to prevent or treat hyperlipidemia, fatty liver disease or arteriosclerosis.

[172] As used herein, the term "administration" means introducing a given material into a patient by any appropriate method. The derivative of the present invention may be administered by any general route, as long as it can reach a target tissue. Specifically, the derivative of the present invention may be administered intraperitoneally, intravenously, intramuscularly, subcutaneously, intradermally, orally, locally, intranasally, intrapulmonarily or intrarectally, but is not limited thereto. However, because the peptide is digested when being administered orally, the oral composition is preferably formulated so that the active ingredient is coated or protected from degradation in the stomach. Preferably, the composition of the present invention may be administered in an injectable form. In addition, the pharmaceutical composition of the present invention may be administered using any system capable of delivering the active ingredient to target cells.

[173] The pharmaceutical composition comprising the oxyntomodulin derivative of the present invention may further comprise a pharmaceutically acceptable carrier. For oral administration, pharmaceutically acceptable carriers include a binder, a lubricant, a disintegrant, an excipient, a solubilizer, a dispersing agent, a stabilizer, a suspending agent, a colorant, and a flavorant. For injectable preparations, pharmaceutically acceptable carriers include a buffer, a preservative, an analgesic, a solubilizer, an isotonic agent, and a stabilizer. For topical administration, pharmaceutically acceptable carriers include a base, an excipient, a lubricant, and a preservative.

[174] The pharmaceutical composition of the present invention may be formulated in various dosage forms using the aforementioned pharmaceutically acceptable carriers. For example, for oral administration, the pharmaceutical composition may be

formulated into tablets, troches, capsules, elixirs, suspensions, syrups, wafers or the like. For injectable preparations, the pharmaceutical composition may be provided in the form of a unit dosage ampoule or a multiple dosage container. The pharmaceutical composition may also be formulated into solutions, suspensions, tablets, pills, capsules and sustained-release preparations.

[175] Meanwhile, examples of the carrier, excipient and diluent suitable for formulation include lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methylcellulose, microcrystalline cellulose, polyvinylpyrrolidone, water, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate and mineral oils. In addition, the pharmaceutical composition of the present invention may further include fillers, anti-coagulating agents, lubricants, wetting agents, flavors, preservative and the like.

[176] The dose of the pharmaceutical composition of the present invention is determined according to the kind of active ingredient, together with various factors such as the disease to be treated, the route of administration, the patient's age, sex and weight, and the severity of the disease.

[177] The pharmaceutical composition of the present invention has a long *in vivo* half-life and excellent-potency, and thus the number and frequency of administration of the pharmaceutical composition can be significantly reduced.

[178] In another embodiment, the present invention provides a method for treating hyperlipidemia, fatty liver disease or arteriosclerosis, the method comprising a step of administering the oxyntomodulin derivative of the present invention to a subject.

[179] The above oxyntomodulin, hyperlipidemia, fatty liver disease and arteriosclerosis are as described above.

[180] As used herein, the term "subject" refers to a subject suspected of having hyperlipidemia, fatty liver disease or arteriosclerosis. Specifically, the term means mammals, including humans, rats and domestic animals, which have or are at the risk of developing the above disease. In addition, the subject may be any subject that can be treated by the oxyntomodulin derivative of the present invention.

[181] The therapeutic method of the present invention may comprise administering a pharmaceutically effective amount of the pharmaceutical composition comprising the conjugate. The total daily dose of the composition can be determined through appropriate medical judgment by a physician, and the composition may be administered once or several times. However, in view of the purpose of the present invention, the specific therapeutically effective dose of the composition for any particular patient may vary depending on various factors well known in the medical field, including the kind and degree of response to be achieved, concrete compositions according to

whether other agents are used therewith or not, the patient'age, body weight, health condition, sex and diet, the time and route of administration, the secretion rate of the composition, the duration of treatment, other drugs used in combination or coincident with the composition of the present invention, and other factors known in the medical field.

[182] In still another aspect, the present invention provides a method for preparing an oxyntomodulin derivative conjugate.

[183] The preparation method may comprise the steps of: (1) covalently linking a non-peptidyl polymer having a reactive aldehyde, maleimide or succinimide group to the amine or thiol group of an oxyntomodulin derivative peptide; (2) separating the oxyntomodulin derivative peptide, having the non-peptidyl polymer covalently bonded thereto at positions other than the amino terminal end, from the reaction mixture of step (1); and (3) covalently an immunoglobulin Fc region to the other end of the linked non-peptidyl polymer, thereby producing a peptide conjugate comprising the immunoglobulin Fc region and the oxyntomodulin derivative peptide, linked to one and the other end of the non-peptidyl polymer, respectively.

[184] More specifically, the preparation method may comprise the steps of: 1) covalently linking a non-peptidyl polymer, having a reactive aldehyde group and a reactive maleimide group at one and the other end thereof, respectively, to the cysteine residue of an oxyntomodulin derivative; (2) separating the oxyntomodulin derivative, having the non-peptidyl polymer covalently linked to the cysteine residue, from the reaction mixture of step (1); and (3) covalently an immunoglobulin Fc region to the other end of the linked non-peptidyl polymer, thereby producing a peptide conjugate comprising the immunoglobulin Fc region and the oxyntomodulin derivative peptide, linked to one and the other end of the non-peptidyl polymer, respectively.

[185] In still another aspect, the present invention provides the use of the oxyntomodulin derivative in the preparation of a medicament for preventing or treating hyperlipidemia, fatty liver disease or arteriosclerosis.

Advantageous Effects of Invention

[186] The oxyntomodulin derivative of the present invention has a high ability to activate GLP-1 receptor and glucagon receptor compared to native oxyntomodulin and exhibits the effects of reducing the blood total cholesterol, low-density cholesterol and triglyceride levels that were increased by high-fat diet, and increasing high-density cholesterol levels and the high-density cholesterol/low-density cholesterol ratio. Thus, the oxyntomodulin derivative of the present invention can be effectively used for the treatment of hyperlipidemia and related diseases.

Brief Description of Drawings

[187] FIG. 1 is a graph showing the change in blood triglyceride levels caused by administration of a long-acting oxyntomodulin derivative to high-fat diet-induced hyperlipidemia hamsters (#: indicates a significant increase compared to a general diet group within a confidence of 99.9% ($p<0.001$);*:indicates a significant decrease compared to a high-fat diet group within a confidence of 99.9% ($p<0.001$).

[188] FIG. 2 is a graph showing the change in blood total cholesterol levels caused by administration of a long-acting oxyntomodulin derivative to high-fat diet-induced hyperlipidemia hamsters (#: indicates a significant increase compared to a general diet group within a confidence of 99.9% ($p<0.001$);*:indicates a significant decrease compared to a high-fat diet group within a confidence of 99.9% ($p<0.001$).

[189] FIG. 3 is a graph showing the change in blood LDL-cholesterol levels caused by administration of a long-acting oxyntomodulin derivative to high-fat diet-induced hyperlipidemia hamsters (#: indicates a significant increase compared to a general diet group within a confidence of 99.9% ($p <0.001$);*:indicates a significant decrease compared to a high-fat diet group within a confidence of 99.9% ($p<0.001$).

[190] FIG. 4 is a graph showing the change in blood HDL-cholesterol levels caused by administration of a long-acting oxyntomodulin derivative to high-fat diet-induced hyperlipidemia hamsters (*: indicates a significant increase compared to a high-fat diet group within a confidence of 99% ($p<0.01$).

[191] FIG. 5 is a graph showing the change in blood HDL/LDL-cholesterol levels caused by administration of a long-acting oxyntomodulin derivative to high-fat diet-induced hyperlipidemia hamsters (*: shows a significant increase compared to a high-fat diet group within a confidence of 95% ($p<0.05$).

[192] FIG. 6 is a graph showing the change in blood total cholesterol levels caused by administration of VICTOZA® or a long-acting oxyntomodulin derivative to high-fat diet-induced hyperlipidemia hamsters (***: indicates a significant decrease compared to a high-fat diet group within a confidence of 99.9% ($p<0.001$).

[193] FIG. 7 is a graph showing the change in blood LDL-cholesterol levels caused by administration of VICTOZA® or a long-acting oxyntomodulin derivative to high-fat diet-induced hyperlipidemia hamsters (***: indicates a significant decrease compared to a high-fat diet group within a confidence of 99.9% ($p<0.001$).

[194] FIG. 8 is a graph showing the change in blood HDL-cholesterol levels caused by administration of VICTOZA® or a long-acting oxyntomodulin derivative to high-fat diet-induced hyperlipidemia hamsters (*: indicates a significant decrease compared to a high-fat diet group within a confidence of 95% ($p<0.05$).

[195] FIG. 9 is a graph showing the change in blood HDL/LDL-cholesterol levels caused by administration of VICTOZA® or a long-acting oxyntomodulin derivative to high-fat diet-induced hyperlipidemia hamsters (**: indicates a significant decrease compared to

a high-fat diet group within a confidence of 99% ($p<0.01$).

[196] FIG. 10 is a graph showing the change in blood triglyceride levels caused by administration of VICTOZA® or a long-acting oxyntomodulin derivative to high-fat diet-induced hyperlipidemia hamsters (***: indicates a significant decrease compared to a high-fat diet group within a confidence of 99.9% ($p<0.001$).

Mode for the Invention

[197] Hereinafter, the present invention will be described in further detail with reference to examples. It is to be understood, however, that these examples are for illustrative purposes only and are not intended to limit the scope of the present invention.

[198]

[199] **Example 1: Production for cell line for *in vitro* activation**

[200] Example 1-1: Production of cell line showing cAMP response to GLP-1

[201] Using a portion corresponding to the ORF (open reading frame) of cDNA (OriGene Technologies, Inc. USA) of the human GLP-1 receptor gene as a template, PCR was performed using reverse and forward primers including a HindIII cleavage site and an EcoRI cleavage site, respectively, thereby obtaining a PCR product.

[202] Forward primer: 5'-CCCGGCCCCCGCGGCCGCTATTCGAAATAC-3' (SEQ ID NO: 50)

[203] Reverse primer: 5'-GAACGGTCCGGAGGACGTCGACTCTTAAGATAG-3' (SEQ ID NO: 51)

[204] The PCR product was cloned into the known animal cell expression vector x0GC/dhfr, thereby constructing the recombinant vector x0GC/GLP-1R.

[205] The recombinant vector x0GC/GLP-1R was introduced into a CHO DG44 cell line, cultured in DMEM/F12 (10% FBS) medium, using lipofectamine (Invitrogene, USA), to obtain a transformant. The transformant was incubated in a selective medium containing 1 mg/mL G418 and 10 nM methotraxate, and monoclonal cell lines were selected therefrom. Then, a cell line showing a good concentration-dependent cAMP response to GLP-1 was finally selected from the monoclonal cell lines.

[206]

[207] Example 1-2: Production of cell line showing cAMP response to glucagon

[208] Using a portion corresponding to the ORF (open reading frame) of cDNA (OriGene Technologies, Inc. USA) of the human glucagon receptor gene as a template, PCR was performed using reverse and forward primers including an EcoRI cleavage site and a XhoI cleavage site, respectively, thereby obtaining a PCR product.

[209] Forward primer: 5'-CAGCGACACCGACCGTCCCCCCGTACTTAAGGCC-3' (SEQ ID NO: 52)

[210] Reverse Primer: 5'-CTAACCGACTCTCGGGGAAGACTGAGCTCGCC-3' (SEQ

ID NO: 53)

[211] The PCR product was cloned into the known animal cell expression vector x0GC/dhfr, thereby constructing the recombinant vector x0GC/GCGR.

[212] The recombinant vector x0GC/GCGR was introduced into a CHO DG44 cell line, cultured in DMEM/F12 (10% FBS) medium, using lipofectamine (Invitrogene, USA), to obtain a transformant. The transformant was incubated in a selective medium containing 1 mg/mL G418 and 10 nM methotraxate, and monoclonal cell lines were selected therefrom. Then, a cell line showing a good concentration-dependent cAMP response to glucagon was finally selected from the monoclonal cell lines.

[213]

[214] **Example 2: *In vitro* activity of oxyntomodulin derivatives**

[215] Example 2-1: Synthesis of oxyntomodulin derivatives

[216] In order to measure the in vitro activities of oxyntomodulin derivatives, oxyntomodulin derivatives having the amino acid sequences shown in Table 1 below.

[217] Table 1

[Table 1]

Oxyntomodulin and oxyntomodulin derivatives

SEQ ID NOs	Sequences
SEQ ID NO: 1	HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIA
SEQ ID NO: 2	CA-SQGTFTSDYSKYLDEEAVRLFIEWLMNTKRNRNNIA
SEQ ID NO: 3	CA-SQGTFTSDYSKYLDERRAQDFVAWLKNTGPSSGAPPPS
SEQ ID NO: 4	CA-GQGTFTSDYSRYLEEEAVRLFIEWLKNGGPSSGAPPPS
SEQ ID NO: 5	CA-GQGTFTSDYSRQMEEEAVRLFIEWLKNGGPSSGAPPPS
SEQ ID NO: 6	CA-GEGTFTSDLSRQMEEEAVRLFIEWAAHSQGTFTSDYSK YLD
SEQ ID NO: 7	CA-SQGTFTSDYSRYLDEEAVRLFIEWLMNTK
SEQ ID NO: 8	CA-SQGTFTSDLSRQLEEEAVRLFIEWLMNK
SEQ ID NO: 9	CA-GQGTFTSDYSRYLDEEAVXLFIEWLMNTKRNRNNIA
SEQ ID NO: 10	CA-SQGTFTSDYSRQMEEEAVRLFIEWLMNGGPSSGAPPPSK
SEQ ID NO: 11	CA-GEGTFTSDLSRQMEEEAVRLFIEWAAHSQGTFTSDYSRY LDK
SEQ ID NO: 12	CA-SQGTFTSDYSRYLDGGGHGEGTFTSDLSKQMEEEAVK
SEQ ID NO: 13	CA-SQGTFTSDYSRYLDXEAVXLFIEWLMNTK
SEQ ID NO: 14	CA-GQGTFTSDYSRYLDEEAVXLFIXWLMNTKRNRNNIA
SEQ ID NO: 15	CA-GQGTFTSDYSRYLDEEAVRLFIXWLMNTKRNRNNIA
SEQ ID NO: 16	CA-SQGTFTSDLSRQLEGGGHSQGTFTSDLSRQLEK
SEQ ID NO: 17	CA-SQGTFTSDYSRYLDEEAVRLFIEWIRNTKRNRNNIA
SEQ ID NO: 18	CA-SQGTFTSDYSRYLDEEAVRLFIEWIRNGGPSSGAPPPSK
SEQ ID NO: 19	CA-SQGTFTSDYSRYLD**E**EAV**K**LFIEWIRNTKRNRNNIA
SEQ ID NO: 20	CA-SQGTFTSDYSRYLD**E**EAV**K**LFIEWIRNGGPSSGAPPPSK
SEQ ID NO: 21	CA-SQGTFTSDYSRQLEEEAVRLFIEWVRNTKRNRNNIA
SEQ ID NO: 22	DA-SQGTFTSDYSKYLD**E**KRA**K**EFVQWLMNTK
SEQ ID NO: 23	HAibQGTFTSDYSKYLDEKRAKEFVCWLMNT
SEQ ID NO: 24	HAibQGTFTSDY SKYLDEKRAK EFVQWLMNTC
SEQ ID NO: 25	HAibQGTFTSDYSKYLD**E**KRA**K**EFVQWLMNTC

SEQ ID NO: 26	HAibQGTFTSDYS**K**YLD**E**KRAKEFVQWLMNTC
SEQ ID NO: 27	HAibQGTFTSDYSKYLD**E**QAA**K**EFICWLMNT
SEQ ID NO: 28	HAibQGTFTSDY SKYLDEKRAK EFVQWLMNT
SEQ ID NO: 29	H(d)SQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIA
SEQ ID NO: 30	CA-SQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIA
SEQ ID NO: 31	CA-(d)SQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIA
SEQ ID NO: 32	CA-AibQGTFTSDYSKYLDEKRAKEFVQWLMNTC
SEQ ID NO: 33	HAibQGTFTSDYAKYLDEKRAKEFVQWLMNTC
SEQ ID NO: 34	YAibQGTFTSDYSKYLDEKRAKEFVQWLMNTC

[218] In Table 1 above, the amino acids indicated by the bold letters mean ring formation, and the amino acids indicated by X mean alpha-methyl-glutamic acid that is a non-native amino acid. In addition, CA indicates 4-imidazoacetyl, DA indicates desamino-histidyl, Aib indicates aminosiobutyric acid, and (d)S indicates d-serine.

[219]

[220] Example 2-2: Measurement of in vitro activities of oxyntomodulin derivatives

[221] In order to measure the effects of anti-obesity peptides, the *in vitro* activities of cells were measured using the transformants prepared in Examples1-1 and 1-2.

[222] Each of the transformants was transformed so as to express each of human GLP-1 receptor and glucagon receptor genes in CHO (Chinese hamster ovary) and was suitable for measuring the activities of GLP-1 and glucagon. Thus, the activity of each of the oxyntomodulin derivatives was measured using each of the transformants.

[223] Specifically, each of the transformants was subcultured twice or three times a week, and the cells were dispensed into each well of a 96-well plate at a density of 1 X 10^5 cells/well and cultured for 24 hours.

[224] The cultured cells were washed with KRB buffer, suspended in 40 ml of 1 mM IBMX-containing KRB buffer, and then allowed to stand at room temperature for 5 minutes. Each of oxyntomodulin and the oxyntomodulin derivatives (SEQ ID NOs: 2-6, 8, 10-13, 17, 18, 23-25, 27, 28 and 32-34) was serially diluted by five-fold from 1000 nM to 0.02 nM, and 40 ㎖ of each of the dilutions was added to the cells, which were then incubated in a CO_2incubatorat37℃ for 1 hour. Then, 20 ml of cell lysis buffer was added to lyse the cells, and the concentration of cAMP in each of the cell lysates was measured using a cAMP assay kit (Molecular Device, USA). From the results of the measurement, EC_{50}values were calculated and compared with each other (Table2).

[225] Table 2

[Table 2]

Comparison of *in vitro* activities of GLP-1 receptor and glucagon receptor between oxyntomodulin derivatives

SEQ ID NOs	EC_{50}(nM)	
	CHO/GLP-1R	CHO/GCGR
SEQ ID NO: 1	50 - 210	10 - 43
SEQ ID NO: 2	51.8	12.8
SEQ ID NO: 3	>1,000	637.7
SEQ ID NO: 4	5.5	>1,000
SEQ ID NO: 5	5.9	>1,000
SEQ ID NO: 6	500.1	>1,000
SEQ ID NO: 8	419.6	>1,000
SEQ ID NO: 10	>1,000	>1,000
SEQ ID NO: 11	>1,000	>1,000
SEQ ID NO: 12	>1,000	>1,000
SEQ ID NO: 13	>1,000	>1,000
SEQ ID NO: 17	97.9	>1,000
SEQ ID NO: 18	96.3	>1,000
SEQ ID NO: 23	2.46	5.8
SEQ ID NO: 24	1.43	6.95
SEQ ID NO: 25	1.9	1.3
SEQ ID NO: 27	2.8-5.5	3.1-5.6
SEQ ID NO: 28	3.1	0.3
SEQ ID NO: 32	41.3	17.7
SEQ ID NO: 33	2.2	80.2
SEQ ID NO: 34	12.5	1.04

[226] As can be seen in Table 2 above, the oxyntomodulin derivatives showed excellent *in vitro* GLP-1 and glucagon activities compared to the oxyntomodulin of SEQ ID NO:1.

[227] Oxyntomodulin is known to have the effect of treating hyperlipidemia, fatty liver disease or arteriosclerosis by activating GLP-1 receptor and glucagon receptor. The oxyntomodulin derivatives according to the present invention have an excellent

activity to activate GLP-1 receptor and glucagon receptor compared to native oxyntomodulin, and thus can be used to treat hyperlipidemia and the fatty liver disease and arteriosclerosis related to hyperlipidemia, in place of native oxyntomodulin.

[228]

[229] Example 3: Preparation of a conjugate comprising an oxyntomodulin derivative (SEP ID NO : 23) with immunoglobulin Fc (immunoglobulin Fc –conjugated oxyntomodulin derivative 23)

[230]

[231] In order to pegylate a cysteine residue at position 24 of an oxyntomodulin derivative of SEQ ID NO: 23 with MAL-IOK-ALD PEG (NOF., Japan), the oxyntomodulin derivative (SEQ ID NO: 23) and MAL-IOK-ALD PEG were allowed to react with each other at molar ratio of 1:3 at a protein concentration of 3mg/m*l* at room temperature for 3 hours. The reaction was performed in 50mM Tris buffer (pH 8.0) containing 1M guanidine. After completion of the reaction, the reaction solution was purified using SOURCE S under the following conditions, thereby obtaining an oxyntomodulin mono-pegylated into the cysteine: column: SOURCE S, flow rate: 2.0 m*l*/min, gradient: A 0 ⟶100% 50 min B (A: 20 mM Na-citrate, pH 3.0 + 45% ethanol, B: A + 1M KCl)).

[232] Then, the purified mono-pegylated oxyntomodulin derivative (SEQ ID NO: 23) and immunoglobulin Fc were allowed to react with each other at a molar ratio of 1:5 at a protein concentration of 20 mg/m*l* at 4 °C for 16 hours. The reaction was performed in 100 mM potassium phosphate buffer (pH 6.0) containing 20mM SCB as a reducing agent. After completion of the reaction, the reaction solution was purified under the following conditions, thereby obtaining a conjugate comprising the oxyntomodulin derivative (SEQ ID NO: 23) and immunoglobulin: column: SOURCE 15Q, flow rate: 2.0 m*l*/min, gradient: A 0 ⟶ 4% 1 min, B ⟶ 20% 80 min B (A: 20mM Tris-HCl, pH 7.5, B: A + 1M NaCl)); source ISO column: SOURCE ISO, flow rate: 2.0 m*l*/min, gradient: B 0 ⟶ 100% 100 min A, (A: 20mM Tris-HCl, pH 7.5, B: A + 1.1M AS).

[233]

[234] Example 4: Preparation of a conjugate comprising an oxyntomodulin derivative (SEQ ID NO : 25) with immunoglobulin Fc (immunoglobulin Fc –conjugated oxyntomodulin derivative 25)

[235]

[236] In order to pegylate a cysteine residue at position 30 of an oxyntomodulin derivative of SEQ ID NO: 25 with MAL-10K-ALD PEG, the oxyntomodulin derivative (SEQ ID NO: 25) and MAL-10K-ALD PEG were allowed to react with each other at molar ratio of 1:3 at a protein concentration of 3 mg/m*l* at room temperature for 3 hours. The reaction was performed in 50mM Tris buffer (pH 8.0) containing 1M guanidine. After

completion of the reaction, the reaction solution was purified using SOURCE S under the following conditions, thereby obtaining an oxyntomodulin mono-pegylated into the cysteine: column: SOURCE S, flow rate: 2.0 mℓ/min, flow rate: A 0 →100% 50 min B (A: 20 mM Na-citrate, pH 3.0 + 45% ethanol, B: A + 1M KCl).

[237] Then, the purified mono-pegylated oxyntomodulin derivative (SEQ ID NO: 25) and immunoglobulin Fc were allowed to react with each other at a molar ratio of 1:5 at a protein concentration of 20 mg/mℓ at 4 °C for 16 hours. The reaction was performed in 100 mM potassium phosphate buffer (pH 6.0) containing 20mM SCB as a reducing agent. After completion of the reaction, the reaction solution was purified under the following conditions, thereby obtaining a conjugate comprising the oxyntomodulin derivative (SEQ ID NO: 25) and immunoglobulin: SOURCE 15Q column: SOURCE 15Q, flow rate: 2.0 mℓ/min, flow rate: A 0 → 4% 1 min B → 20% 80 min B (A: 20 mM Tris-HCl, pH 7.5, B: A + 1M NaCl); and Source ISO column: SOURCE ISO, flow rate: 2.0 mℓ/min, flow rate: B 0 → 100% 100 min A (A: 20mM Tris-HCl, pH 7.5, B: A + 1.1M AS).

[238]

[239] **Example 5: Effect of long-acting oxyntomodulin on reduction in lipid in hyperlipidemia model hamsters**

[240]

[241] Example 5-1: Grouping of test animals

[242] 8 week-old male hamsters (Golden Syrian hamsters, 120-130 g) were purchased from Vital River China. It is known that hamsters show blood lipid profiles similar to humans, unlike other rodents, and are sensitive to high-fat diets.

[243] The animals were allowed access to a sterilized high-fat diet (Purina 5001 containing 11.5% maize oil, 11.5% coconut oil, 0.5% cholesterol, and 0.25% deoxycholate; Dyets, Bethlehem, PA) or a standard rodent diet (low fat, 2018; Harlan Teklad, Madison, WI). A normal diet group was allowed access to filtered and UV-sterilized tap water, and a high-fat diet group was allowed access to water containing 10% fructose. The animals were kept in a breeding chamber satisfying GLP standards under a 12-hr light/12-hr dark cycle (lighting: am 6 to pm 6), and all the experimental procedures were performed according to the standard guideline for animal experiments. Drug administration was started after 3 weeks of hyperlipidemia induction, and the animals were divided into four groups (n=6) as shown in Table 3 below.

[244] Table 3

[Table 3]

Groups	Drugs administered	Method of administration
Normal group	Vehicle (DPBS)	Administered subcutaneously once a week
Hyperlipidemia-induced group	Vehicle (DPBS)	
	3.25 nmol/kg of SEQ ID NO: 25-Fc conjugate	
	8.96 nmo/kg of SEQ ID NO: 23-Fc conjugate	

[245] Specifically, group 1 (normal group) was fed with a normal feed and administered subcutaneously with 5 ml/kg of Dulbecco's phosphate buffered saline (DPBS, Sigma) once or more a week.

[246] Group 2 (hyperlipidemia-induced group) was fed with a high-fat diet to induce hyperlipidemia, and then administered subcutaneously with 5 ml/kg of Dulbecco's phosphate buffered saline (DPBS, Sigma) once or more a week.

[247] Group 3 (hyperlipidemia-induced group + group administered with 3.25 nmol/kg of SEQ ID NO: 25-Fc conjugate) was fed with a high-fat diet to induce hyperlipidemia, and then administered with 3.25 nmol/kg of the SEQ ID NO: 25-Fc conjugate (prepared in Example 4) once a week at an injection dose of 5 ml/kg.

[248] Group 4 (hyperlipidemia-induced group + group administered with 8.96 nmol/kg of SEQ ID NO: 23-Fc conjugate) was fed with a high-fat diet to induce hyperlipidemia, and then administered with 8.96 nmol/kg of the SEQ ID NO: 23-Fc conjugate (prepared in Example 3) once a week at an injection dose of 5 ml/kg.

[249] Saline or the drug was administered into each group (n=6) for 2 weeks,and then the effects thereof on a reduction in lipid levels were analyzed.

[250]

[251] Example 5-2: Analysis of effect of long-acting oxyntomodulin derivative conjugate on reduction in lipid levels

[252] In order to examine the effect of the long-acting oxyntomodulin derivative conjugate on a reduction in lipid levels in hamsters, the following experiment was performed.

[253] Blood was collected from the hamsters which were administered or not administered with the long-acting oxyntomodulin derivative as described in Example 5-1, and the lipid levels of the blood were analyzed using HITACHI 7020. The results of the analysis are shown in FIGS. 1 to 5.

[254] FIGS. 1 to 5 show the change in blood triglyceride levels (FIG. 1), the change in blood total cholesterol levels (FIG. 2), the change in LDL-cholesterol levels (FIG. 3), blood HDL-cholesterol levels (FIG. 4), and the change in the blood HDL/

LDL-cholesterol ratio (FIG. 5). The obtained results were statistically processed, and the mean values and the standard deviations of the mean values were calculated. In the verification of significance between the groups (n=6),data were statistically processed using Dunnett's test of one-way ANOVA, and a value of $p<0.05$ was considered statistically significant.

[255] Specifically, in the results of measurement of blood triglyceride levels, it was seen that, in the case of hamsters fed with a high-fat diet, the triglyceride levels were significantly increased, but when the long-acting oxyntomodulin derivative (SEQ ID NO: 25-Fc conjugate or SEQ ID NO: 23-Fc conjugate) was administered into the hamsters, the triglyceride levels were significantly decreased (FIG. 1).

[256] In the results of measurement of blood total cholesterol levels, it was seen that, in the case of hamsters fed with a high-fat diet, the blood total cholesterol levels were significantly increased, but when the long-acting oxyntomodulin derivative (SEQ ID NO: 25-Fc conjugate or SEQ ID NO: 23-Fc conjugate) was administered into the hamsters, the blood total cholesterol levels were significantly decreased (FIG. 2).

[257] In the results of measurement of blood LDL-cholesterol levels, it was seen that, in the case of hamsters fed with a high-fat diet, the blood LDL-cholesterol levels were significantly increased, but when the long-acting oxyntomodulin derivative (SEQ ID NO: 25-Fc conjugate or SEQ ID NO: 23-Fc conjugate) was administered into the hamsters, the blood LDL-cholesterol cholesterol levels were significantly decreased (FIG. 3).

[258] In the results of measurement of blood HDL-cholesterol levels, the group administered with the SEQ ID NO: 25-Fc conjugate or the SEQ ID NO: 23-Fc conjugate showed a significant increase in the blood HDL-cholesterol levels compared to the high-fat diet hamster group (FIG. 4).

[259] In the results of measurement of blood HDL/LDL-cholesterol levels, the group administered with the SEQ ID NO: 25-Fc conjugate or the SEQ ID NO: 23-Fc conjugate showed a significant increase in the blood HDL/LDL-cholesterol ratio compared to the high-fat diet hamster group (FIG. 5).

[260] From the above results, it could be seen that the inventive oxyntomodulin derivative conjugate comprising the immunoglobulin Fc region covalently linked to the oxyntomodulin derivative by PEG prevents the accumulation of blood triglyceride and low-density (LDL) cholesterol, and thus can be effectively used for the treatment of hyperlipidemia or related fatty liver disease or arteriosclerosis.

[261]

[262] **Example 6: Analysis of effects of known long-acting GLP-1 analog and long-acting oxyntomodulin derivative conjugate**

[263]

[264] VICTOZA® is a long-acting glucagon-like peptide-1, GLP-1 analog which is currently marketed as an agent for treating diabetes and is known to have the effects of treating obesity and increasing HDL cholesterol levels.

[265] The effect of reducing lipid levels was compared between the oxyntomodulin derivative conjugate and known VICTOZA®.

[266] As described in Example 5, hamsters were divided into a normal hamster group and hamster groups fed with a high-fat diet. The normal hamster group was administered subcutaneously with 5 ml/kg of DPBS once or more a week. The hamster groups fed with high-fat diet were divided into a group administered subcutaneously with 5 ml/kg of DPBS once or more a week, a group administered subcutaneously with 35.5 nmol/ kg of VICTOZA® once or more a week,a group administered subcutaneously with 3.25 nmol/kg of the SEQ ID NO: 25-Fc conjugate,and a group administered subcutaneously with 8.96 nmol/kg ofthe SEQ ID NO:23-Fc conjugate,and the blood lipid levels of the groups were analyzed.

[267] As a result, it could be seen that administration of the inventive long-acting oxyntomodulin derivative conjugate (SEQ ID NO: 25-Fc conjugate or SEQ ID NO: 23-Fc conjugate) showed a decrease in blood total cholesterol levels (FIG. 6) and a decrease in blood LDL-cholesterol level (FIG. 7) compared to administration of commercial VICTOZA®.

[268] In addition, it could be seen that administration of the inventive long-acting oxyntomodulin derivative conjugate (SEQ ID NO: 25-Fc conjugate or SEQ ID NO: 23-Fc conjugate) showed increases in blood HDL-cholesterol levels and the HDL/ LDL-cholesterol ratio compared to administration of VICTOZA®(FIGS.8 and 9). Particularly, the long-acting SEQ ID NO: 25-Fc conjugate showed significant increases in blood HDL-cholesterol levels and the HDL/LDL-cholesterol ratio compared to VICTOZA®.

[269] In addition, administration of the inventive long-acting oxyntomodulin derivative conjugate (SEQ ID NO: 25-Fc conjugate or SEQ ID NO: 23-Fc conjugate) showed a decrease in blood triglyceride levels compared to administration of VICTOZA®.

[270] From the above results, it can be seen that the long-acting oxyntomodulin derivative conjugate of the present invention exhibits a lipid-lowering effect that is equal to or higher than that of known VICTOZA®, and thus the conjugate can be effectively used as an agent for treating hyperlipidemia, fatty liver disease or arteriosclerosis.

[271]

[272]

Claims

[Claim 1] A composition for preventing or treating hyperlipidemia, fatty liver disease or arteriosclerosis, comprising an oxyntomodulin derivative having the amino acid sequence selected from the group consisting of SEQ ID NOs: 24-26 and 28.

[Claim 2] The composition according to claim 1, wherein the oxyntomodulin derivative is in the form of a conjugate linked to one selected from the group consisting of an immunoglobulin fragment, an antibody, elastin, albumin and fibronectin.

[Claim 3] The composition according to claim 2, wherein the conjugate is a conjugate in which the oxyntomodulin derivative whose amino acid sequence is selected from the group consisting of SEQ ID NOs: 24-26 and 28 is linked to an immunoglobulin Fc region via a non-peptidyl polymer.

[Claim 4] The composition according to claim 3, wherein the non-peptidyl polymer is selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol/propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, polysaccharides, dextran, polyvinyl ethyl ether, PLA (polylactic acid), PLGA (polylactic-glycolic acid), lipid polymers, chitin, hyaluronic acid, polysaccharide and combinations thereof.

[Claim 5] The composition according to claim 3, wherein one end of the non-peptidyl polymer and the other are linked to an amine group or a thiol group of the immunoglobulin Fc region and the oxyntomodulin derivative, respectively.

[Claim 6] The composition according to claim 1, further comprising a pharmaceutical agent showing preventive or therapeutic effects against hyperlipidemia, fatty liver disease or arteriosclerosis.

[Claim 7] The composition according to claim 1, wherein the fatty liver disease is non-alcoholic fatty liver disease, alcoholic fatty liver disease, nutritional fatty liver disease,

starvation fatty liver disease, obesity fatty liver disease, diabetic fatty liver disease, or steatohepatitis.

[Claim 8] The composition according to claim 7, wherein the non-alcoholic fatty liver disease results from hyperlipidemia, diabetes, or obesity.

[Claim 9] The composition according to claim 7, wherein the non-alcoholic fatty liver disease is selected from the group consisting of simple steatosis, non-alcoholic steatohepatitis, liver fibrosis and liver cirrhosis.