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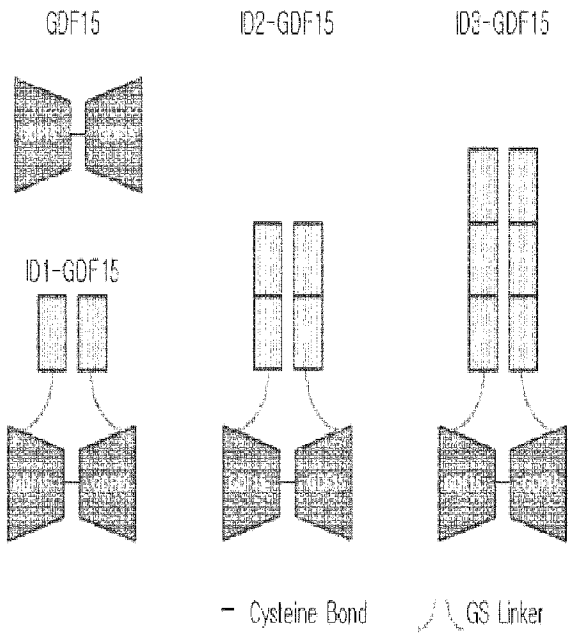
(54) Titre : POLYPEPTIDE DE FUSION COMPRENANT LE GDF15 ET REGION POLYPEPTIDIQUE APTE A L'O-GLYCOSYLATION

(54) Title: FUSION POLYPEPTIDE COMPRISING GDF15 AND POLYPEPTIDE REGION CAPABLE OF O-GLYCOSYLATION

[Fig. 2b]



ID: ESPKAQASSVPTAQPQAEGSLAKATTAPATT RNT (SEQ ID NO: 1)
GS Linker: GGGGSGGGGS GGGGSGGGGS (SEQ ID NO: 17)
GDF15: SEQ ID NO: 3

(57) Abrégé/Abstract:
Disclosed are: a fusion polypeptide comprising growth differentiation factor 15 (GDF15) and a polypeptide region capable of O-glycosylation; a pharmaceutical composition comprising the fusion polypeptide; and a method for increasing the in vivo duration of

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(57) Abrégé(suite)/Abstract(continued):
GDF15, comprising the step of fusing a polypeptide region capable of O-glycosylation.

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Abstract:

Disclosed are: a fusion polypeptide comprising growth differentiation factor 15 (GDF15) and a polypeptide region capable of O-glycosylation; a pharmaceutical composition comprising the fusion polypeptide; and a method for increasing the in vivo duration of GDF15, comprising the step of fusing a polypeptide region capable of O-glycosylation.

【DESCRIPTION】

【TITLE OF THE INVENTION】

FUSION POLYPEPTIDE COMPRISING GDF15 AND POLYPEPTIDE REGION CAPABLE OF O-GLYCOSYLATION

【TECHNICAL FIELD】

The present invention relates to a fusion polypeptide comprising GDF15 (Growth differentiation factor 15) and a polypeptide region capable of O-glycosylation, a pharmaceutical composition comprising the fusion polypeptide, and a method for increasing in vivo duration of GDF15 comprising the step of fusing a polypeptide region capable of O-glycosylation.

【BACKGROUND ART】

Most protein or peptide drugs have a short duration of activity in the body, and their absorption rate is low when administered by methods other than intravenous administration, and therefore, there is an inconvenience of having to continuously inject these drugs repeatedly at short administration intervals when treatment of long-term drug administration is required. In order to solve such inconvenience, it is required to develop a technology for continuously releasing a drug with single administration. As a part to meet these needs, a sustained-release formulation for sustained release is being developed.

For examples, research on a sustained-release formulation in which the drug is slowly released while the matrix substance is slowly decomposed in vivo when it is administered, by preparing a microparticle in the form of a protein or peptide drug surrounded by a biodegradable polymer matrix is actively progressed.

For example, U.S. Patent NO. 5,416,017 discloses a sustained-release injection of erythropoietin using a gel having a hyaluronic acid concentration of 0.01 to 3 %, and Japanese Patent Publication No. 1-287041 discloses a sustained-release injection in which insulin is contained in a gel having a hyaluronic acid concentration of

1%, and Japanese Patent Publication No. 2-213 discloses a sustained-release formulation in which calcitonin, elcatonin or human GDF15 is contained in hyaluronic acid having a concentration of 5%. In such a formulation, the protein drug dissolved in the gel of hyaluronic acid passes through the gel matrix with high viscosity at a slow speed, so it can exhibit a sustained release effect, but there are disadvantages in that it is not easy to administer by injection due to high viscosity, and it is difficult to release the drug for more than 1 day as the gel is easily diluted or decomposed by body fluids after injection.

On the other hand, there are examples of preparing solid microparticles by emulsion solvent extraction using a hyaluronic acid derivative having hydrophobicity (for example, hyaluronic acid-benzyl ester) (N.S. Nightlinger, et al., Proceed. Intern. Symp. Control. Rel. Bioact. Mater., 22nd, Paper No. 3205 (1995); L. Ilum, et al., J. Controlled Rel., 29, 133(1994)). Since it is necessary to use an organic solvent in preparation of the drug release formulation particles using a hydrophobic hyaluronic acid derivative, there is a risk of denaturation of the protein drug by contact with the organic solvent, and the possibility of denaturation of the protein due to the hydrophobicity of the hyaluronic acid derivative is high.

Therefore, in order to improve in vivo persistence of a protein or peptide drug, an approach different from the conventional studies is required.

On the other hand, GDF15 (Growth differentiation factor 15) is a member of the TGF-beta family, and is a 25kDa homodimer, and is a secretory protein circulating in plasma. The plasma level of GDF15 is related to BMI (body mass index) and GDF15 plays a role as a long-term regulator of energy homeostasis. GDF15 also has protective actions in pathological conditions such as cardiovascular disease, myocardial hypertrophy and ischemic injury. In addition, GDF15 plays a protective role against renal tubular and renal interstitial damage in models of type 1 diabetes and type 2 diabetes. Furthermore, GDF15 has a protective effect against age-related sensory and motor nerve loss, and can contribute to peripheral nerve damage recovery. Moreover, GDF15 has effects of weight loss and body fat reduction and glucose tolerance, and has an effect of increasing systemic energy consumption and oxidative metabolism. GDF15 exhibits an effect of glycemic control through body weight-

dependent and non-dependent mechanisms.

The development of a technology for improving in vivo persistence of GDF15 protein exhibiting such various pharmacological effects is required.

【DISCLOSURE】

【TECHNICAL PROBLEM】

In the present description, provided is a technology of increasing an in vivo half-life of GDF15 to enhance the in vivo duration and thereby, increasing the administration interval, by linking a polypeptide capable of O-glycosylation (for example, immunoglobulin hinge region, etc.) to GDF15 (Growth differentiation factor 15) to form a fusion polypeptide, compared to the case where it is not fused with a polypeptide region capable of O-glycosylation.

One embodiment provides a fusion polypeptide comprising GDF15 and a polypeptide region capable of O-glycosylation.

In the fusion polypeptide, the polypeptide region capable of O-glycosylation may be comprised in the N-terminus of the GDF15.

The total number of the polypeptide region capable of O-glycosylation comprised in the fusion polypeptide may be 1 or more, for example, 1 to 10, 1 to 8, 1 to 6, 1 to 4, 2 to 10, 2 to 8, 2 to 6, 2 to 4 (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10).

In one embodiment, the fusion polypeptide may be represented by the following general formula:

N'-(Z)n-Y -C' [general formula]

in the formula,

N' is the N-terminus of the fusion polypeptide, and C' is the C-terminus of the fusion polypeptide, and

Y is GDF15, and

Z is a polypeptide region capable of O-glycosylation, and

n is the number of the polypeptide region capable of O-glycosylation positioned at the N-terminus of the fusion polypeptide (bound to the N-terminus of GDF15) and an integer of 1 to 10 (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10), 1 to 7, 1 to 5, or 1 to 3.

The n polypeptide regions capable of O-glycosylation comprised in the fusion polypeptide may be each independently selected among polypeptide regions comprising amino acid residues capable of O-glycosylation. For example, the polypeptide regions comprising amino acid residues capable of O-glycosylation may be immunoglobulin hinge regions. In one embodiment, the polypeptide regions capable of O-glycosylation may be selected from the group consisting of immunoglobulin D (IgD) hinge regions and immunoglobulin A (IgA, for example, IgA1) hinge regions (i.e., n immunoglobulin hinge regions may be same or different each other).

In the fusion polypeptide, the GDF15 fused with the polypeptide region capable of O-glycosylation, is characterized by having increased in vivo (or in blood) stability (duration), compared to the GDF15 not fused with the polypeptide region capable of O-glycosylation (for example, in vivo or blood half-life increase).

Another embodiment provides a nucleic acid molecule encoding the fusion polypeptide.

Another embodiment provides a recombinant vector comprising the nucleic acid molecule.

Another embodiment provides a recombinant cell comprising the recombinant vector.

Another embodiment provides a method for preparation of GDF15 with an increased in vivo (or in blood) half-life, or a method for preparation of a fusion polypeptide comprising the GDF15 with an increased in vivo (or in blood) half-life, comprising expressing the recombinant vector in a cell.

Another embodiment provides a method for increasing in vivo duration of GDF15, or a method for enhancing in vivo (or in blood) stability of a GDF15 (protein or peptide) drug and/or increasing an in vivo (or in blood) half-life, comprising fusing (or linking or binding) GDF15 and a polypeptide region capable of O-glycosylation. In one specific example, the fusing may comprise fusing (or linking or binding) one or more

polypeptide regions capable of O-glycosylation at the N-terminus of GDF15 through or not through a linker. The fusing (or linking or binding) may be performed in vitro.

Another embodiment provides a fusion polypeptide dimer, comprising two of the fusion polypeptides. The fusion polypeptide dimer may be formed by being linked by a bond (for example, disulfide bond) between GDF15 comprised in each fusion polypeptide. The fusion polypeptide dimer may be a homodimer.

Another embodiment provides a pharmaceutical composition comprising one or more selected from the group consisting of the fusion polypeptide, a fusion polypeptide dimer comprising the fusion polypeptide, a nucleic acid molecule encoding the fusion polypeptide, a recombinant vector comprising the nucleic acid molecule and a recombinant cell comprising the recombinant vector.

Another embodiment provides a method for preparing a pharmaceutical composition using one or more selected from the group consisting of the fusion polypeptide, a fusion polypeptide dimer comprising the fusion polypeptide, a nucleic acid molecule encoding the fusion polypeptide, a recombinant vector comprising the nucleic acid molecule and a recombinant cell comprising the recombinant vector.

Another embodiment provides a use of one or more selected from the group consisting of the fusion polypeptide, a fusion polypeptide dimer comprising the fusion polypeptide, a nucleic acid molecule encoding the fusion polypeptide, a recombinant vector comprising the nucleic acid molecule and a recombinant cell comprising the recombinant vector, for preparing a pharmaceutical composition.

Another embodiment provides a use of a polypeptide region capable of O-glycosylation for enhancing in vivo (or in blood) stability and/or increasing an in vivo (or in blood) half-life of a GDF15 (protein or peptide) drug. Specifically, an embodiment provides a composition for enhancing in vivo (or in blood) stability and/or increasing an in vivo (or in blood) half-life of a GDF15 (protein or peptide) drug, the composition comprising a polypeptide region capable of O-glycosylation.

【TECHNICAL SOLUTION】

The present description provides a technology capable of enhancing in vivo (or in blood) stability and/or in vivo (or in blood) duration in case of in vivo application of GDF15, by providing a fusion polypeptide form in which a polypeptide region capable of O-glycosylation such as an immunoglobulin hinge region is fused to GDF15.

One embodiment provides a fusion polypeptide comprising GDF15 and a polypeptide region capable of O-glycosylation.

In the fusion polypeptide, the polypeptide region capable of O-glycosylation may be comprised at the N-terminus of the GDF15.

The total number of the polypeptide region capable of O-glycosylation comprised in the fusion polypeptide may be 1 or more, for example, 1 to 10, 1 to 8, 1 to 6, 1 to 4, 2 to 10, 2 to 8, 2 to 6, 2 to 4 (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10).

In one embodiment, the fusion polypeptide may be represented by the following general formula:

N'-(Z)n-Y -C' [general formula]

in the formula,

N' is the N-terminus of the fusion polypeptide, and C' is the C-terminus of the fusion polypeptide, and

Y is GDF15, and

Z is a polypeptide region capable of O-glycosylation, and

n is the number of the polypeptide region capable of O-glycosylation positioned at the N-terminus of the fusion polypeptide (bound to the N-terminus of GDF15) and an integer of 1 to 10 (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10), 1 to 7, 1 to 5, or 1 to 3.

In one embodiment, in the fusion polypeptide, when the active site of GDF15 is positioned at the C-terminus, the polypeptide region capable of O-glycosylation may be fused to the N-terminus.

The n polypeptide regions capable of O-glycosylation comprised in the fusion polypeptide may be each independently selected among polypeptide regions comprising amino acid residues capable of O-glycosylation. For example, the polypeptide regions comprising amino acid residues capable of O-glycosylation may

be immunoglobulin hinge regions. In one embodiment, the polypeptide regions capable of O-glycosylation may be selected from the group consisting of immunoglobulin D (IgD) hinge regions and immunoglobulin A (IgA, for example, IgA1) hinge regions (i.e., n immunoglobulin hinge regions may be same or different each other).

In one specific embodiment, the polypeptide region capable of O-glycosylation positioned (comprised) at the N-terminus of the fusion polypeptide may be 1 or 2, and in case of 2 or more, each of the polypeptide regions capable of O-glycosylation may be same or different each other. In one specific embodiment, one or more (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) polypeptide regions capable of O-glycosylation positioned at the N-terminus may be all IgD hinge regions or IgA (for example, IgA1) hinge regions, or comprise one or more (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) IgD hinge regions and one or more (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) IgA (for example, IgA1) hinge regions in various orders.

In other specific embodiment, when all the n polypeptide regions capable of O-glycosylation comprised in the fusion polypeptide are positioned only at the N-terminus of the fusion polypeptide (in other words, when one or more polypeptide regions capable of O-glycosylation are present only at the N-terminus of the fusion polypeptide), the one or more (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) polypeptide regions capable of O-glycosylation may be all IgD hinge regions or IgA hinge regions, or comprise one or more (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) IgD hinge regions and one or more (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) IgA hinge regions in various orders.

The polypeptide region capable of O-glycosylation (each region when the polypeptide region capable of O-glycosylation is 2 or more) may comprise 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, or 7 or more O-glycosylation residues (the upper limit is 100, 50, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, or 8) (for example, 1, 2, 3, 4, 5, 6, 7 or 8). For example, the polypeptide region capable of O-glycosylation (each region when the polypeptide region capable of O-glycosylation is 2 or more) may comprise 1 to 10 or 3 to 10 O-glycosylation residues (amino acid residues capable of O-glycosylation).

In one embodiment, the polypeptide region capable of O-glycosylation may be one or more selected from immunoglobulin (for example, human immunoglobulin)

hinge regions, and for example, may be IgD hinge regions, IgA hinge regions or a combination thereof.

Since hinge regions such as IgD hinge regions (for example, human IgD hinge regions) and/or IgA hinge regions (for example, human hinge regions) among the regions of immunoglobulin (for example, human immunoglobulin) comprise a residue capable of O-glycosylation, the polypeptide region capable of O-glycosylation may necessarily comprise one or more (human) IgD hinge regions and/or one or more (human) IgA hinge regions, or necessarily consist of the hinge regions. In one specific embodiment, the polypeptide region capable of O-glycosylation may not comprise one or more (e.g., 1, 2, or all 3) selected from the group consisting of CH1, CH2, and CH3 of immunoglobulin regions not comprising a residue capable of O-glycosylation (for example, IgD and/or IgA).

In addition, considering the number of appropriate residues capable of O-glycosylation in the fusion polypeptide provided in the present description, the polypeptide capable of O-glycosylation may comprise one or more, more specifically, 2 or more (for example, 2, 3, 4, 5, 6, 7, 8, 9, or 10) IgD hinge regions (for example, human IgD hinge regions) and/or IgA hinge regions (for example, human IgA hinge regions).

More specifically, the IgD may be human IgD (for example, UniProKB P01880 (invariant domain; SEQ ID NO: 7), etc.), and the hinge region of IgD may be one or more selected from the group consisting of

a polypeptide comprising an amino caid sequence of "N'-ESPKAQA**SS**VP**T**AQPQAEGSLAKA**TT**APA**TT**RNT-C' (SEQ ID NO: 1); amino acid residues in bold are residues capable of O-glycosylation (7 in total)" or essentially consisting of the amino acid sequence ("IgD hinge"),

a polypeptide comprising 5 or more, 7 or more, 10 or more, 15 or more, 20 or more, 22 or more, or 24 or more (the upper limit is 34 or 33) consecutive amino acids comprising one or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, or 7 or more O-glycosylation residues in the amino acid sequence of SEQ ID NO: 1, or essentially consisting of the amino acids ("a part of IgD hinge"; for example, a polypeptide comprising 5 or more continuous amino acids comprising "SSVPT" (SEQ

ID NO: 9) in SEQ ID NO: 1 or a polypeptide comprising 7 or more continuous amino acids comprising "TTAPATT" (SEQ ID NO: 10)), and

a polypeptide comprising 34 or more or 35 or more continuous amino acids comprising the amino acid sequence (IgD hinge) of SEQ ID NO: 1, in the IgD (for example, SEQ ID NO: 7) or 7 or more, 10 or more, 15 or more, 20 or more, 22 or more or 24 or more continuous amino acids comprising a part of the IgD hinge, or essentially consisting of the amino acids ("extension of IgD hinge"; for example, a polypeptide comprising 34 or more or 35 or more continuous amino acids comprising SEQ ID NO: 1 in "ESPKAQASS VPTAQPQAEG SLAKATTAPA TTRNTGRGGE EKKKEKEKEE QEERETKTP" (SEQ ID NO: 11) in the IgD (SEQ ID NO: 7) or a part of the IgD hinge).

The IgA may be human IgA (for example, IgA1 (UniProKB P01876, invariant domain; SEQ ID NO: 8), etc.), and the hinge region of the IgA may be one or more selected from the group consisting of

a polypeptide comprising an amino acid sequence of "N'-VP**ST**PP**T**P**S**P**S**TPP**T**P**S**P**S**-C' (SEQ ID NO: 2); amino acid residues in bold are residues capable of O-glycosylation (8 in total)" or essentially consisting of the amino acid sequence ("IgA hinge"),

a polypeptide comprising 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 12 or more, 15 or more, 17 or more or 18 continuous amino acids comprising 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more or 8 O-glycosylation residues in the amino acid sequence of SEQ ID NO: 2, or essentially consisting of the amino acid sequence ("a part of IgA hinge"; for example, a polypeptide comprising 8 or more or 9 or more amino acids comprising "STPPTPSP" (SEQ ID NO: 12) in SEQ ID NO: 2), and

19 or more or 20 or more continuous amino acids comprising the amino acid sequence (IgA (for example, IgA1) hinge), in IgA (for example, IgA1 (SEQ ID NO: 8)), or a polypeptide comprising 7 or more, 10 or more, 12 or more, 15 or more, 17 or more, or 18 continuous amino acids comprising a part of the IgA (for example, IgA1) hinge, or essentially consisting of the amino acid sequence ("extension of IgA hinge").

In other embodiment, the polypeptide region capable of O-glycosylation may be a polypeptide region comprising 5 or more, 7 or more, 10 or more, 12 or more, 15 or more, 17 or more, 20 or more, 22 or more, 25 or more, 27 or more, 30 or more, 32 or more or 35 or more (the upper limit is 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300 or the total amino acid number of each protein) continuous amino acids comprising 1 or more, 2 or more, 5 or more, 7 or more, 10 or more, 12 or more, 15 or more, 17 or more, 20 or more, or 22 or more (for example, 1 to 10, 3 to 10; or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25) amino acid residues capable of O-glycosylation in the proteins indicated in the following Table 1 (for example, proteins comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 23 to 113) or essentially consisting of the amino acids. In the present description, it is preferred that the polypeptide region capable of O-glycosylation does not affect the function of GDF15. The polypeptide region capable of O-glycosylation of the proteins indicated in Table below may be selected from among regions not involved in the original function of a full-length protein, and thereby, the polypeptide region capable of O-glycosylation may only serve to increase the half-life without affecting the function of GDF15:

【Table 1】

UniProtKB Entry No.	UniProtKB Entry name	Protein names	Gene names	Length	O-Glycosylation (site)	SEQ ID NO:
Q96DR8	MUCL1_HUMAN	Mucin-like protein 1	MUCL1 SBEMUNQ590/PRO1160	90	23T, 24T, 30T, 34T, 46T, 47T, 51T, 52T, 54T, 55T, 59T, 60T, 62T, 63T, 66S, 67T, 68T	23
Q0VAQ4	SMAGP_HUMAN	Small cell adhesion glycoprotein	SMAGP	97	2T, 3S, 6T, 7T, 9S, 16T, 17T, 23T	24
P04921	GLPC_HUMAN	Glycophorin-C	GYPC GLPC GPC	128	3S, 4T, 6S, 9S, 10T, 15S, 24S, 26S, 27T, 28T, 31T, 32T, 33T, 42S	25
P16860	ANFB_HUMAN	Natriuretic peptides B	NPPB	134	62T, 63S, 70S, 74T, 79S, 84T, 97T	26

P04141	CSF2_HUMAN	Granulocyte-macrophage colony-stimulating factor	CSF2 GMCSF	144	22S, 24S, 26S, 27T	27
P02724	GLPA_HUMAN	Glycophorin-A	GYPA GPA	150	21S, 22T, 23T, 29T, 30S, 31T, 32S, 36T, 38S, 41S, 44T, 52T, 56T, 63S, 66S, 69T	28
P10124	SRGN_HUMAN	Serglycin	SRGN PRG PRG1	158	94S, 96S, 100S, 102S, 104S, 106S, 108S, 110S	29
Q86YL7	PDPN_HUMAN	Podoplanin	PDPN GP36 PSEC0003 PSEC0025	162	25T, 32T, 34T, 35T, 52T, 55T, 65T, 66T, 76T, 85T, 86S, 88S, 89T, 96S, 98S, 100T, 102S, 106T, 107S, 109S, 110T, 117T, 119T, 120T	30
P0DN87	CGB7_HUMAN	Choriogonadotropin subunit beta 7	CGB7	165	139S, 141S, 147S, 150S, 152S, 158S	31
P0DN86	CGB3_HUMAN	Choriogonadotropin subunit beta 3	CGB3 CGB; CGB5; CGB8	165	139S, 141S, 147S, 150S, 152S, 158S	32
P01344	IGF2_HUMAN	Insulin-like growth factor II	IGF2 PP1446	180	96T, 99T, 163T	33
P07498	CASK_HUMAN	Kappa-casein	CSN3 CASK CSN10 CSNK	182	133T, 143T, 148T, 151T, 157T, 167T, 169T, 178T	34
P31431	SDC4_HUMAN	Syndecan-4	SDC4	198	39S, 61S, 63S	35
P34741	SDC2_HUMAN	Syndecan-2	SDC2 HSPG1	201	41S, 55S, 57S, 101T	36
Q99075	HBEGF_HUMAN	Proheparin-binding EGF-like growth factor	HBEGF DTR DTS HEGFL	208	37T, 38S, 44T, 47T, 75T, 85T	37
P13727	PRG2_HUMAN	Bone marrow proteoglycan (BMPG)	PRG2 MBP	222	23T, 24S, 25T, 34T, 62S	38
P24592	IBP6_HUMAN	Insulin-like growth factor-binding protein 6 (IBP-6)	IGFBP6 IBP6	240	126T, 144S, 145T, 146T, 152S	39
Q9UHG2	PCSK1_HUMAN	ProSAAS (Proprotein convertase subtilisin/kexin	PCSK1N	260	53T, 228S, 247T	40

		type 1 inhibitor)				
P01589	IL2RA_HUMAN	Interleukin-2 receptor subunit alpha (IL-2 receptor subunit alpha)	IL2RA	272	218T, 224T, 229T, 237T	41
P21583	SCF_HUMAN	Kit ligand (Mast cell growth factor) (MGF)	KITLG MGF SCF	273	167S, 168T, 180T	42
A1E959	ODAM_HUMAN	Odontogenic ameloblast-associated protein (Apin)	ODAM APIN	279	115T, 119T, 244T, 249S, 250T, 251T, 255T, 256S, 261T, 263T, 273T, 275S	43
P10451	OSTP_HUMAN	Osteopontin	SPP1 BNSP OPN PSEC 0156	314	134T, 138T, 143T, 147T, 152T	44
P21815	SIAL_HUMAN	Bone sialoprotein 2 (Bone sialoprotein II) (BSP II)	IBSP BNSP	317	119T, 122T, 227T, 228T, 229T, 238T, 239T	45
P02649	APOE_HUMAN	Apolipoprotein E (Apo-E)	APOE	317	26T, 36T, 212T, 307T, 308S, 314S	46
Q99645	EPYC_HUMAN	Epiphycan (Dermatan sulfate proteoglycan 3)	EPYC DSPG 3 PGLB SLRR3 B	322	60T, 64S, 96S	47
Q6UXG3	CLM9_HUMAN	CMRF35-like molecule 9 (CLM-9)	CD300 LG CLM9 TREM 4 UNQ4 22/PR O846	332	137T, 143T, 144T, 155T, 161T, 170T, 171T, 177T, 187T, 195T, 196S, 199T, 201T, 202S, 207T, 208S, 213S, 214S, 222S, 223T, 224S, 228T, 229S, 237S	48
Q9GZM5	YIPF3_HUMAN	Protein YIPF3 (Killer lineage protein 1)	YIPF3 C6orf1 09 KLIP1	350	333T, 334T, 339T, 346T	49
P51681	CCR5_HUMAN	C-C chemokine receptor type 5 (C-C CKR-5)	CCR5 CMKB R5	352	6S, 7S, 16T, 17S	50
P40225	TPO_HUMAN	Thrombopoietin (C-mpl ligand) (ML)	THPO MGDF	353	22S, 58T, 131T, 179T, 180T, 184S, 213T, 265S	51
P01876	IGHA1_HUMAN	Immunoglobulin heavy constant alpha	IGHA1	353	105S, 106T, 109T, 111S, 113S, 117T, 119S, 121S	8

		1 (Ig alpha-1 chain C region)				
P02765	FETUA_HUMAN	Alpha-2-HS-glycoprotein (Alpha-2-Z-globulin)	AHSG FETUA PRO2743	367	270T, 280S, 293S, 339T, 341T, 346S	52
P21810	PGS1_HUMAN	Biglycan	BGN SLRR1A	368	42S, 47S, 180S, 198S	53
P01860	IGHG3_HUMAN	Immunoglobulin heavy constant gamma 3 (HDC)	IGHG3	377	122T, 137T, 152T	54
P80370	DLK1_HUMAN	Protein delta homolog 1 (DLK-1)	DLK1 DLK	383	94S, 143T, 163S, 214S, 222T 251S 256T, 260S	55
P01880	IGHD_HUMAN	Immunoglobulin heavy constant delta (Ig delta chain C region)	IGHD	384	109S, 110S, 113T, 126T, 127T, 131T, 132T	7
P15529	MCP_HUMAN	Membrane cofactor protein (TLX)	CD46 MCP MIC10	392	290S, 291S, 292T, 298S, 300S, 302S, 303T, 304S, 305S, 306T, 307T, 309S, 312S, 313S, 315S, 320T, 326S	56
P04280	PRP1_HUMAN	Basic salivary proline-rich protein 1	PRB1	392	40S, 87S, 150S, 330S	57
P78423	X3CL1_HUMAN	Fractalkine (C-X3-C motif chemokine 1)	CX3CL1 FKN NTT SCYD1 A-152E5.2	397	183T, 253S, 329T	58
P16150	LEUK_HUMAN	Leukosialin (GPL115)	SPN CD43	400	21T, 22T, 26T, 28T, 29S, 35S, 36T, 37S, 41S, 42S, 46T, 47T, 48S, 50T, 58T, 69T, 99S, 103S, 109T, 113T, 114S, 136T, 137T, 173T, 178T	59
P13473	LAMP2_HUMAN	Lysosome-associated membrane glycoprotein 2 (LAMP-2)	LAMP2	410	195S, 196T, 200T, 203T, 204T, 207S, 209T, 210T, 211T, 213T	60
P11279	LAMP1_HUMAN	Lysosome-associated membrane glycoprotein 1 (LAMP-1)	LAMP1	417	197S, 199T, 200T, 207S, 209S, 211S,	61

P21754	ZP3_HUMAN	Zona pellucida sperm-binding protein 3 (Sperm receptor)	ZP3 ZP3A ZP3B ZPC	424	156T, 162T, 163T	62
P05783	K1C18_HUMAN	Keratin, type I cytoskeletal 18	KRT18 CYK18 PIG46	430	30S, 31S, 49S	63
Q08629	TICN1_HUMAN	Testican-1 (Protein SPOCK)	SPOCK1 SPOCK TIC1 TICN1	439	228T, 383S, 388S	64
O75056	SDC3_HUMAN	Syndecan-3 (SYND3)	SDC3 KIAA0468	442	80S, 82S, 84S, 91S, 314S, 367S	65
P10645	CMGA_HUMAN	Chromogranin-A (CgA)	CHGA	457	181T, 183T, 251T	66
P15169	CBPN_HUMAN	Carboxypeptidase N catalytic chain (CPN)	CPN1 ACBP	458	400T, 402T, 409T	67
P00740	FA9_HUMAN	Coagulation factor IX (EC 3.4.21.22)	F9	461	85T, 99S, 107S	68
P20333	TNR1B_HUMAN	Tumor necrosis factor receptor superfamily member 1B	TNFRSF1B TNFBR TNFR2	461	30T, 206T, 221S, 222T, 224S, 230T, 234S, 235T, 239T, 240S, 248S	69
P08670	VIME_HUMAN	Vimentin	VIM	466	7S, 33T, 34S	70
Q8WXD2	SCG3_HUMAN	Secretogranin-3 (Secretogranin III) (SgIII)	SCG3 UNQ2502/PRO5990	468	216T, 231T, 359S	71
Q16566	KCC4_HUMAN	Calcium/calmodulin-dependent protein kinase type IV (CaMK IV) (EC 2.7.11.17)	CAMK4 CAMK CAMK-GR CAMKIV	473	57T, 58S, 137S, 189S, 344S, 345S, 356S	72
P31749	AKT1_HUMAN	RAC-alpha serine/threonine-protein kinase (EC 2.7.11.1)	AKT1 PKB RAC	480	126S, 129S, 305T, 312T, 473S	73
P31751	AKT2_HUMAN	RAC-beta serine/threonine-protein kinase (EC 2.7.11.1)	AKT2	481	128S, 131S, 306T, 313T	74
O60883	G37L1_HUMAN	G-protein coupled	GPR37L1	481	79T, 85T, 86S, 95T, 107T	75

		receptor 37-like 1	ETBRLP2			
Q9BXF9	TEKT3_HUMAN	Tektin-3	TEKT3	490	7T, 9T, 10T	76
P05155	IC1_HUMAN	Plasma protease C1 inhibitor (C1 Inh)	SERPING1 C1IN C1NH	500	47T, 48T, 64S, 71T, 83T, 88T, 92T, 96T	77
P11831	SRF_HUMAN	Serum response factor (SRF)	SRF	508	277S, 307S, 309S, 316S, 383S	78
P0DOX3	IGD_HUMAN	Immunoglobulin delta heavy chain		512	238S, 255T, 256T, 260T, 261T,	79
O75487	GPC4_HUMAN	Glypican-4 (K-glypican)	GPC4 UNQ474/PRO937	556	494S, 498S, 500S	80
P35052	GPC1_HUMAN	Glypican-1	GPC1	558	486S, 488S, 490S	81
P78333	GPC5_HUMAN	Glypican-5	GPC5	572	441S, 486S, 495S, 507S, 509S	82
Q8N158	GPC2_HUMAN	Glypican-2	GPC2	579	55S, 92S, 155S, 500S, 502S	83
P00748	FA12_HUMAN	Coagulation factor XII (EC 3.4.21.38)	F12	615	109T, 299T, 305T, 308S, 328T, 329T, 337T	84
P01042	KNG1_HUMAN	Kininogen-1 (Alpha-2-thiol proteinase inhibitor)	KNG1 BDK KNG	644	401T, 533T, 542T, 546T, 557T, 571T, 577S, 628T	85
P51693	APLP1_HUMAN	Amyloid-like protein 1 (APLP) (APLP-1)	APLP1	650	215T, 227S, 228T	86
Q9NQ79	CRAC1_HUMAN	Cartilage acidic protein 1 (68 kDa chondrocyte-expressed protein) (CEP-68) (ASPIC)	CRTAC1 ASPIC 1 CEP68	661	608T, 618T, 619T, 621T, 626T	87
Q14515	SPRL1_HUMAN	SPARC-like protein 1 (High endothelial venule protein) (Hevin) (MAST 9)	SPARCL1	664	31T, 40T, 44S, 116T	88
Q16820	MEP1B_HUMAN	Meprin A subunit beta (EC 3.4.24.63)	MEP1B	701	593S, 594T, 599T, 603S	89
P17600	SYN1_HUMAN	Synapsin-1 (Brain protein	SYN1	705	55S, 87T, 96S, 103S, 261S, 432S, 526T, 564T, 578S	90

		4.1) (Synapsin I)				
P19835	CEL_HUMAN	Bile salt-activated lipase (BAL) (EC 3.1.1.13) (EC 3.1.1.3)	CEL BAL	753	558T, 569T, 579T, 607T, 618T, 629T, 640T, 651T, 662T, 673T	91
Q9HCU0	CD248_HUMAN	Endosialin (Tumor endothelial marker 1) (CD antigen CD248)	CD248 CD164L1 TEM1	757	60T, 401T, 428T, 448T, 456T, 459T, 472T, 519T, 541T, 543T, 544T, 545T, 587T, 593T, 594T, 595T, 598S, 601S, 612T, 619T, 623S, 625S, 627T, 630T, 631S, 636T, 640S,	92
P05067	A4_HUMAN	Amyloid-beta precursor protein (APP)	APP A4 AD1	770	633T, 651T, 652T, 656S, 659T, 663T, 667S,	93
Q9NR71	ASAH2_HUMAN	Neutral ceramidase (N-CDase) (NCDase) (EC 3.5.1.-) (EC 3.5.1.23)	ASAH2 HNAC1	780	62T, 67S, 68T, 70T, 73S, 74T, 76T, 78S, 79S, 80T, 82T, 84T	94
P08047	SP1_HUMAN	Transcription factor Sp1	SP1 TSFP1	785	491S, 612S, 640T, 641S, 698S, 702S	95
Q17R60	IMPG1_HUMAN	Interphotoreceptor matrix proteoglycan 1	IMPG1 IPM150 SPACR	797	403T, 421T, 432T, 442T	96
P19634	SL9A1_HUMAN	Sodium/hydrogen exchanger 1 (APNH)	SLC9A1 APNH1 NHE1	815	42T, 56S, 61T, 62T, 68T	97
P12830	CADH1_HUMAN	Cadherin-1 (CAM 120/80)	CDH1 CDHEUVO	882	280S, 285T, 358T, 470T, 472T, 509T, 576T, 578T, 580T	98
Q14118	DAG1_HUMAN	Dystroglycan (Dystrophin-associated glycoprotein 1)	DAG1	895	63T, 317T, 319T, 367T, 369T, 372T, 379T, 388T, 455T	99
Q14624	ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4 (ITI heavy chain H4) (ITI-HC4)	ITIH4 IHRP ITIHL1 PK120 PRO1851	930	719T, 720T, 722T	100
P19823	ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2 (ITI heavy chain H2) (ITI-HC2)	ITIH2 IGHEP2	946	666T, 673S, 675T, 691T	101

Q9UPV9	TRAK1_HUMAN	Trafficking kinesin-binding protein 1	TRAK1 KIAA1042 OIP106	953	447S, 680S, 719S, 935T	102
P15941	MUC1_HUMAN	Mucin-1 (MUC-1)	MUC1 PUM	1255	131T, 139T, 140S, 144T	103
Q7Z589	EMSY_HUMAN	BRCA2-interacting transcriptional repressor EMSY	EMSY C11orf30 GL002	1322	228S, 236S, 271T, 501T, 506T, 557S, 1120T	104
Q92954	PRG4_HUMAN	Proteoglycan 4 (Lubricin)	PRG4 MSF SZP	1404	123S, 136S, 240T, 253T, 277T, 291T, 305T, 306S, 310T, 317S, 324T, 332T, 338T, 367T, 373S, 376T, 384T, 385T, 388S, 391T, 399T, 400T, 407T, 408T, 415T, 423T, 427S, 430T, 438T, 439T, 446T, 447T, 454T, 455T, 477T, 478T, 485T, 493T, 494T, 501T, 502T, 509T, 525T, 529S, 532T, 540T, 541T, 553S, 555T, 563T, 564T, 571T, 572T, 579T, 580T, 587T, 588T, 595T, 603T, 604T, 611T, 612T, 616T, 619T, 627T, 676T, 683T, 684T, 691T, 692T, 699T, 700T, 704T, 707T, 723T, 724T, 736T, 768T, 769T, 776T, 777T, 792T, 793T, 805T, 812S, 829T, 837T, 838T, 892S, 900T, 930T, 931T, 962S, 963T, 968T, 975T, 978T, 979T, 980T, 1039T, 1161T	105
Q76LX8	ATS13_HUMAN	A disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAM-TS 13)	ADAMTS13 C9orf8 UNQ6102/PRO20085	1427	399S, 698S, 757S,907S, 965S, 1027S, 1087S	106
P49790	NU153_HUMAN	Nuclear pore complex protein Nup153 (153 kDa nucleoporin) (Nucleoporin Nup153)	NUP153	1475	534S, 544S, 908S, 909S, 1113S, 1156T	107
P31327	CPSM_HUMAN	Carbamoyl-phosphate	CPS1	1500	537S, 1331S, 1332T	108

		synthase [ammonia], mitochondrial (EC 6.3.4.16)				
Q8N6G6	ATL1_HUMAN	ADAMTS-like protein 1 (ADAMTSL-1) (Punctin-1)	ADAMTSL1 ADAMTSR1 C9orf94 UNQ528/PRO1071	1762	48T, 312T, 391S, 451T	109
P46531	NOTC1_HUMAN	Neurogenic locus notch homolog protein 1 (Notch 1) (hN1)	NOTCH1 TAN1	2555	65S, 73T, 116T, 146S, 194T, 232T, 311T, 341S, 349T, 378S, 435S, 458S, 466T, 496S, 534S, 609S, 617T, 647S, 692T, 722S, 759S, 767T, 784S, 797S, 805T, 921S, 951S, 997T, 1027S, 1035T, 1065S, 1159T, 1189S, 1197T, 1273S, 1362T, 1379T, 1402T,	110
P04275	VWF_HUMAN	von Willebrand factor (vWF)	VWF F8VWF	2813	1248T, 1255T, 1256T, 1263S, 1468T, 1477T, 1486S, 1487T,	111
Q9UPA5	BSN_HUMAN	Protein bassoon (Zinc finger protein 231)	BSN KIAA0434 ZNF231	3926	1343T, 1384T, 2314T, 2691T, 2936T	112
Q86WI1	PKHL1_HUMAN	Fibrocystin-L (Polycystic kidney and hepatic disease 1-like protein 1) (PKHD1-like protein 1)	PKHD1L1	4243	122T, 445T, 1803T, 1839T, 2320T, 3736T	113

The fusion polypeptide may have the total number of actually comprised O-glycan of 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, or 21 or more (the maximum value is determined by the number of the disclosed polypeptide region capable of O-glycosylation and the number of O-glycosylation residues comprised in each of the polypeptide region capable of O-glycosylation), or have the total number of theoretically comprised O-glycan of 20 or more, 21 or more, 23 or 24 or more (the maximum value is determined by the number

of the disclosed polypeptide region capable of O-glycosylation and the number of O-glycosylation residues comprised in each of the polypeptide region capable of O-glycosylation). In addition, in the fusion polypeptide, the total number of actually comprised O-glycan may be related to stability upon administration in the body (for example, in blood), and specifically, in the fusion polypeptide, as the total number of the actually comprised O-glycan increases, the in vivo stability of the fusion polypeptide or GDF15 comprised in the fusion polypeptide may increases (in other words, in vivo (in blood) half-life increase and/or in vivo (in blood) concentration increase and/or in vivo (in blood) rate of degradation decrease, etc.).

The fusion polypeptide may further comprise a peptide linker between GDF15 and a polypeptide region capable of O-glycosylation and/or between polypeptide regions capable of O-glycosylation when 2 or more of the polypeptide regions capable of O-glycosylation are comprised. In one embodiment, the peptide linker may be a GS linker repeatedly comprising one or more Gly(G) and one or more Ser(S), and for example, it may be (GGGGS)n (n is a repetition time of GGGGS (SEQ ID NO: 13) and is an integer of 1 to 10 or 1 to 5 (for example, 1, 2, 3, 4, or 5)), but not limited thereto.

Other embodiment provides a fusion polypeptide dimer, comprising 2 of the fusion polypeptides. The fusion polypeptide dimer may be formed by being linked by a bond (for example, disulfide bond) between GDF15 comprised in each of the fusion polypeptides. The fusion polypeptide dimer may be a homodimer.

In the fusion polypeptide and/or fusion polypeptide dimer, the GDF15 fused with the polypeptide region capable of O-glycosylation is characterized by increased in vivo (or in blood) stability, compared to GDF15 in which the polypeptide region capable of O-glycosylation is not fused (for example, in vivo or in blood half-life increase).

Other embodiment provides a nucleic acid molecule encoding the fusion polypeptide.

Other embodiment provides a recombinant vector comprising the nucleic acid molecule.

Other embodiment provides a recombinant cell comprising the recombinant vector.

Other embodiment provides a method for preparation of GGF15 with increased in vivo (or in blood) half-life, or a method for preparation of a fusion polypeptide comprising the GDF15 with increased in vivo (or in blood) half-life, comprising expressing the recombinant vector in a cell.

Other embodiment provides a method for increasing in vivo duration of GDF15 comprising fusing (or linking or binding) GDF15 and a polypeptide region capable of O-glycosylation. In one specific embodiment, the fusing may comprise fusing (or linking or binding) one or more polypeptide regions capable of O-glycosylation at the N-terminus, C-terminus or both terminuses of GDF15 through or not through a linker. The fusing (or linking or binding) may be progressed in vitro.

Other embodiment provides a pharmaceutical composition comprising one or more selected from the group consisting of the fusion polypeptide, a fusion polypeptide dimer comprising the fusion polypeptide, a nucleic acid molecule encoding the fusion polypeptide, a recombinant vector comprising the nucleic acid molecule and a recombinant cell comprising the recombinant vector.

Other embodiment provides a use of a polypeptide region capable of O-glycosylation for enhancing in vivo (or in blood) stability and/or increasing in vivo (or in blood) half-life of a polypeptide (protein or peptide) drug. Specifically, one embodiment provides a composition for enhancing in vivo (or in blood) stability and/or increasing in vivo (or in blood) half-life of a polypeptide (protein or peptide) drug comprising a polypeptide region capable of O-glycosylation. As used in the present description, enhancing stability and/or increasing half-life mean that the stability is enhanced and/or the half-life is increased, compared to a polypeptide (protein or peptide) not comprising a polypeptide region capable of O-glycosylation.

Hereinafter, the present invention will be described in more detail:

In the present description, GDF15 (Growth differentiation factor 15) (corresponding to Y in the general formula) is a soluble polypeptide, and consists of amino acids from the 197th (A) to 308th (I) except for a signal peptide and a propeptide in total 308 amino acids (UniProt Q99988) (SEQ ID NO: 3; See FIG. 1; mature form):

In the present description, GDF15 means, unless otherwise mentioned,

(1) the amino acid sequence from 197th (A) to 308th (I) of the full-length protein (UniProt Q99988) (SEQ ID NO: 3, See FIG. 1; ARNG DHCPLGPGRC CRLHTVRASL EDLGWADWVL SPREVQVTMC IGACPSQFRA ANMHAQIKTS LHRLKPDTVP APCCVPASYN PMVLIQKTDT GVSLQTYDDL LAKDCHCI);

(2) a functional variant of GDF15; and/or

(3) a polypeptide essentially comprising the amino acid sequence having the sequence homology of 80% or more, 85% or more, 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more to the amino acid sequence of the (1) and/or (2) in a range of maintaining the intrinsic activity and structure.

In the present description, the functional variant of GDF15 may be a variant mutated to be advantageous for dimer structure formation, while maintaining the intrinsic activity and structure. In one embodiment, the functional variant of GDF15 may be a N-terminal deletion variant in which one or more (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14) (for example, one or more from the N-terminus in order) at the N-terminus of the amino acid sequence of GDF15 of SEQ ID NO: 3 (in other words, 14 amino acid residues in total from the 1st to 14th) in SEQ ID NO: 1), for example, all the 14 amino acid residues are deleted. In one specific embodiment, the functional variant of GDF15 may be a polypeptide essentially comprising the amino acid sequence of SEQ ID NO: 4 (CRLHTVRASL EDLGWADWVL SPREVQVTMC IGACPSQFRA ANMHAQIKTS LHRLKPDTVP APCCVPASYN PMVLIQKTDT GVSLQTYDDL LAKDCHCI) or the amino acid sequence having the sequence homology of 80% or more, 85% or more, 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more to the amino acid sequence in a range of maintaining the intrinsic activity and structure of GDF15.

In the fusion polypeptide comprising GDF15 and a polypeptide region capable of O-glycosylation provided in the present description, the GDF15 and polypeptide region capable of O-glycosylation and/or 2 or more of polypeptide regions capable of O-glycosylation may be linked directly (for example, without a linker) or linked through an appropriate linker (for example, peptide linker) covalently or non-covalently. The peptide linker may be a polypeptide consisting of any amino acids of 1 to 20, 1 to 15,

1 to 10, 2 to 20, 2 to 15 or 2 to 10, and the kind of the comprised amino acids is not limited. The peptide linker may comprise, for example, Gly, Asn and/or Ser residues, and may also comprise neutral amino acids such as Thr and/or Ala, but not limited thereto, and the amino acid sequence suitable for a peptide linker is known in the art. In one embodiment, the peptide linker may be a GS linker repeatedly comprising one or more Gly(G) and one or more Ser(S), and for example, may be (GGGGS)n (n is a repetition time of GGGGS (SEQ ID NO: 13) and is an integer of 1 to 10 or 1 to 5 (for example, 1, 2, 3, 4, or 5)), but not limited thereto.

In addition, the fusion polypeptide may comprise total 1 or more or total 2 or more (for example, 2 to 10, 2 to 8, 2 to 6, 2 to 5, 2 to 4, 2 or 3) polypeptide regions capable of O-glycosylation. When 2 or more of the polypeptide regions capable of O-glycosylation are comprised, in the fusion polypeptide, 2 or more of the polypeptide regions capable of O-glycosylation are linked to the N-terminus of GDF15 and each of the polypeptide regions capable of O-glycosylation may be same or different each other. Then, between the polypeptide regions capable of O-glycosylation and/or between the polypeptide region capable of O-glycosylation and human GDF15, the aforementioned peptide linker may be further comprised.

The fusion polypeptide provided in the present description may be recombinantly or synthetically produced, and it may not be naturally occurring.

The in vivo (in blood) half-life in a mammal of GDF15 comprised in the fusion polypeptide provided in the present description may be increased about 1.1 time or more, about 1.15 times or more, about 1.2 times or more, about 1.5 times or more, about 2 times or more, about 2.5 time or more, about 3 times or more, about 3.5 times or more, about 4 times or more, about 5 times or more, about 6 times or more, about 7 times or more, about 8 times or more, about 9 times or more, or about 10 times or more, compared to the GDF15 in which the polypeptide region capable of O-glycosylation is not fused. Otherwise, the highest blood concentration in case of administration in a mammal body of GDF15 comprised in the fusion polypeptide provided in the present description may be higher about 1.2 times or more, about 1.5 times or more, about 2 times or more, about 2.5 times or more, about 3 times or more, about 3.5 times or more, or about 4 times or more, compared to the not fused GDF15.

Otherwise, the time of reaching the highest blood concentration in case of administration in a mammal body of the GDF15 comprised in the fusion polypeptide provided in the present description may be extended about 2 times or more, about 3 times or more, about 4 times or more, about 5 times or more, about 6 times or more, about 7 times or more, about 8 times or more, about 9 times or more, about 10 times or more, about 11 times or more, about 12 times or more, about 13 times or more, about 14 times or more, about 15 times or more, about 18 times or more, about 20 times or more, or about 22 times or more, compared to the not fused GDF15. Otherwise, the area under the blood concentration-time curve up to the measurable last blood gathering time (AUC_{last}) and/or the area under the blood concentration-time curve calculated by extrapolating from the measurable last blood gathering time to the infinite time (AUC_{inf}), in case of administration in a mammal body of the GDF15 comprised in the fusion polypeptide provided in the present description may be increased about 2 times or more, about 2.5 times or more, about 3 times or more, about 3.5 times or more, about 4 times or more, about 4.5 times or more, about 5 times or more, about 6 times or more, about 7 times or more, about 8 times or more, about 9 times or more, about 10 times or more, about 11 times or more, about 12 times or more, about 13 times or more, about 14 times or more, or about 15 times or more, compared to the GDF15 not fused with the polypeptide region capable of O-glycosylation.

As such, due to the increased GDF15 half-life, the GDF15 in a fusion polypeptide form to which a polypeptide region capable of O-glycosylation is linked, has an advantage of having a longer administration interval, compared to the GDF15 in a form to which a polypeptide region capable of O-glycosylation is not linked.

The fusion polypeptide comprising GDF15 and a polypeptide region capable of O-glycosylation may be prepared by a common chemical synthesis method or recombinant method.

In the present description, the term “vector” means an expression means to express a target gene in a host cell, and for example, may be selected from the group consisting of a plasmid vector, a cosmid vector and a virus vector such as a bacteriophage vector, an adenovirus vector, a retrovirus vector, and an adeno-related

virus vector, and the like. In one embodiment, the vector which can be used for the recombinant vector may be produced on the basis of a plasmid (for example, pcDNA series, pSC101, pGV1106, pACYC177, ColE1, pKT230, pME290, pBR322, pUC8/9, pUC6, pBD9, pHC79, pIJ61, pLAFR1, pHV14, pGEX series, pET series, pUC19, etc.), phage (for example, λgt4λB, λ-Charon, λΔz1, M13, etc.) or virus (for example, SV40, etc.), but not limited thereto.

The nucleic acid molecule encoding the fusion polypeptide in the recombinant vector may be operatively linked to a promoter. The term "operatively linked" means functional binding between a nucleic acid expression regulatory sequence (for example, promoter sequence) and other nucleic acid sequence. The regulatory sequence may regulate transcription and/or translation of other nucleic acid sequence by being "operatively linked".

The recombinant vector may be typically constructed as a vector for cloning or an expression vector for expression. As the expression vector, common ones used for expressing a foreign protein in a plant, animal or microorganism may be used. The recombinant vector may be constructed by various methods known in the art.

The recombinant vector may be expressed using a eukaryote as a host. When an eukaryote is to be expressed as a host, the recombinant vector may comprise a replication origin such as f1 replication origin, SV40 replication origin, pMB1 replication origin, adeno replication origin, AAV replication origin and/or BBV replication origin, and the like, but not limited thereto, in addition to a nucleic acid molecule to be expressed and the aforementioned promoter, a ribosome binding site, a secretory signal sequence (See Patent Publication No. 2015-0125402) and/or a transcription/translation termination sequence. In addition, a promoter derived from genome of a mammal cell (for example, metallothionein promoter) or a promoter derived from a mammal virus (for example, adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, cytomegalovirus and *tk* promoter of HSV) may be used and all secretory signal sequences commonly available as a secretory signal sequence may be used, and for example, the secretory signal sequence disclosed in Patent Publication No. 2015-0125402 may be used, but not limited thereto, and as a transcription termination sequence, a polyadenylation sequence may be comprised.

The recombinant cell may be obtained by introducing (transforming or transfecting) the recombinant vector into an appropriate host cell. The host cell may be selected from all eukaryotes which can stably and continuously clone or express the recombinant vector. The eukaryote available as a host includes a yeast (*Saccharomyces cerevisiae*), an insect cell, a plant cell and an animal cell, and the like, and for example, includes mice (for example, COP, L, C127, Sp2/0, NS-0, NS-1, At20, or NIH3T3), rats (for example, PC12, PC12h, GH3, or MtT), hamsters (for example, BHK, CHO, GS genetic defect CHO, or DHFR genetic defect CHO), monkeys (for example, COS (COS1, COS3, COS7, etc.), CV1 or Vero), humans (for example, HeLa, HEK-293, retina-derived PER-C6, cell derived from diploid fibroblast, myeloma cell or HepG2), other animal cells (for example, MDCK, etc.), insect cells (for example, Sf9 cell, Sf21 cell, Tn-368 cell, BTI-TN-5B1-4 cell, etc.), hybridoma, and the like, but not limited thereto.

By expressing a nucleic acid molecule encoding the fusion polypeptide provided in the present description in the aforementioned appropriate host cell, GDF15 with enhanced in vivo stability compared to the not fused form and a fusion polypeptide comprising thereof may be prepared. The method for preparation of the fusion polypeptide may comprise culturing a recombinant vector comprising the nucleic acid molecule. The culturing may be performed under a common culturing condition. In addition, the method for preparation may further comprise separating and/or purifying the fusion polypeptide from the culture, after the culturing.

For delivery (introduction) of the nucleic acid molecule or recombinant vector comprising the same, a delivery method widely known in the art may be used. As the delivery method, for example, when the host cell is a eukaryote, microinjection, calcium phosphate precipitation, electroporation, liposome-mediated transfection and gene bombardment, and the like may be used, but not limited thereto.

The method for selecting the transformed (recombinant vector-introduced) host cell may be easily conducted according to a method widely known in the art, using a phenotype expressed by a selection marker. For example, when the selection marker is a specific antibiotic resistant gene, a recombinant cell in which a recombinant vector is introduced may be easily selected by culturing in a medium containing the antibiotic.

The fusion polypeptide may be used in prevention and/or treatment of all diseases which are related to GDF15 deficiency and/or dysfunction or can be treated, alleviated or improved by GDF15 activity.

Accordingly, in one embodiment, a pharmaceutical composition comprising one or more selected from the group consisting of the fusion polypeptide, a nucleic acid molecule encoding the fusion polypeptide, a recombinant vector comprising the nucleic acid molecule and a recombinant cell comprising the recombinant vector is provided. The pharmaceutical composition may be a pharmaceutical composition for prevention and/or treatment of diseases related to deficiency and/or dysfunction of GDF15 comprised in the fusion protein or diseases having a therapeutic and/or preventive effect of the GDF15.

Other embodiment provides a method for prevention and/or treatment of diseases related to deficiency and/or dysfunction of GDF15 comprised in the fusion protein or diseases having a therapeutic and/or preventive effect of the GDF15, comprising administering one or more selected from the group consisting of the fusion polypeptide, a nucleic acid molecule encoding the fusion polypeptide, a recombinant vector comprising the nucleic acid molecule and a recombinant cell comprising the recombinant vector, into a patient in need of prevention and/or treatment of diseases related to deficiency and/or dysfunction of GDF15 comprised in the fusion protein or diseases having a therapeutic and/or preventive effect of the GDF15. The method may further comprise confirming a patient in need of prevention and/or treatment of diseases related to deficiency and/or dysfunction of GDF15 comprised in the fusion protein or diseases having a therapeutic and/or preventive effect of the GDF15, before the administering.

The example of the diseases related to deficiency and/or dysfunction of GDF15 comprised in the fusion protein or diseases (or symptoms) having a therapeutic and/or preventive effect of the GDF15 may include obesity, diabetes (type 1 diabetes, type 2 diabetes), cardiovascular disease, myocardial hypertrophy, liver disease (e.g., nonalcoholic steatohepatitis (NASH), etc.), ischemic injury (ischemic brain damage, ischemic retina injury), peripheral nerve injury, age-related sensory and/or motor nerves loss, renal tubular and/or renal epileptic injury, but not limited thereto.

In other embodiment, the pharmaceutical composition or method comprising administering the same provided in the present description may have one or more effects selected from the group consisting of body weight loss, diet control (intake reduction), body fat reduction, and giving and/or enhancing glucose tolerance, and in this case, the pharmaceutical composition or method may be applied as a use for reducing body weight, reducing body fat and/or giving and/or enhancing glucose tolerance.

Therefore, in one embodiment, it may be a pharmaceutical composition or food composition (health functional food) for reducing a body weight, regulating a diet (reducing an amount of food), reducing body fat, or giving and/or enhancing glucose tolerance, as a composition comprising one or more selected from the group consisting of the fusion polypeptide, a nucleic acid molecule encoding the fusion polypeptide, a recombinant vector comprising the nucleic acid molecule, and a recombinant cell comprising the recombinant vector.

Other embodiment provides a method for reducing a body weight, regulating a diet (reducing an amount of food), reducing body fat, or giving and/or enhancing glucose tolerance, comprising administering one or more selected from the group consisting of the fusion polypeptide, a nucleic acid molecule encoding the fusion polypeptide, a recombinant vector comprising the nucleic acid molecule and a recombinant cell comprising the recombinant vector, into a patient in need of reducing a body weight, regulating a diet (reducing an amount of food), reducing body fat, or giving and/or enhancing glucose tolerance. The method may further comprise confirming the patient in need of reducing a body weight, regulating a diet (reducing an amount of food), reducing body fat, or giving and/or enhancing glucose tolerance, before the administering.

The pharmaceutical composition may comprise one or more of active ingredients selected from the group consisting of the fusion polypeptide, a fusion polypeptide dimer, a nucleic acid molecule, a recombinant vector and a recombinant cell comprising the fusion polypeptide in a pharmaceutically effective dose. The pharmaceutically effective dose means a contained amount or a dosage of the active ingredient capable of obtaining a desired effect. The contained amount or dosage of

the active ingredient may be variously prescribed by factors such as preparation method, administration method, patient's age, body weight, gender, morbid condition, food, administration time, administration interval, administration route, excretion rate and reaction sensitivity. For example, the single dosage of the active ingredient may be in a range of 0.001 to 1000 mg/kg, 0.01 to 100 mg/kg, 0.01 to 50 mg/kg, 0.01 to 20 mg/kg, or 0.01 to 1mg/kg, but not limited thereto.

Furthermore, the pharmaceutical composition may further comprise a pharmaceutically acceptable carrier, in addition the active ingredients. The carrier is one commonly used in preparation of a drug comprising a protein, nucleic acid or cell, and may be one or more selected from the group consisting of lactose, dextrose, sucrose, sorbitol, mannitol, starch, acacia gum, calcium phosphate, alginate, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate, mineral oil and the like, but not limited thereto. The pharmaceutical composition may also comprise one or more selected from the group consisting of diluents, excipients, lubricants, wetting agents, sweeteners, flavoring agents, emulsifiers, suspending agents, preservatives, and the like, commonly used in preparation of pharmaceutical compositions additionally.

The administration subject of the pharmaceutical composition may be a mammal including primates such as humans and monkeys, rodents such as mice and rats, and the like, or a cell, tissue, cell culture or tissue culture derived therefrom.

The pharmaceutical composition may be administered by oral administration or parenteral administration, or may be administered by contacting it to a cell, tissue or body fluid. Specifically, in case of parenteral administration, it may be administered by intravenous injection, subcutaneous injection, intramuscular injection, intraperitoneal injection, endothelial administration, local administration, intranasal administration, intrapulmonary administration and intrarectal administration, and the like. In case of oral administration, since proteins or peptides are digested, an oral composition should be formulated to coat an active agent or to protect it from degradation in the stomach.

In addition, the pharmaceutical composition may be formulated in a form of solution, suspension, syrup or emulsion in an oil or aqueous medium, or in a form of extract, powder, granule, tablet or capsule, or the like, and for formulation, it may further comprise a dispersing agent or stabilizing agent.

【ADVANTAGEOUS EFFECTS】

The GDF15 fused with a polypeptide region capable of O-glycosylation provided in the present description has a long duration when administered in vivo, so it is possible to increase the administration interval and thereby reduce the administration dose, and therefore, it has an advantageous effect in terms of administration convenience and/or economics and can be usefully applied to fields requiring GDF15 treatment.

【BRIEF DESCRIPTION OF THE DRAWINGS】

FIG. 1 schematically shows the amino acid sequence of GDF15.

FIG. 2a schematically shows the fusion polypeptide comprising His Tag according to one example.

FIG. 2b schematically shows the structure of the fusion polypeptide according to one example.

FIG. 3 schematically shows the structures of the various types of fusion polypeptides.

FIG. 4 shows the result of analyzing fusion polypeptides HT-ID1-GDF15, HT-ID2-GDF15 and HT-ID3-GDF15 synthesized in one example by SDS-PAGE.

FIG. 5 shows the result of analyzing fusion polypeptides HT-ID1-GDF15, HT-ID2-GDF15 and HT-ID3-GDF15 synthesized in one example by Q-TOF Mass Spectrometry.

FIG. 6 is a graph showing the body weight change when each of the fusion polypeptides HT-ID1-GDF15, HT-ID2-GDF15 and HT-ID3-GDF15 is administered to mice once.

FIG. 7 is a graph extracting and showing the result at Day 4 among the result of FIG. 6.

FIG. 8 is a graph showing the feed intake change of mice administered with the fusion polypeptides HT-ID1-GDF15, HT-ID2-GDF15 and HT-ID3-GDF15.

FIG. 9 is a graph showing the body weight change upon repeated administration of each of the fusion polypeptides HT-ID2-GDF15 and HT-ID3-GDF15 to mice.

FIG. 10a is a graph extracting and showing the result at Day 7 and Day 14 among the result of FIG. 9.

FIG. 10b is a graph extracting and showing the result at Day 21 and Day 28 among the result of FIG. 9.

FIG. 11 is a graph showing the cumulative feed intake up to Day 7, Day 14, Day 21 and Day 28 when the fusion polypeptides HT-ID2-GDF15 and HT-ID3-GDF15 are repeatedly administered to mice, respectively.

FIG. 12 is a graph showing the change in the blood fusion polypeptide concentration with time when the fusion polypeptides HT-ID1-GDF15, HT-ID2-GDF15 and HT-ID3-GDF15 are administered to SD Rat.

【MODE FOR INVENTION】

Hereinafter, the present invention will be described in more detail by the following examples. However, they are intended to illustrate the present invention only, but the scope of the present invention is not limited by these examples.

Example 1: Preparation of fusion polypeptide

1.1. Preparation of fusion polypeptide comprising GDF15

Fusion polypeptides IgD-GDF15 (ID1-GDF15), IgD-IgD-GDF15 (ID2-GDF15), IgD-IgD-IgD-GDF15 (ID3-GDF15) (See FIGs. 2a and 2b) in which a combination of IgD hinge (ESPKAQA**SS**VP**T**AQPQAEGSLAKA**TT**APA**TT**RNT; SEQ ID NO: 1; the underlined parts were sites capable of O-glycosylation) or several (1, 2 or 3) IgD hinges were fused with GDF15 (SEQ ID NO: 3, FIG. 1) were prepared. For convenience of

purification, a fusion polypeptide comprising His-tag (SEQ ID NO: 15) and TEV cleavage Site (SEQ ID NO: 16) was also prepared. The amino acid sequences of each part comprised in the fusion polypeptide were summarized in Table 2 below.

【Table 2】

	Amino acid sequence (N terminus→C terminus)	SEQ ID NO:
Signal Peptide (SP7.2)	MHRPEAMLLL LTLALLGGPT WA	14
Target polypeptide (GDF15)	ARNGDHCPLG PGRCCRLHTV RASLEDLGWA DWVLSPREVQ VTMCIGACPS QFRAANMHAQ IKTSLHRLKP DTVPAPCCVP ASYNPMVLIQ KTDTGVSLQT YDDLLAKDCH CI	3
Hinge region of immunoglobulin IgD (ID)	ESPKAQASSV PTAQPQAEGS LAKATTAPAT TRNT	1
His-Tag	HHHHHHHH	15
TEV Cleavage Site	ENLYFQG	16
GS Linker	GGGGSGGGGS GGGGSGGGGS	17

1.1.1. Preparation of recombinant expression vector

1.1.1.1. Mature GDF15

In order to obtain a gene encoding mature GDF15, referring to the amino acid sequence information of UniprotKB Q99968, a gene encoding mature GDF15 (SEQ ID NO: 5) was synthesized (Bioneer).

SEQ ID NO: 5 (339bp)

1 GCCCGGAACG GCGACCACTG CCCCCTGGGG CCCGGACGGT GCTGCCGGCT

51 GCACACCGTG CGGGCCTCCC TGGAGGACCT GGGCTGGGCC GACTGGGTGC

101 TGTCCCCAAG GGAGGTGCAA GTGACCATGT GCATCGGCGC CTGCCCATCT

151 CAGTTCCGGG CCGCCAACAT GCACGCTCAG ATCAAGACCA GCCTGCACCG

201 GCTGAAGCCC GACACCGTGC CCGCCCCCTG CTGCGTGCCC GCCTCCTACA

251 ACCCCATGGT GCTGATTCAG AAGACCGACA CCGGCGTGAG CCTGCAGACC
301 TACGACGACC TGCTGGCCAA GGACTGCCAC TGCATCTAA

1.1.1.2. IgD Hinge (ID)

In order to obtain a gene encoding Human IgD Hinge, referring to the amino acid sequence information of UniprotKB P01880, a gene encoding 3 Human IgD Hinges (hereinafter, referred to as 'ID3') (SEQ ID NO: 6) was synthesized in Bioneer.

SEQ ID NO: 6 (306bp)

1 GAGAGCCCTA AGGCTCAGGC CTCTAGCGTG CCAACAGCTC AGCCACAAGC
51 TGAAGGAAGC CTGGCCAAGG CTACAACCGC CCCTGCCACA ACACGGAATA
101 CA**GAGTCCCC CAAGGCCCAG GCTAGCAGCG TGCCTACCGC CCAGCCTCAG**
151 **GCCGAGGGCT CCCTGGCTAA GGCCACAACC GCTCCCGCTA CAACCAGGAA**
201 **CACCGAGTCT CCAAAGGCAC AGGCCTCCTC CGTGCCCACT GCACAACCCC**
251 **AAGCAGAGGG CAGCCTCGCC AAGGCAACCA CAGCCCCAGC CACCACCCGG**
301 **AACACA**

(1-102 polynucleotide (underlined), 103-204 polynucleotide (bold), and 205-306 polynucleotide (bold + underlined) encode IgD Hinge, respectively)

1.1.1.3. Preparation of expression vector

A variant of pcDNA3.1(+) (Invitrogen, Cat. No. V790-20), pDHDD-D1G1 (comprising the promoter of KR10-1868139B1) was cut with BamHI and NotI, and a gene designed to encode a fusion protein having the structure below (See FIG. 3) by combining the above genes (mature GDF15 encoding gene and ID3 encoding gene) was inserted thereto to prepare each recombinant vector.

pGDF15

'(N-terminus)-[BamHI restriction site (GGATCC)-signal peptide (SEQ ID NO: 14)-Mature GDF15 (SEQ ID NO: 3)- NotI restriction site (GCGGCCGC)]-(C-terminus)'

pHT-GDF15

'(N-terminus)-[BamHI restriction site-signal peptide (SEQ ID NO: 14)-His-Taq (SEQ ID NO: 15)-TEV Cleavage Site (SEQ ID NO: 16)-Mature GDF15 (SEQ ID NO: 3)- NotI restriction site]-(C-terminus)'

pID1-GDF15

'(N-terminus)-[BamHI restriction site-signal peptide (SEQ ID NO: 14)-IgD Hinge (SEQ ID NO: 1)-GS Linker (SEQ ID NO: 17)-Mature GDF15 (SEQ ID NO: 3)- NotI restriction site]-(C-terminus)'

pHT-ID1-GDF15

'(N-terminus)-[BamHI restriction site-signal peptide (SEQ ID NO: 14)-His-Taq (SEQ ID NO: 15)-TEV Cleavage Site (SEQ ID NO: 16)- IgD Hinge (SEQ ID NO: 1)-GS Linker (SEQ ID NO: 17)-GDF15 (SEQ ID NO: 3)- NotI restriction site]-(C-terminus)'

pID2-GDF15

'(N-terminus)-[BamHI restriction site-signal peptide (SEQ ID NO: 14)-IgD Hinge (SEQ ID NO: 1)- IgD Hinge (SEQ ID NO: 1)-GS Linker (SEQ ID NO: 17)-GDF15 (SEQ ID NO: 3)- NotI restriction peptide]-(C-terminus)'

pHT-ID2-GDF15

'(N-terminus)-[BamHI restriction site-signal peptide (SEQ ID NO: 14)- His-Taq (SEQ ID NO: 15)-TEV Cleavage Site (SEQ ID NO: 16)-IgD Hinge (SEQ ID NO: 1)-IgD Hinge (SEQ ID NO: 1)-GS Linker (SEQ ID NO: 17)-GDF15 (SEQ ID NO: 3)- NotI restriction site]-(C-terminus)'

pID3-GDF15

'(N-terminus)-[BamHI restriction site-signal peptide (SEQ ID NO: 14)-IgD Hinge (SEQ ID NO: 1)-IgD Hinge (SEQ ID NO: 1)-IgD Hinge (SEQ ID NO: 1)-GS Linker (SEQ ID NO: 17)-GDF15 (SEQ ID NO: 3)- NotI restriction site]-(C-terminus)'

pHT-ID3-GDF15

'(N-terminus)-[BamHI restriction site-signal peptide (SEQ ID NO: 14)- His-Taq (SEQ ID NO: 15)-TEV Cleavage Site (SEQ ID NO: 16)-IgD Hinge (SEQ ID NO: 1)-IgD Hinge (SEQ ID NO: 1)-IgD Hinge (SEQ ID NO: 1)-GS Linker (SEQ ID NO: 17)-GDF15 (SEQ ID NO: 3)- NotI restriction site]-(C-terminus)'

1.1.2. Expression of fusion polypeptide

The prepared recombinant expression vectors, pGDF15, pHT-GDF15, pID1-GDF15, pHT-ID1-GDF15, pID2-GDF15, pHT-ID2-GDF15, pID3-GDF15, and pHT-ID3-GDF15 were introduced into ExpiCHO-S™ cell (Thermo Fisher Scientific) and cultured (Fed-Batch Culture; Day 1 & Day 5 Feeding) in ExpiCHO Expression Medium (Thermo

Fisher Scientific; 400 mL) for 12 days, to express the fusion polypeptides GDF15, HT-GDF15, ID1-GDF15, HT-ID1-GDF15, ID2-GDF15, HT-ID2-GDF15, ID3-GDF15, and HT-ID3-GDF15.

1.1.3. Purification of fusion polypeptide

The fusion polypeptides HT-ID1-GDF15, HT-ID2-GDF15 and HT-ID3-GDF15 produced through the recombinant expression vector were purified and O-Glycan site Occupancy was analyzed using Sialic Acid content analysis and Q-TOF Mass Spectrometry.

Specifically, the fusion polypeptides were purified by continuously performing ultrafiltration/diafiltration, Immobilized Metal Affinity Chromatography (IMAC), and Anion Exchange Chromatography (AEX). At first, the culture solution of the fusion protein in which cells were removed was filtered with a 0.22 μm filter. For the filtered solution, concentration was performed using TFF System and then buffer exchange was conducted with a tromethamine buffer solution. A column in which HiTrap™ Chelating HP (GE Healthcare Life Sciences) resin was packed was equipped and an equilibrium buffer (20 mM Tris pH 8.0, 0.5 M NaCl, 5 mM Imidazole) was flowed to equilibrate the column. The process solution in which the ultrafiltration/diafiltration was completed previously was injected into the column, and then the equilibrium buffer was flowed again to wash the column. After completing the washing operation of the column, the elution buffer (20 mM Tris pH 8.0, 0.5 M NaCl, 0.5 M Imidazole) was flowed into the column to elute a target protein.

For the obtained eluted solution, concentration was performed using Amicon Ultra Filter Device (MWCO 10K, Merck) and a centrifuge, and then buffer exchange was conducted with a tromethamine buffer solution. The process solution prepared as such was injected into the equilibrated anion exchange column, and the equilibrium buffer (20 mM Tris pH 8.0) was flowed and the column was washed. After completing the washing operation of the column, the elution buffer (20 mM Tris pH 8.0, 0.5 M NaCl) was flowed into the column under a concentration gradient condition to elute the target protein. Among eluted fractions, fractions with high concentration and high purity of the fusion polypeptide were collected and kept frozen.

For an animal experiment, concentration and buffer exchange for samples were performed with Phosphate Buffered Saline (PBS, 10 mM Sodium Phosphate, 150 mM NaCl pH 7.4) using Amicon Ultra Filter Device (MWCO 10K, Merck) and a centrifuge.

The quantitative analysis of the fusion polypeptide was conducted by measuring the absorbance at 280 nm and 340 nm in UV Spectrophotometer (G113A, Agilent Technologies) by the following equation. As the extinction coefficient, a value theoretically calculated using the amino acid sequence was used.

$$\text{Protein concentration (mg/mL)} = \frac{\text{Absorbance } (A_{280\ nm} - A_{340\ nm})}{\text{*Extinction Coefficient}} \times \text{Dilution Factor}$$

*Extinction coefficient (0.1%): theoretical absorbance at 280 nm, assuming that the protein concentration is 0.1% (1g/L), and all cysteines in Primary Sequence are oxidized to form disulfide bonds. Calculated via ProtParam tool (https://web.expasy.org/protparam/).

【Table 3】

Extinction coefficient of fusion polypeptide

Sample name	Extinction coefficient (0.1%, 1 mg/mL)
HT-ID1-GDF15	0.833
HT-ID2-GDF15	0.706
HT-ID3-GDF15	0.612

For the purified fusion polypeptides HT-ID1-GDF15, HT-ID2-GDF15 and HT-ID3-GDF15, after Sialic Acid content analysis and reducing, O-Glycan site Occupancy was analyzed using Q-TOF Mass Spectrometry.

The result of analyzing the fusion polypeptides HT-ID1-GDF15, HT-ID2-GDF15 and HT-ID3-GDF15 by SDS-PAGE was shown in FIG. 4, and the result of analyzing them by Q-TOF Mass Spectrometry was shown in FIG. 5 and Table 5. In addition, the sialic acid content was also shown in Table 4.

【Table 4】

Average O-Glycan number and Sialic Acid content

Sample	Theoretical O-Glycan number (One Chain)	Average O-Glycan number	O-Glycan distribution (main)	Sialic Acid content (mol/mol)
HT-ID1-GDF15	7	5.4	2-7 (6)	14.4
HT-ID2-GDF15	14	9.7	3-15* (10)	16.3
HT-ID3-GDF15	21	13.5	5-21 (13)	18.9

* More than the theoretical O-glycan number is presumed that O-glycan is attached to the GS Linker (Spahr et al., 2014, mAbs, 6: 904)

Example 2. Pharmacological effect of fusion polypeptide (in vivo)

2.1. Single administration

2.1.1 Test process

The pharmacological effect of the fusion polypeptides produced and purified in Example 1 above was tested in mice (C57BL/6J, 6-week-old, male, 100 mice; Raonbio).

In the present example, DIO mouse model (Mouse, C57BL/6J –DIO, male, 100 mice, 14-week-old (obesity feed feeding for 8 weeks)) in which obesity was induced by feeding a high-fat diet into the C57BL/6J mice for 8 weeks was used. The DIO mouse model is an animal model widely used for evaluation of diabetes and insulin improvement efficacy, as it exhibits clinical characteristics of type 2 diabetes such as hyperlipidemia, insulin resistance, and hyperglycemia, and a lot of comparable basic data have been accumulated for the study of metabolic diseases such as obesity, diabetes and hyperlipidemia, and therefore, it was suitable for the pharmacological effect test of the present example, and thus this model was selected.

The mouse model fed with the obesity feed for 8 weeks was subjected to a quarantine and acclimatization period of 2 weeks, and during this period, general symptoms were observed once a day, and healthy animals were selected by confirming whether they were healthy and suitable for conducting the experiment. During the acclimatization period, the animal's tail was marked with a red oil pen at the time of acquisition (tail marking), and temporary individual identification cards (test

name, individual number, stocking time) were attached to the breeding box during the quarantine acclimatization period. At the time of group separation, individuals were marked on the tails of animals using a black oil pen and individual identification cards (test name, group information, individual number, gender, stocking time, administration period) were attached to each cage.

In order to minimize stress experienced by the experimental animals due to subcutaneous administration of the test substance (fusion polypeptide), 200 uL/head of sterile distilled physiological saline was administered subcutaneously to all animals using a 1 mL syringe from 3 days before the administration of the test substance. Pre-adaptation training for subcutaneous administration was conducted.

For healthy animals with no abnormalities found during the quarantine and acclimatization period, the body weight and feed intake were measured for all individuals after the acclimatization period.

The body weight and feed intake were measured, and group separation was performed so that the averages of the two measured values were similar between groups based on body weight. Test substance administration was started from the day after group separation. Remaining animals that were not selected were excluded from the test system after group separation was terminated.

The information of the high fat diet (obesity feed; HFD) fed to the C57BL/6J – DIO was as follows:

5.24 kcal/g, fat 60 % by weight, protein 20 % by weight, and carbohydrate-derived calories 20 % by weight; Research Diet Inc., U.S.A.; Product No. High fat diet (Fat 60 kcal%, D12492).

The feed was fed by a free feeding (feeding during the acclimatization and test period) method.

The drinking water method was that tap water was filtered with a filter oil-water sterilizer and then ultraviolet rays were irradiated and it was freely ingested using a polycarbonate drinking water bottle (250 mL).

The administration of the test substances HT-ID1-GDF15, HT-ID2-GDF15, and HT-ID3-GDF15 and the control substance Semaglutide (Bachem) was conducted from the next day after group separation, and the administration time was performed

at 9 AM every day. Subcutaneous administration was conducted for all the control substance and test substances. For the administration route of the control substance and test substances, subcutaneous administration was selected depending on the clinically scheduled administration route.

For all the control substance and test substances, the amount of the administration solution was set to 5 mL/kg and the administration liquid by individual was calculated on the basis of the recently measured body weight and it was administered by subcutaneous injection once on the start day of the test using a disposable syringe (1 mL). The test substances were administered only once. For comparison, the control group in which the control substance Semaglutide was administered was prepared, and the comparison group in which Semaglutide was administered was administered once a day, and all the administration was progressed from 9 AM.

The composition of the test groups and administration dose were summarized in Table 5 below:

【Table 5】

Fusion polypeptide administration group composition

High Fat Diet	Test substance	Administration route	Administration dose (nmol/kg)	Administration volume (mL/kg)	Animal number
O	Vehicle, qw	Subcutaneous	-	5	5
O	Semaglutide, qd	Subcutaneous	3	5	5
O	HT-ID1-GDF15	Subcutaneous	10	5	5
O	HT-ID2-GDF15	Subcutaneous	10	5	5
O	HT-ID3-GDF15	Subcutaneous	10	5	5

As for the observation, measurement and test schedule for the test groups, the administration start date was set to Day 0, and 7 days from the administration start date were set to one week of administration.

The test schedule was summarized in Table 6:

【Table 6】

Test schedule

Observation item	Acclimatization period (week)		Period (day)									
	1	2	0	1	2	3	4	5	6	7	8	9
High fat feed feeding	●	●	●	●	●	●	●	●	●	●	●	●
Adaptation to oral and subcutaneous administration		●										
Administration			●									
Body weight measurement		●	●	●	●	●	●	●	●	●	●	●
Feed intake measurement			●	●	●	●	●	●	●	●	●	●

General clinical symptoms were observed once a day for all animals, and the presence or absence of moribund and dead animals was checked twice a day, and these observations were conducted from the 1st day of administration to the end of administration. Only when there were abnormal symptoms during observation, it was recorded on the recording sheet.

The body weight of each mouse was measured on the day of the start of administration of the test substances (before administration), and thereafter, the body weight was measured every day (measured up to 9 days), and the amount of the administration solution of the test substances was determined on the basis of the most recently measured body weight.

In addition, after administering the test substances into mice, the daily feed intake was measured, and the feeding amount was measured using an electronic scale for each breeding box, and the remaining amount was measured to calculate the daily feed intake. In case of an individual that gnawed heavily on heed, it was excluded from the measurement.

All the experimental results obtained in the present example were expressed as mean ± standard error and tested using Prism5 (version 5.01). One-way analysis of variance (ANOVA) was performed on all data, and when significance was observed, Dunnett's test was performed to find out the test groups with a significant difference from the control group (significance level: two-sided 5% and 1%, 0.1%).

2.1.2. Body weight loss test result

The change in the body weight measured in Example 2.1.1 above was shown in FIG. 6 and FIG. 7, and Table 7 (Body Weight (Group, % of initial).

【Table 7】

Group	Day	0	1	2	3	4	5	6	7	8	9
DIO Vehicle Control (Daily Inj.)	Mean	100	100	100	99	100	100	100	100	101	101
	S.E.	0	0	1	0	0	0	0	1	0	1
DIO Semaglutide 3 nmol/kg (Daily Inj.)	Mean	100	94	91	90	87	87	85	86	83	85
	S.E.	0	0	1	2	2	3	3	3	3	3
HT-ID1-GDF15 10 nmol/kg (Single Inj.)	Mean	100	99	98	97	96	**95**	95	96	96	97
	S.E.	0	0	0	1	1	1	1	1	1	1
HT-ID2-GDF15 10 nmol/kg (Single Inj.)	Mean	100	98	97	96	**94**	94	94	93	93	94
	S.E.	0	0	1	1	1	2	2	2	2	2
HT-ID3-GDF15 10 nmol/kg (Single Inj.)	Mean	100	98	97	96	95	**94**	95	95	95	96
	S.E.	0	0	0	1	0	1	1	1	1	1

FIG. 6 and Table 7 shows the change in the body weight when the fusion polypeptides HT-ID1-GDF15, HT-ID2-GDF15 and HT-ID3-GDF15 were administered once, respectively, compared to the negative control group (vehicle administration group) and positive control group (Semaglutide daily administration group). In addition, FIG. 7 is a graph showing the result at Day 4 by extracting it among the result of FIG. 6.

As shown in the above result, it could be confirmed that there was little change in the body weight in case of the negative control group (vehicle administration group), while the body weight loss effect was continuously shown from Day 1 after administration in case of the positive control group (Semaglutide daily administration group). In addition, it could be confirmed that the body weight loss effect was shown immediately after single administration at Day 0 in case of the fusion polypeptide in which GDF15 was fused with IgD Hinge, and the body weight loss effect was not reduced and appeared continuously until 3-4 days.

2.1.3. Diet intake test result

The change in the feed intake measured in Example 2.1.1 above was shown in Table 8 and FIG. 8 (cumulative intake up to Day 6), respectively.

【Table 8】

Group	Day	0	1	2	3	4	5	6	7	8	9
DIO Vehicle Control (Daily Inj.)	Mean	3.3	2.7	3.0	3.0	3.1	3.5	3.0	3.5	3.3	3.4
	S.E.	0.1	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.2
DIO Semaglutide 3nmol/kg(Daily Inj.)	Mean	3.7	2.1	3.3	2.8	2.3	2.9	2.7	3.2	2.4	3.7
	S.E.	0.3	0.7	1.4	0.6	0.4	0.9	0.6	0.3	0.4	0.1
HT-ID1-GDF15 10nmol/kg(Single Inj.)	Mean	3.3	2.2	2.9	2.3	2.8	3.1	3.1	3.7	3.4	3.5
	S.E.	0.2	0.3	0.2	0.5	0.2	0.3	0.1	0.3	0.3	0.5
HT-ID2-GDF15 10nmol/kg(Single Inj.)	Mean	2.7	1.7	2.3	2.2	2.6	2.4	2.8	3.1	2.7	3.3
	S.E.	0.2	0.2	0.1	0.1	0.3	0.2	0.1	0.1	0.2	0.2
HT-ID3-GDF15 10nmol/kg(Single Inj.)	Mean	3.2	2.0	2.5	2.3	2.3	2.7	2.8	3.3	3.3	3.6
	S.E.	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.3	0.2	0.2

As shown in the above result, in case of the fusion polypeptide administration group in which GDF15 was fused with IgD Hinge, compared to the negative control group (vehicle administration group) administration group, the feed intake reduction effect was shown up to Day 6 at maximum depending on the fusion polypeptide, and this feed intake reduction effect of the fusion polypeptide can be said to be comparable to the case of administering Semaglutide, the positive control group, once a day throughout the test period.

2.2. Repeated administration

2.2.1 Test process

Except for the administration dose, animal number and administration cycle, most of the test processes were the same as in Example 2.1.1 above.

The test substances were administered twice a week (Days 0, 4, 7, 11, 14, 18, 21, 25) for a total of 8 times. For comparison, the control group in which the control substance Semaglutide was administered every day was prepared, and the comparison group in which Semaglutide was administered every day was administered once a day daily, and all the administration was progressed from 9 AM.

The composition of the test groups and administration dose, and the like were summarized in Table 9 below:

【Table 9】

Fusion polypeptide administration group composition

High Fat Diet	Test substance	Administration route	Administration cycle	Administration dose (nmol/kg)	Administration volume (mL/kg)	Animal number
O	Lean Vehicle Control	Subcutaneous	Once a week	-	5	4
O	DIO Vehicle Control	Subcutaneous	Once a week	-	5	8
O	Semaglutide	Subcutaneous	Once a day	3	5	8
O	HT-ID2-GDF15	Subcutan	Twice a	3	5	8

		eous	week			
O	HT-ID2-GDF15	Subcutaneous	Twice a week	10	5	8
O	HT-ID2-GDF15	Subcutaneous	Twice a week	30	5	8
O	HT-ID3-GDF15	Subcutaneous	Twice a week	3	5	8
0	HT-ID3-GDF15	Subcutaneous	Twice a week	10	5	8
0	HT-ID3-GDF15	Subcutaneous	Twice a week	30	5	8

2.2.2. Body weight loss test result

The change in the body weight measured in Example 2.2.1 above was shown in FIG. 9, FIG. 10a, FIG. 10b and Table 10 (Body Weight (Group, % of initial).

【Table 10】

Group	Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Lean Vehicle Control	Mean	100	100	100	100	100	100	101	99	100	102	102	101	101	102	101
	S E	0	0	0	0	1	1	1	1	1	1	1	0	1	1	1
DIO Vehicle Control	Mean	100.0	99.4	99.1	98.6	98.6	98.3	98.6	97.7	96.8	96.2	97.3	99.0	99.8	100.2	100.5
	S E	0.0	0.4	0.4	0.5	0.6	0.6	0.7	1.1	1.5	1.5	1.1	1.1	1.0	1.0	1.0
DIO Semaglutide 3 nmol/kg	Mean	100.0	93.9	92.5	90.4	88.4	86.2	84.1	82.6	81.9	80.7	80.3	80.3	79.1	78.8	78.7
	S E	0.0	0.3	0.6	0.8	1.1	1.5	1.5	1.6	1.6	1.6	2.2	1.9	2.0	2.0	2.0
HT-ID2-GDF15 3 nmol/kg	Mean	**100.0**	**98.0**	**96.5**	**95.0**	**93.9**	**91.8**	**90.8**	**90.3**	**89.2**	**89.2**	90.1	92.1	92.6	92.7	93.3
	S E	**0.0**	**0.3**	0.4	**0.4**	0.4	**0.4**	**0.4**	**0.5**	0.6	**0.6**	0.7	0.8	0.8	0.7	0.7
HT-ID2-GDF15 10 nmol/kg	Mean	**100.0**	**98.3**	**96.7**	**95.4**	**94.6**	**93.0**	**91.7**	**90.8**	**90.1**	**90.3**	90.6	92.1	91.2	92.0	92.2
	S E	**0.0**	**0.3**	0.5	**0.5**	0.4	**0.5**	**0.6**	**0.7**	0.7	0.7	0.7	0.8	0.8	0.9	0.9
HT-ID2-GDF15 30 nmol/kg	Mean	**100.0**	**98.4**	**97.3**	**96.2**	**95.2**	**94.0**	**93.1**	**92.3**	**91.5**	**91.2**	91.3	93.2	91.9	92.1	93.0
	S E	**0.0**	**0.3**	0.5	**0.6**	0.6	**0.8**	**0.9**	**1.1**	**1.2**	**1.2**	1.6	1.6	1.7	1.9	2.1
HT-ID3-GDF15 3 nmol/kg	Mean	**100.0**	**98.2**	**96.9**	**95.6**	**94.4**	**92.8**	**91.9**	**91.0**	**90.0**	**89.6**	**89.1**	91.5	91.5	92.3	92.5
	S E	**0.0**	**0.3**	0.3	**0.3**	0.5	**0.6**	**0.6**	**0.7**	0.8	**1.0**	**1.4**	1.2	1.6	1.4	1.4
HT-ID3-GDF15 10 nmol/kg	Mean	**100.0**	**98.5**	**97.3**	**95.7**	**94.6**	**92.8**	**91.5**	**90.7**	**89.8**	**89.3**	**88.7**	90.9	89.6	90.1	91.0
	S E	**0.0**	**0.3**	0.4	**0.5**	0.5	**0.6**	**0.6**	**0.7**	0.9	**0.9**	**1.0**	1.0	1.3	1.4	1.3
HT-ID3-GDF15 30 nmol/kg	Mean	**100.0**	**98.2**	**97.1**	**95.8**	**94.8**	**92.8**	**91.7**	**90.8**	**89.8**	**89.8**	**89.7**	92.0	90.0	90.4	90.9
	S E	**0.0**	**0.3**	0.3	**0.4**	0.5	**0.4**	**0.4**	**0.5**	0.6	**0.8**	**1.1**	1.3	1.3	1.6	1.5

Group	Day	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Lean Vehicle Control	Mean	101	102	101	102	102	102	101	101	102	102	101	101	102	101
	S E	2	1	1	1	2	1	1	1	1	1	1	1	1	1
DIO Vehicle Control	Mean	100 8	101 3	102 0	102 1	102 4	101 9	101 5	101 8	102 2	103 3	103 3	104 1	104 3	103 9
	S E	1 0	1 0	1 1	1 0	1 1	1 1	1 7	1 6	1 7	1 6	1 5	1 5	1 7	1 5
DIO Semaglutide 3 nmol/kg	Mean	78 1	78 3	77 2	76 7	77 1	76 1	76 8	75 9	75 6	76 0	76 3	76 5	76 2	75 7
	S E	1 8	2 0	2 3	2 2	2 1	2 3	2 3	2 2	2 3	2 4	2 8	2 7	2 4	2 7
HT-HD2-GDF15 3 nmol/kg	Mean	93 8	94 3	94 7	95 1	95 9	95 7	96 2	96 6	96 8	97 8	98 5	99 3	99 5	99 8
	S E.	0.9	0.7	0.8	0.9	0.8	0 8	1 0	1 1	1 2	1 2	1.2	1.3	1.3	1.4
HT-HD2-GDF15 10 nmol/kg	Mean	92 1	92.7	93.6	94.2	94.0	93 7	94 0	93 8	94.3	95.4	95.8	95.8	96.3	96 6
	S E	0 9	0.9	0 9	0 9	0 9	1 0	0 9	1 0	0 8	0 9	0.9	0 8	0 8	0.7
HT-HD2-GDF15 30 nmol/kg	Mean	92 1	92 8	93 4	93.6	92 9	92 5	93 2	92 4	92 6	93 1	93 9	93 6	93 2	93 4
	S E.	2 2	2 4	2 4	2 4	2 5	2 6	2 6	2 8	2 7	2 7	2 6	2 8	2 7	2 6
HT-HD3-GDF15 3 nmol/kg	Mean	92 6	93 3	94 0	94 4	95 0	95 2	95 8	96 4	97 1	97 4	97 6	98 5	98 0	98 6
	S E	1 3	1 3	1 2	1 2	1 2	1 2	1 3	1 3	1 5	1 4	1 3	1 5	1 7	1 7
HT-HD3-GDF15 10 nmol/kg	Mean	90 0	91 2	91 8	92 3	91 9	91 2	92 1	92 1	92 5	93 2	93 7	94 2	94 4	94 1
	S E	1 5	1 5	1 5	1 5	1 7	1 7	1 7	2 1	2 0	2 0	1 9	2 3	2 3	2 2
HT-HD3-GDF15 30 nmol/kg	Mean	89 3	89 6	90 4	91 0	90 0	89 6	90 4	90 0	90 0	91 0	91 5	91 5	91 2	91 4
	S E	1 4	1 6	1 5	1 7	1 5	1 5	1 4	1 3	1 4	1 5	1 5	1 5	1 7	1 6

FIG. 9 and Table 10 shows the change in the body weight in case of repeated administration of the fusion proteins HT-ID2-GDF15 and HT-ID3-GDF15, respectively (Table 9), compared to the negative control group (vehicle administration group) and positive control group (Semaglutide daily administration group). In addition, FIG. 10a is a graph showing the results at Day 7 and Day 14 by extracting them from the result of FIG. 9 above and FIG. 10b is a graph showing the results at Day 21 and Day 28 by extracting them from the result of FIG. 9.

As shown in the above result, it could be confirmed that there was little change in the body weight in case of the negative control group (vehicle administration group), while the body weight loss effect was continuously shown from Day 1 after administration in case of the positive control group (Semaglutide daily administration group). In addition, it could be confirmed that the body weight loss effect was shown immediately after single administration at Day 0 in case of the fusion polypeptide in which GDF15 was fused with IgD Hinge, and the body weight loss effect was continuously shown without being reduced, and the body weight loss effect was concentration-dependent.

2.2.3. Diet intake test result

The change in the feed intake measured in Example 2.2.1 above was shown in Table 11 and FIG. 11 (cumulative intake up to Day 7, Day 14, Day 21 and Day 28), respectively.

【Table 11】

Group	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Lean Vehicle Control	Mean	4.4	3.9	4.1	4.5	4.4	3.9	4.3	4.1	4.1	4.6	4.5	4.4	4.2	4.3
	SE	0.2	0.1	0.1	0.1	0.3	0.1	0.2	0.1	0.3	0.2	0.1	0.2	0.2	0.2
DIO Vehicle Control	Mean	2.9	2.6	2.7	3.6	3.3	2.5	3.0	2.7	1.7	2.2	3.0	4.0	3.1	3.4
	SE	0.2	0.5	0.3	0.8	0.4	0.2	0.1	0.1	0.4	0.2	0.1	0.2	0.1	0.2
DIO Semaglutide 3 nmol/kg	Mean	2.9	0.7	1.3	1.4	1.7	1.5	1.6	1.9	2.0	2.3	2.2	3.2	2.2	2.5
	SE	0.1	0.1	0.2	0.1	0.1	0.2	0.2	0.4	0.5	0.3	0.3	0.3	0.2	0.2
HT-ID2-GDF15 3 nmol/kg	Mean	3.0	1.5	2.1	2.1	2.4	1.7	2.3	2.3	2.0	2.8	3.3	4.2	3.7	3.4
	SE	0.1	0.1	0.1	0.1	0.4	0.1	0.2	0.2	0.1	0.2	0.1	0.2	0.7	0.1
HT-ID2-GDF15 10 nmol/kg	Mean	2.6	1.5	1.8	2.0	2.0	1.8	2.1	2.1	1.9	2.5	3.1	3.7	2.4	3.0
	SE	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
HT-ID2-GDF15 30 nmol/kg	Mean	3.0	1.7	2.3	2.3	2.3	2.1	2.4	2.3	2.2	2.6	3.0	3.9	2.3	3.2
	SE	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.2	0.2
HT-ID3-GDF15 3 nmol/kg	Mean	2.9	1.5	1.9	1.9	2.0	1.6	2.1	2.1	1.9	2.4	2.5	3.9	2.9	3.2
	SE	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.1	0.1
HT-ID3-GDF15 10 nmol/kg	Mean	2.9	1.7	2.3	2.2	2.1	1.9	2.2	2.1	2.1	2.4	3.0	4.1	2.5	3.3
	SE	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2
HT-ID3-GDF15 30 nmol/kg	Mean	2.5	1.6	2.1	2.2	2.3	1.8	2.2	2.1	2.0	2.5	2.9	4.0	2.1	3.0
	SE	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.2

Group	Day	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Lean Vehicle Control	Mean	4 3	4 1	4 2	4 1	4 9	3 8	4 8	4 2	3 9	4 2	4 2	3 8	3 7	4 3
	S E	0 2	0 2	0 1	0.3	0.2	0 2	0.1	0.3	0 2	0 2	0 2	0.2	0.2	0 3
DIO Vehicle Control	Mean	3 2	3 2	3 1	3.1	3.1	2 7	3.2	2.7	2 8	3 1	3 4	3.5	2 9	3 2
	S E	0 1	0 1	0 1	0.1	0 1	0 1	0.1	0.4	0 1	0 2	0 2	0 5	0 1	0 1
DIO Semaglutide 3 nmol/kg	Mean	2 5	2 2	2 4	2 2	2 6	2 6	2 5	2 9	2 0	2 4	2 9	2 4	2 4	2 7
	S E	0 3	0 2	0 1	0 2	0 1	0 3	0 1	0 4	0 2	0 1	0 3	0 2	0 2	0 3
HT-ID2-GDF15 3 nmol/kg	Mean	3 2	3 4	3 4	3 4	3 7	3 3	3 2	3 4	3 1	3 3	3 4	3 3	3 1	3 4
	S E	0 2	0 5	0 4	0 2	0 4	0 5	0 1	0 3	0 1	0 2	0 1	0 2	0 1	0 1
HT-ID2-GDF15 10 nmol/kg	Mean	3 0	2 4	3 0	3 2	3 3	2 4	2 9	3 1	2 3	3 1	3 3	3 1	2 5	3 0
	S E	0 1	0 1	0 1	0 1	0 1	0 1	0 1	0 1	0 1	0 1	0 1	0 1	0 1	0 1
HT-ID2-GDF15 30 nmol/kg	Mean	3 3	2 4	3 1	3 2	3 1	2 1	3 2	3 1	2 1	3 0	3 1	3 3	2 1	2 9
	S E	0 3	0 2	0 2	0 2	0 1	0 1	0.2	0.1	0 2	0 2	0 1	0.1	0 2	0 2
HT-ID3-GDF15 3 nmol/kg	Mean	3 1	2 7	3 0	3.1	3 3	2 7	3.0	3.1	2 9	3 1	2.9	3 0	2 8	2 7
	S E	0 1	0 1	0 1	0 1	0.1	0 1	0 1	0 1	0 1	0 1	0 1	0 1	0.1	0 1
HT-ID3-GDF15 10 nmol/kg	Mean	3 2	2 2	3 3	3.3	3.4	2 4	3 1	3.2	2 7	3 1	3 3	3 3	2 7	3 2
	S E	0 2	0 3	0 2	0 1	0 1	0 1	0 1	0 1	0 2	0 1	0 1	0 1	0 2	0 2
HT-ID3-GDF15 30 nmol/kg	Mean	3 1	1 9	3 0	3 2	3 3	1 9	3 1	3 4	2 3	3 3	3 2	3 2	2 2	3 2
	S E	0 1	0 0	0 1	0 1	0 1	0 1	0 1	0 2	0 2	0 2	0 1	0 1	0 1	0 2

As shown in the above result, the administration group of the fusion polypeptide in which GDF15 was fused with IgD Hinge showed the feed intake reduction effect throughout the test period depending on the fusion polypeptide, compared to the negative control group (vehicle administration group) administration group, and showed a concentration-dependent tendency.

Example 3. Pharmacokinetic test of fusion polypeptide

3.1. Test group and control group serum preparation

For evaluation of pharmacokinetic characteristics when each fusion polypeptide was subcutaneously administered to rats, the fusion polypeptides HT-ID1-GDF15, HT-ID2-GDF15 and HT-ID3-GDF15 were subcutaneously administered into SD rats (Koatech, male, 7-week-old, about 250g; n=3 each; test group) in an amount of 2 mg/kg, respectively, and about 200 μl of blood was collected through the caudal vein at a predetermined time. The blood collection time was performed before administration, and 1, 2, 4, 8, 24, 48, 72, 96, 168, 240 and 336 hours after administration. As the control group for comparison of pharmacokinetic characteristics, GDF15 (R&D Systems) was subcutaneously administered in an amount of 2 mg/kg by the same method to prepare a GDF15 administration group.

After administering into SD Rats as above, blood collected by time-point was centrifuged to obtain serum, and ELISA was performed using Human GDF15 Immunoassay (SGD150, R&D Systems), and the concentration in the serum was measured depending on the time of each polypeptide. Using this data, values of parameters including AUC (area under the curve) were obtained using a software for PK analysis (WinNonlin (Certara L.P.), etc.).

3.2 Pharmacokinetic test result

The obtained pharmacokinetic parameters of the fusion polypeptide were shown in Table 12, and the change in the concentration of the fusion polypeptide with time was shown in FIG. 12.

【Table 12】

PK parameter	Group 1	Group 2	Group 3	Group 4
	rhGDF15	HT-ID1-GDF15	HT-ID2-GDF15	HT-ID3-GDF15
C_{max} (ug/mL)	0.443	2.09	0.945	1.11
T_{max} (hr)	1	8	24	24
AUC_{last}(ug*hr/mL)	4.86	81.2	53.5	76.2
AUC_{inf}(ug*hr/mL)	4.88	81.3	53.5	76.4
$t_{1/2}$ (hr)	19.0	24.7	22.3	26.2
AUC_{extp} (%)	0.463	0.0700	0.100	0.287

(C_{max}: maximum concentration in blood, T_{max}: reaching time to maximum concentration in blood, AUC_{inf}: area under a blood concentration-time curve by extrapolating from the last measurable blood collecting time to infinity, AUC_{last}: area under a blood concentration-time curve up to the last measurable blood collecting time, $T_{1/2}$: loss half-life, AUC_{Extp}(%): [(AUC_{inf}–AUC_{last})/AUC_{inf}]*100)

As shown in the above result, it could be confirmed that the half-life was increased in case of the fusion polypeptide fused with IgD Hinge, compared to GDF15 (half-life: 19 hours), and in particular, in case of AUC_{last}, it was increased 16.7 times at maximum compared to GDF15.

From the above description, those skilled in the art to which the present invention pertains will understand that the present invention may be embodied in other specific forms without changing the technical spirit or essential characteristics thereof. In this regard, it should be understood that the embodiments described above are illustrative and not restrictive in all respects. The scope of the present invention should be construed that all changes or modifications derived from the meaning and scope of the claims to be described later and their equivalent concepts are included in the scope of the present invention, rather than the above detailed description.

【CLAIMS】

【Claim 1】

A fusion polypeptide, comprising,

GDF15 (Growth differentiation factor 15), and

a total of 1 to 10 polypeptide regions capable of O-glycosylation, which is bound to the N-terminus of the GDF15,

wherein each of the 1 to 10 polypeptide regions capable of O-glycosylation is a polypeptide comprising 3 to 10 amino acid residues capable of O-glycosylation.

【Claim 2】

The fusion polypeptide according to claim 1, represented by the following formula:

N'-(Z)n-Y -C'

in the formula,

N' is the N-terminus of the fusion polypeptide, and C' is the C-terminus of the fusion polypeptide, and

Y is the GDF15, and

Z is a polypeptide region capable of O-glycosylation, and

n is the number of the polypeptide regions capable of O-glycosylation bound to the N-terminus of GDF15 and is an integer of 0 to 10.

【Claim 3】

The fusion polypeptide according to claim 1, wherein the 1 to 10 polypeptide regions capable of O-glycosylation are polypeptide regions comprising 1 to 10 immunoglobulin hinge regions or 10 or more continuous amino acids comprising 3 to 10 amino acid residues capable of O-glycosylation selected from proteins of SEQ ID NOs: 23 to 113.

【Claim 4】

The fusion polypeptide according to claim 3, wherein the 1 to 10 immunoglobulin hinge regions are immunoglobulin D (IgD) hinge regions.

【Claim 5】

The fusion polypeptide according to claim 4, wherein the 1 to 10 immunoglobulin hinge regions are each independently selected from the group consisting of the following:

(1) a polypeptide comprising the amino acid sequence of SEQ ID NO: 1,

(2) a polypeptide comprising 5 or more continuous amino acids comprising 3 to 7 O-glycosylation residues in the amino acid sequence of SEQ ID NO: 1, and

(3) a polypeptide comprising 34 or more continuous amino acids comprising the polypeptide of (1) or (2) in the IgD.

【Claim 6】

The fusion polypeptide according to claim 4, wherein the 1 to 10 immunoglobulin hinge regions are each independently selected from the group consisting of the following:

(1) a polypeptide comprising the amino acid sequence of SEQ ID NO: 1,

(2) a polypeptide comprising 5 or more continuous amino acids comprising SEQ ID NO: 9 or 7 or more continuous amino acids comprising SEQ ID NO: 10 in the amino acid sequence of SEQ ID NO: 1, and

(3) a polypeptide comprising 34 or more continuous amino acids comprising the polypeptide of (1) or (2) in the IgD.

【Claim 7】

The fusion polypeptide according to any one claim of claim 1 to claim 6, wherein the area under the blood concentration-time curve (AUC_{last}) up to the last blood sampling point measurable upon in vivo administration of the GDF15 bound to the polypeptide region capable of O-glycosylation in the fusion polypeptide is, increased at least 2-fold, compared to GDF15 not bound to the polypeptide region capable of O-glycosylation.

【Claim 8】

A nucleic acid molecule encoding the fusion polypeptide of any one claim of claim 1 to claim 6.

【Claim 9】

A recombinant vector comprising the nucleic acid molecule of claim 8.

【Claim 10】

A recombinant cell comprising the recombinant vector of claim 9.

【Claim 11】

A method of preparation of the fusion polypeptide of any one claim of claim 1 to claim 6, wherein the fusion polypeptide comprises GDF15 and a polypeptide region capable of O-glycosylation, and the method comprises culturing the recombinant cell of claim 10.

【Claim 12】

A method for enhancing in vivo stability of GDF15,

comprising linking a total of 1 to 10 polypeptide regions capable of O-glycosylation to the N-terminus of the GDF15,

wherein the 1 to 10 polypeptide regions capable of O-glycosylation are each a polypeptide comprising 3 to 10 amino acid residues capable of O-glycosylation.

【Claim 13】

The method for enhancing in vivo stability of GDF15 according to claim 12, wherein the 1 to 10 polypeptide regions capable of O-glycosylation are polypeptide regions comprising 1 to 10 immunoglobulin hinge regions or 10 or more continuous amino acids comprising 3 to 10 O-glycosylation residues selected from proteins of SEQ ID NOs: 23 to 113.

【Claim 14】

The method for enhancing in vivo stability of GDF15 according to claim 13, wherein the 1 to 10 immunoglobulin hinge regions are immunoglobulin D (IgD) hinge regions.

【Claim 15】

The method for enhancing in vivo stability of GDF15 according to claim 14,

wherein the 1 to 10 immunoglobulin hinge regions are each independently selected from the group consisting of the following:

(1) a polypeptide comprising the amino acid sequence of SEQ ID NO: 1,

(2) a polypeptide comprising 5 or more continuous amino acids comprising 3 to 7 O-glycosylation residues in the amino acid sequence of SEQ ID NO: 1, and

(3) a polypeptide comprising 34 or more continuous amino acids comprising the polypeptide of (1) or (2) in the IgD.

【Claim 16】

The method for enhancing in vivo stability of GDF15 according to claim 14, wherein the 1 to 10 immunoglobulin hinge regions are each independently selected from the group consisting of the following:

(1) a polypeptide comprising the amino acid sequence of SEQ ID NO: 1,

(2) a polypeptide comprising 5 or more continuous amino acids comprising SEQ ID NO: 9 or 7 or more continuous amino acids comprising SEQ ID NO: 10 in the amino acid sequence of SEQ ID NO: 1, and

(3) a polypeptide comprising 34 or more continuous amino acids comprising the polypeptide of (1) or (2) in the IgD.

【Claim 17】

A fusion polypeptide dimer, comprising 2 of the fusion polypeptides of any one claim of claim 1 to claim 6.

【Claim 18】

The fusion polypeptide dimer according to claim 17, wherein the GDF15 of each fusion polypeptide binds to each other to form a dimer.

【Claim 19】

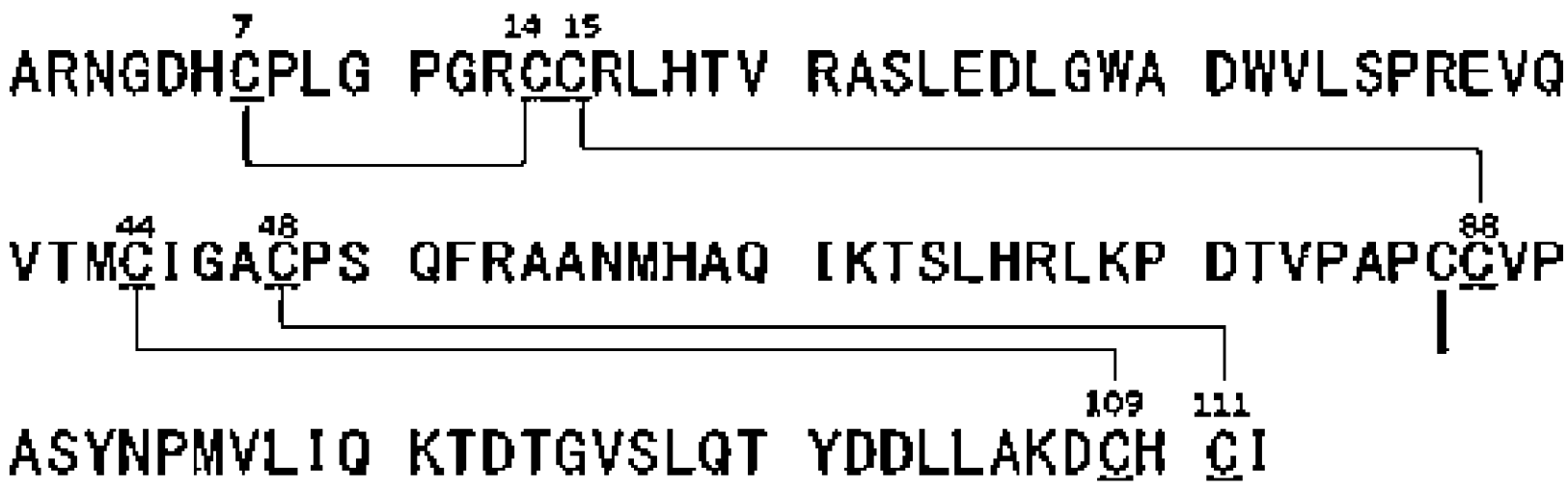
The fusion polypeptide dimer according to claim 17, wherein the dimer is a homodimer.

【Claim 20】

A pharmaceutical composition for preventing or treating diseases related to GDF15 deficiency or dysfunction, comprising the fusion polypeptide of any one claim of claim 1 to claim 6, or a fusion polypeptide dimer in which two of the fusion polypeptides are linked to each other at the GDF15.

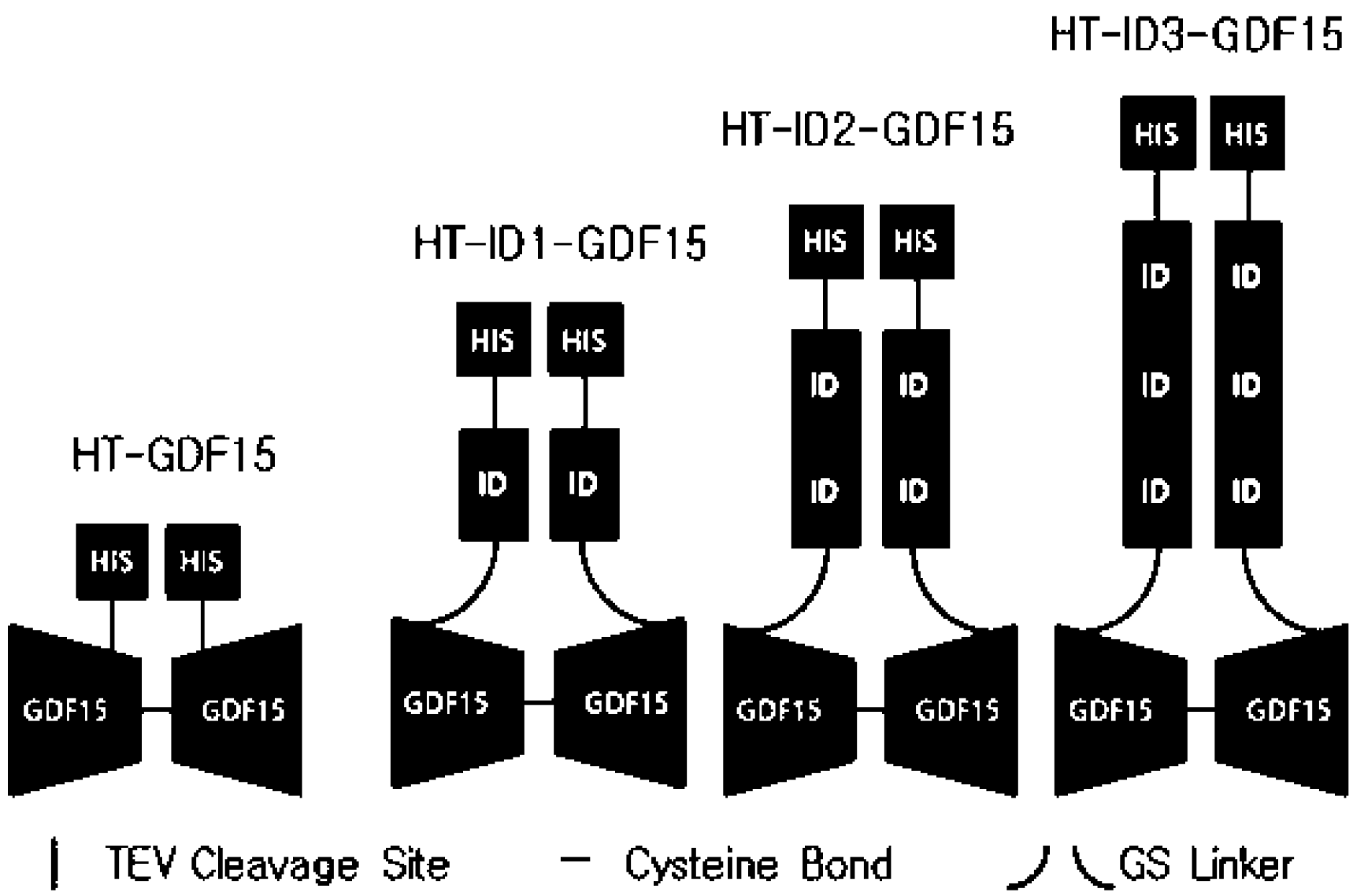
【DRAWINGS】

【FIG. 1】



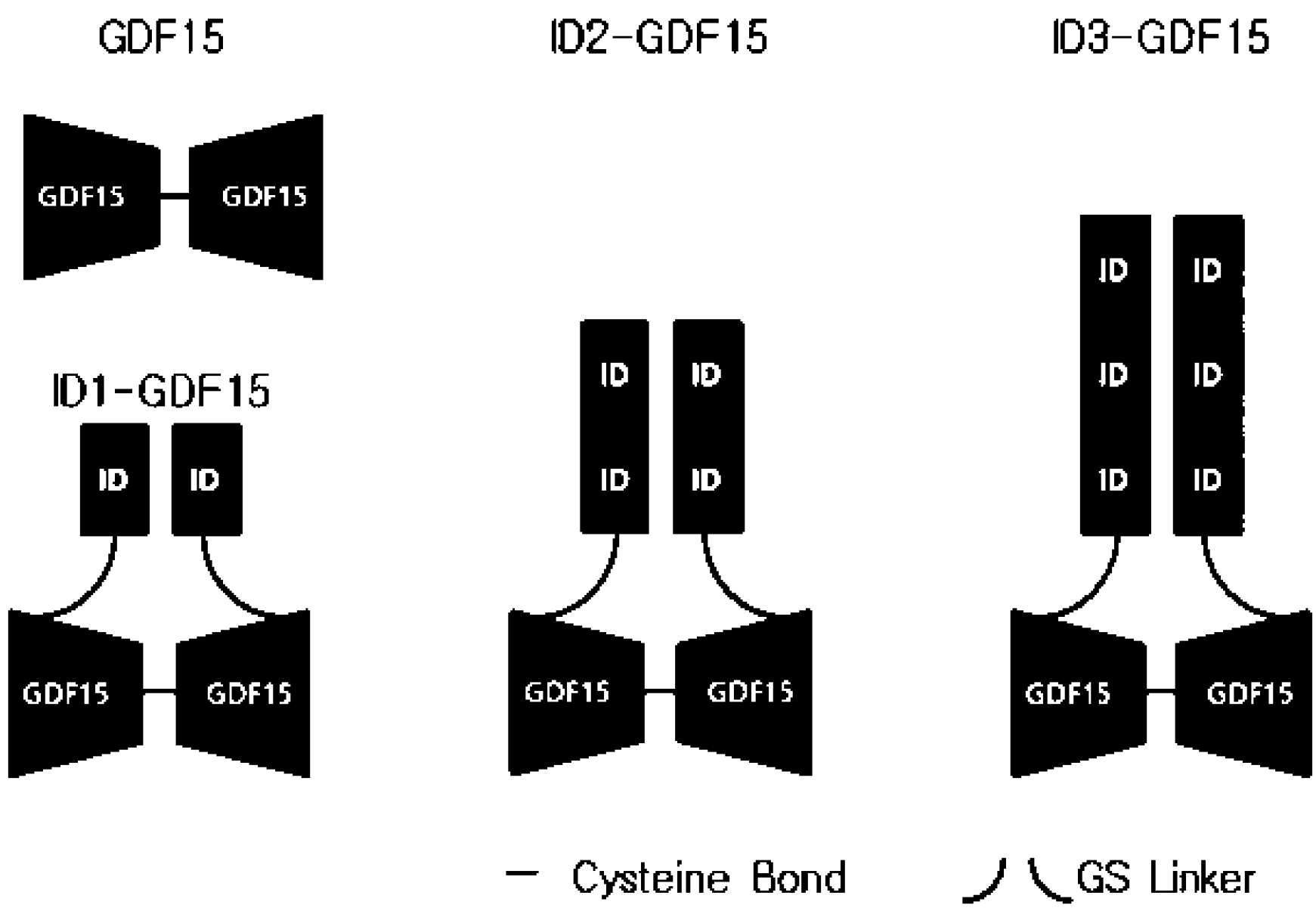
(SEQ ID NO: 3)

【FIG. 2a】



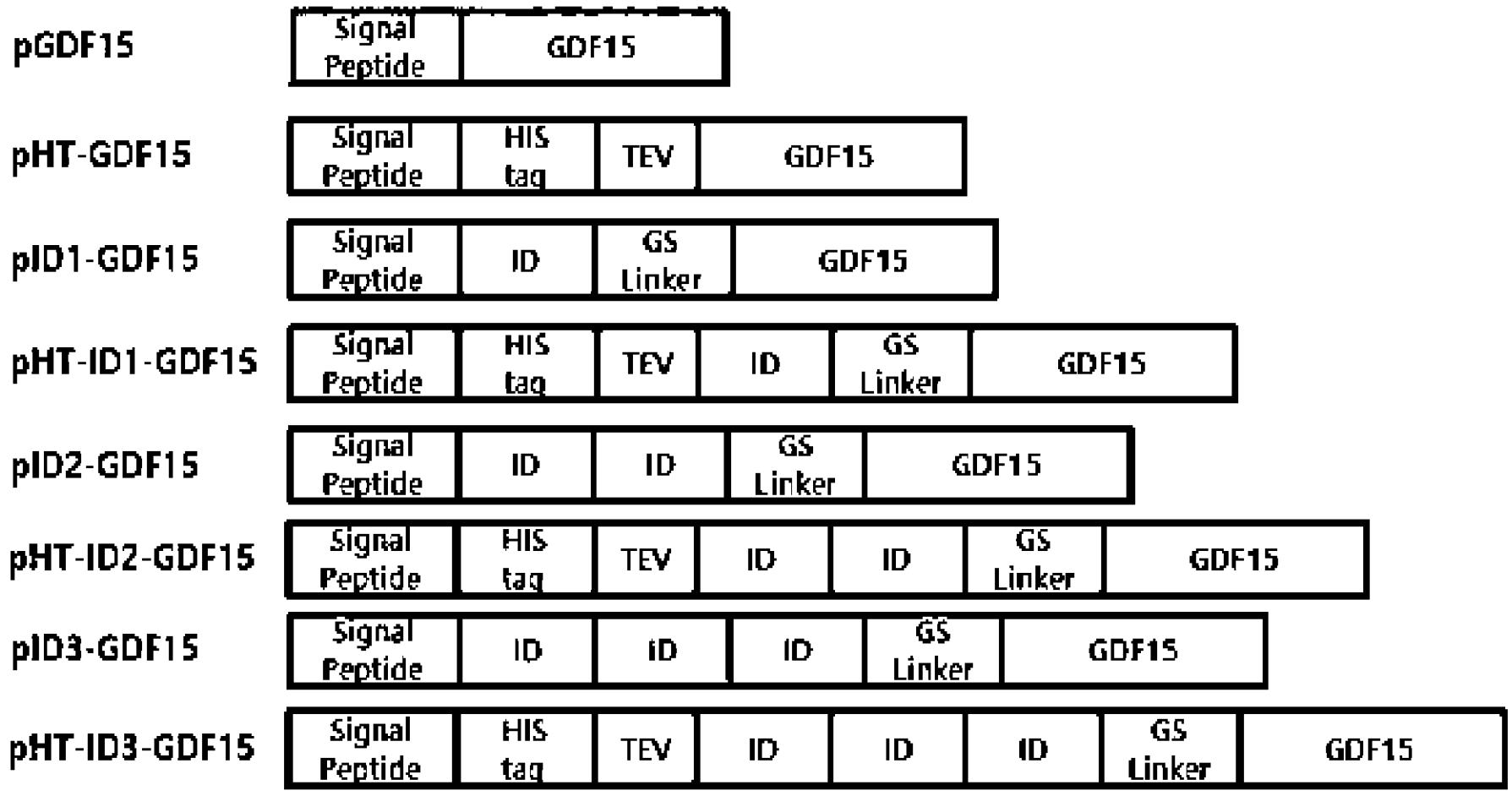
ID: ESPKAQASSVPTAQPQAEGSLAKATTAPATT RNT (SEQ ID NO: 1)
HIS: HHHHHH (SEQ ID NO: 15)
TEV Cleavage Site: ENLYFQG (SEQ ID NO: 16)
GS Linker: GGGGSGGGGS GGGGSGGGGS (SEQ ID NO: 17)
GDF15: SEQ ID NO: 3

【FIG. 2b】

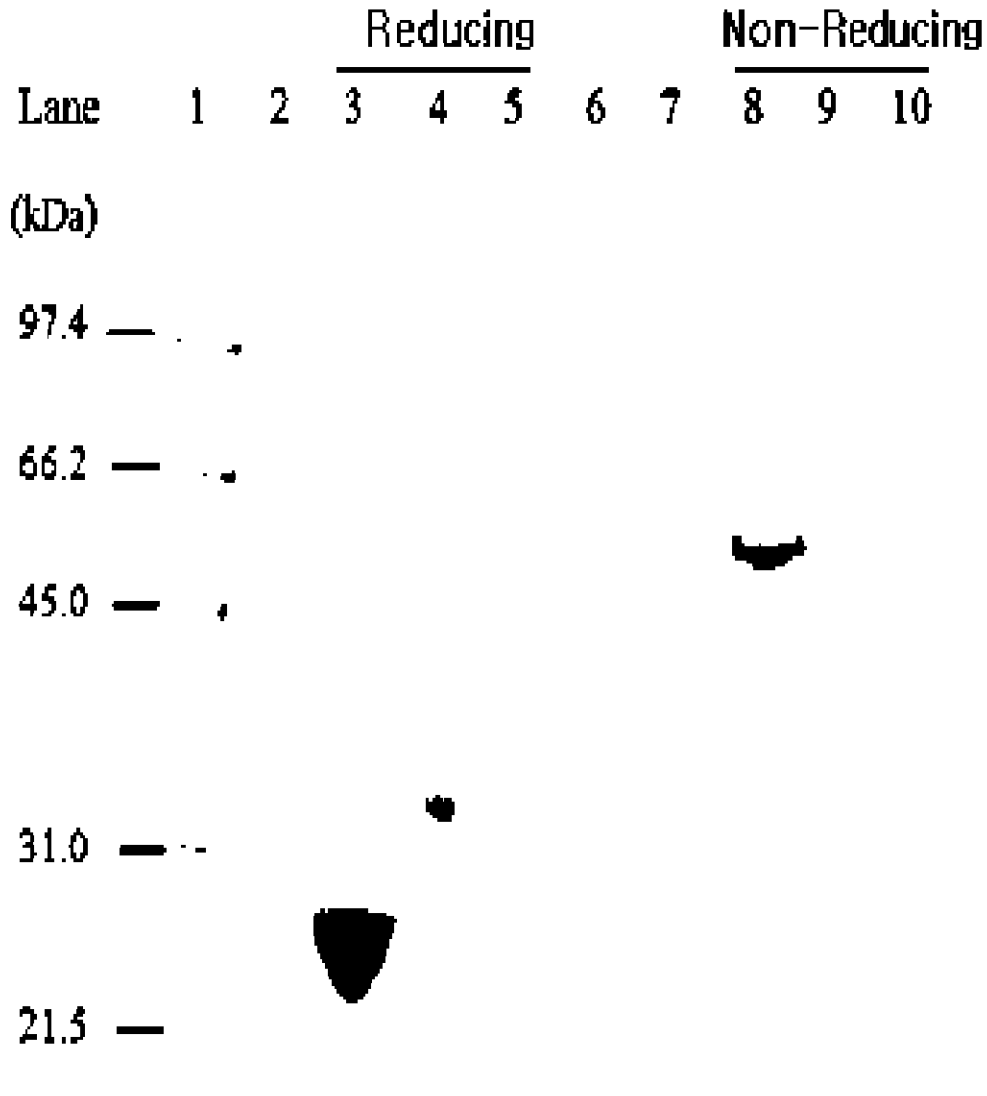


ID: ESPKAQASSVPTAQPQAEGSLAKATTAPATT RNT (SEQ ID NO: 1)
GS Linker: GGGGSGGGGS GGGGSGGGGS (SEQ ID NO: 17)
GDF15: SEQ ID NO: 3

【FIG. 3】

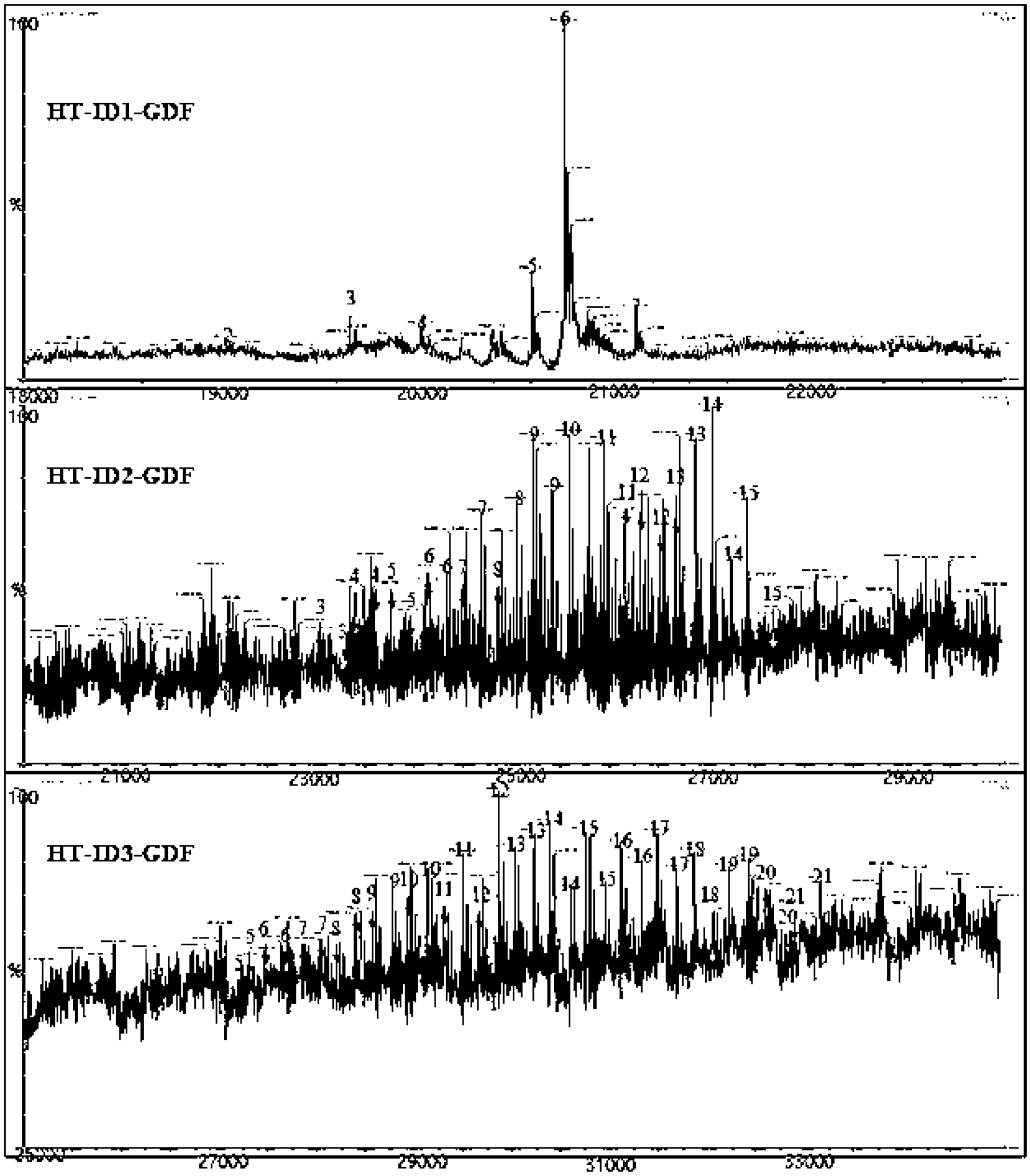

pGDF15
Signal Peptide
GDF15
pHT-GDF15
Signal Peptide
HIS tag
TEV
GDF15
pID1-GDF15
Signal Peptide
ID
GS Linker
GDF15
pHT-ID1-GDF15
Signal Peptide
HIS tag
TEV
ID
GS Linker
GDF15
pID2-GDF15
Signal Peptide
ID
ID
GS Linker
GDF15
pHT-ID2-GDF15
Signal Peptide
HIS tag
TEV
ID
ID
GS Linker
GDF15
pID3-GDF15
Signal Peptide
ID
ID
ID
GS Linker
GDF15
pHT-ID3-GDF15
Signal Peptide
HIS tag
TEV
ID
ID
ID
GS Linker
GDF15

【FIG. 4】

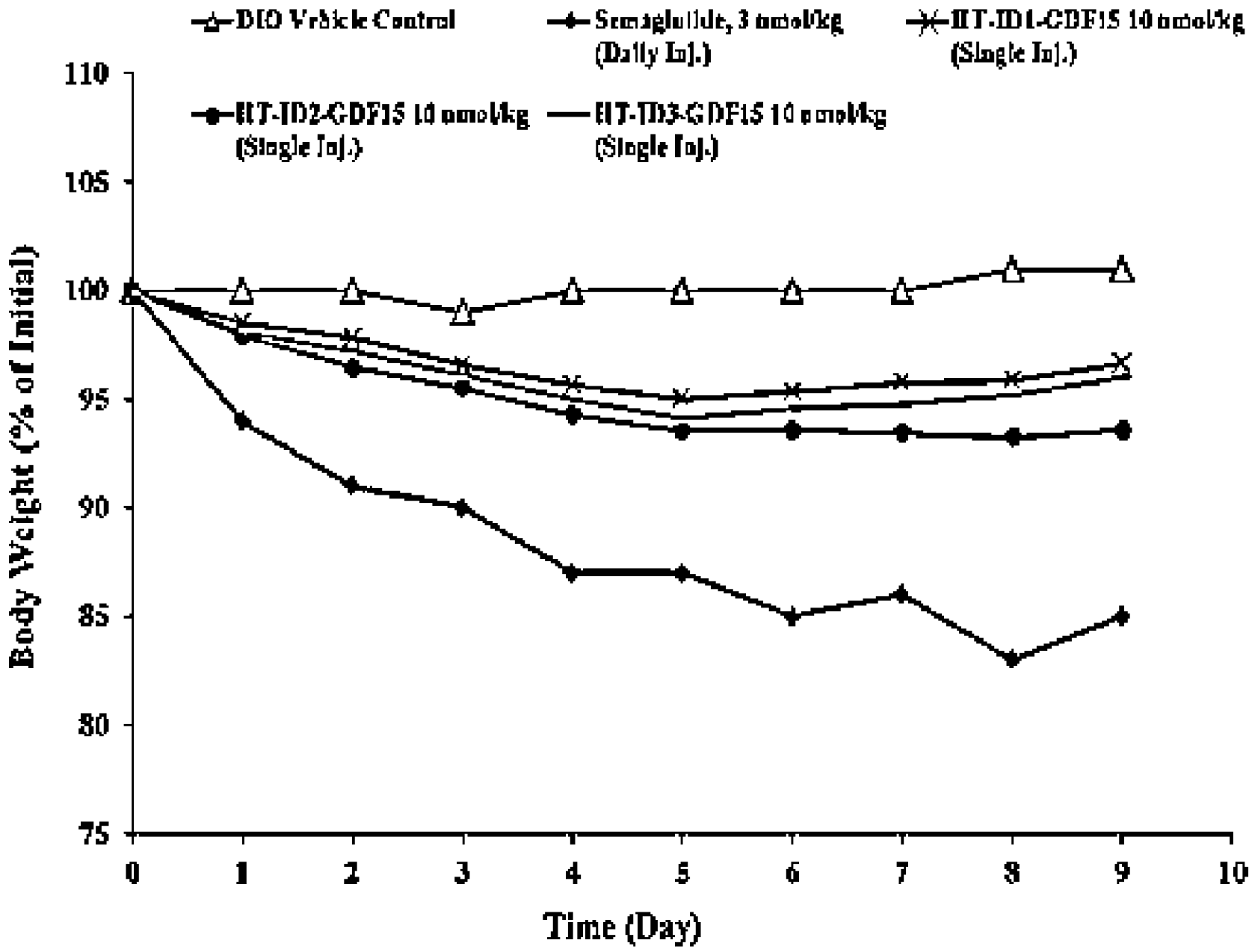


Lane	Sample
1	Low Range MW Standard
2, 6, 7	Empty
3	HT-ID1-GDF15 (R)
4	HT-ID2-GDF15 (R)
5	HT-ID3-GDF15 (R)
8	HT-ID1-GDF15 (NR)
9	HT-ID2-GDF15 NR)
10	HT-ID3-GDF15 NR)

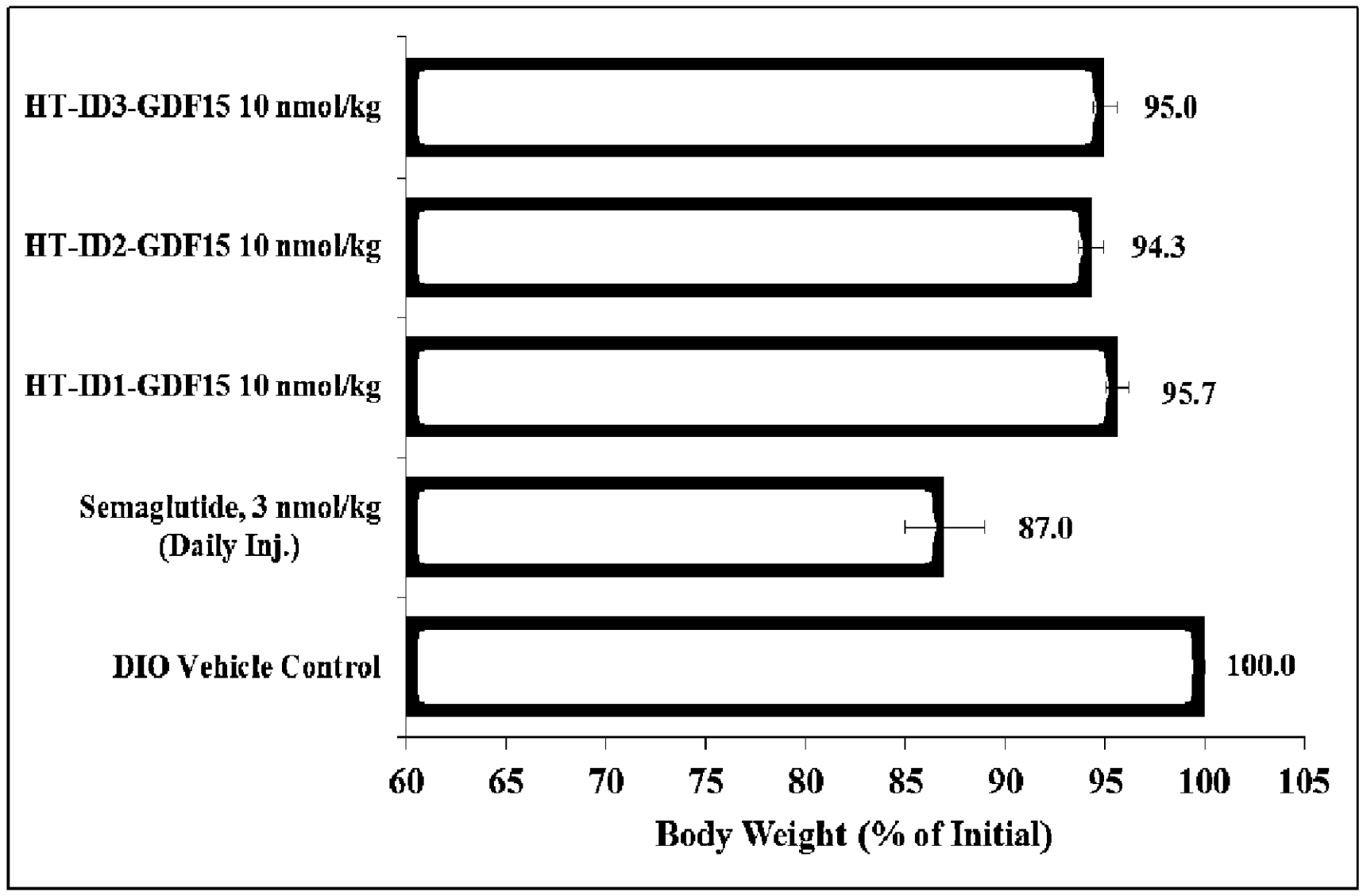
【FIG. 5】



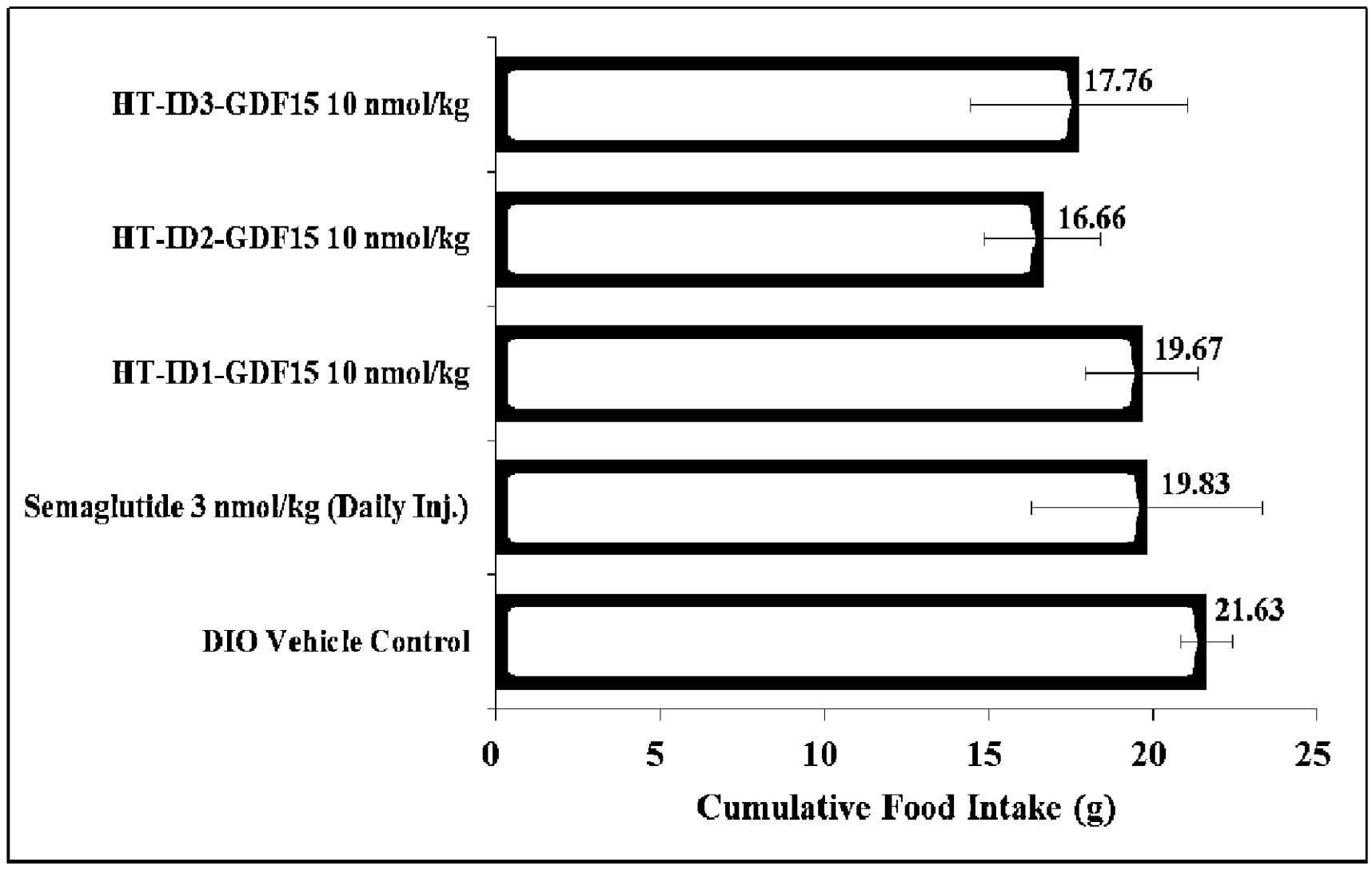
【FIG. 6】


DIO Vehicle Control
Semaglutide, 3 nmol/kg (Daily Inj.)
HT-ID1-GDF15 10 nmol/kg (Single Inj.)
HT-ID2-GDF15 10 nmol/kg (Single Inj.)
HT-ID3-GDF15 10 nmol/kg (Single Inj.)
Body Weight (% of Initial)
110
105
100
95
90
85
80
75
0
1
2
3
4
5
6
7
8
9
10
Time (Day)

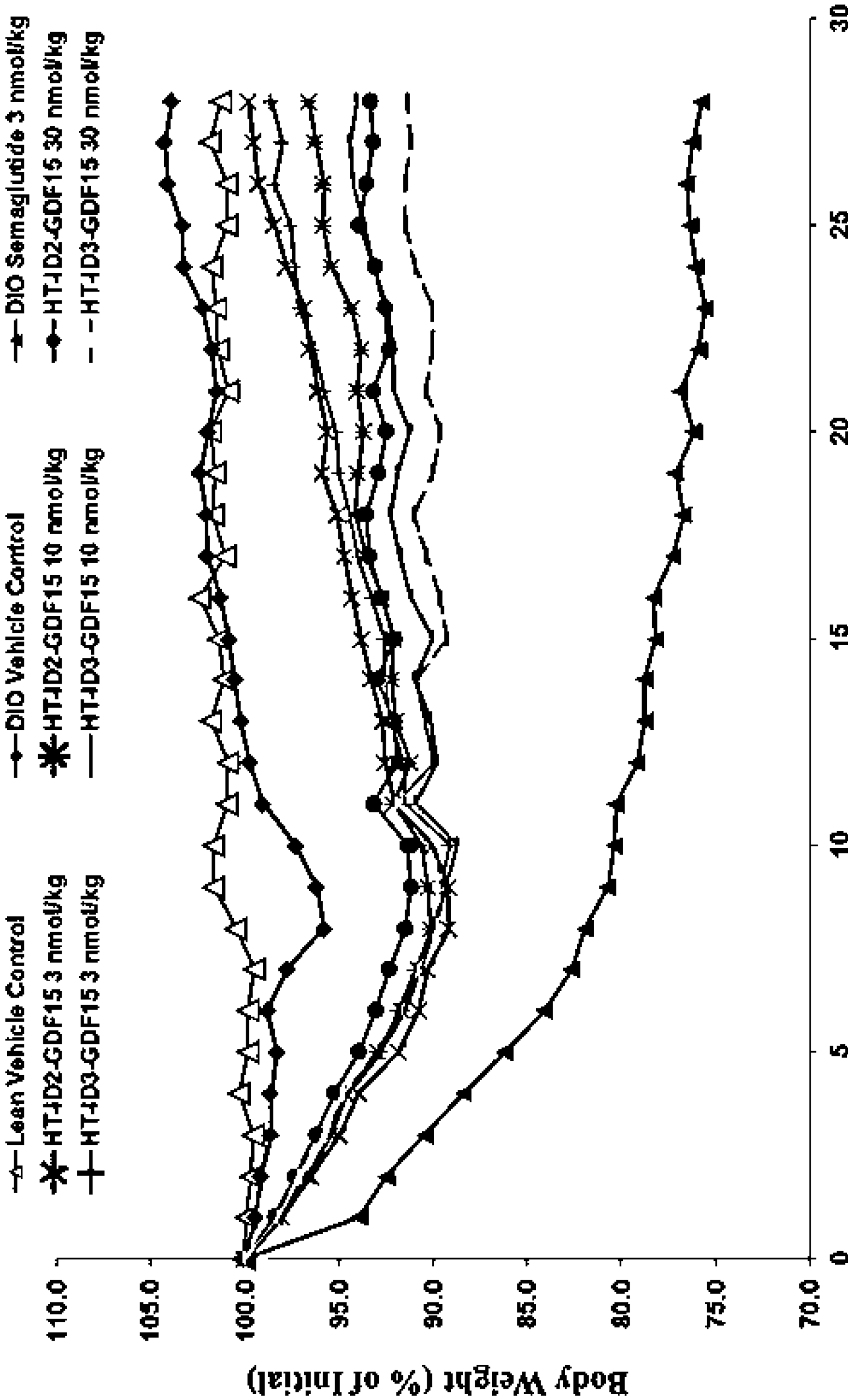
【FIG. 7】


HT-ID3-GDF15 10 nmol/kg
95.0
HT-ID2-GDF15 10 nmol/kg
94.3
HT-ID1-GDF15 10 nmol/kg
95.7
Semaglutide, 3 nmol/kg
(Daily Inj.)
87.0
DIO Vehicle Control
100.0
60
65
70
75
80
85
90
95
100
105
Body Weight (% of Initial)

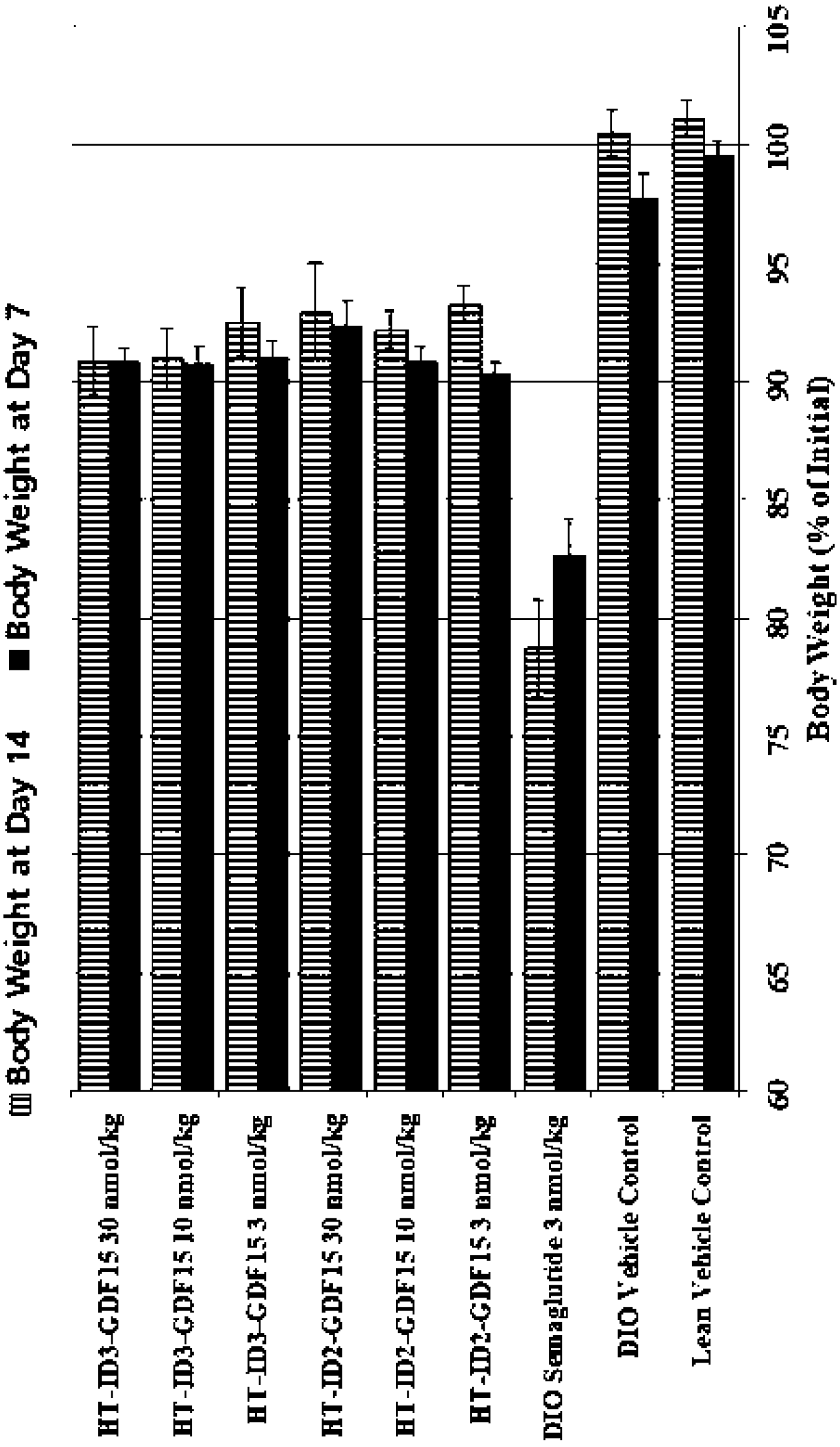
【FIG. 8】


HT-ID3-GDF15 10 nmol/kg
17.76
HT-ID2-GDF15 10 nmol/kg
16.66
HT-ID1-GDF15 10 nmol/kg
19.67
Semaglutide 3 nmol/kg (Daily Inj.)
19.83
DIO Vehicle Control
21.63
0
5
10
15
20
25
Cumulative Food Intake (g)

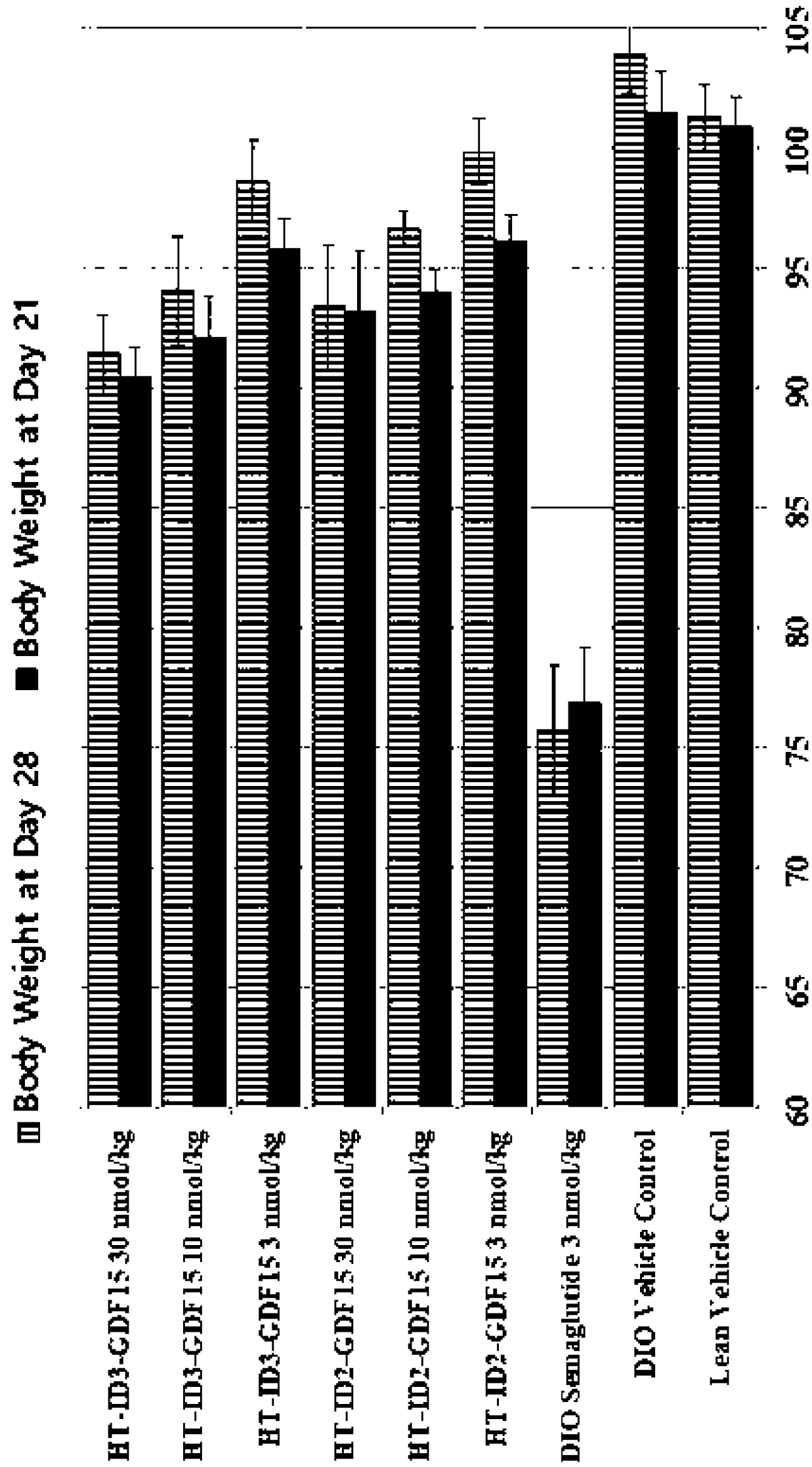
【FIG. 9】


Lean Vehicle Control
HT-ID2-GDF15 3 nmol/kg
HT-ID3-GDF15 3 nmol/kg
DIO Vehicle Control
HT-ID2-GDF15 10 nmol/kg
HT-ID3-GDF15 10 nmol/kg
DIO Semaglutide 3 nmol/kg
HT-ID2-GDF15 30 nmol/kg
HT-ID3-GDF15 30 nmol/kg
Body Weight (% of Initial)
110.0
105.0
100.0
95.0
90.0
85.0
80.0
75.0
70.0
0
5
10
15
20
25
30
Time (Day)

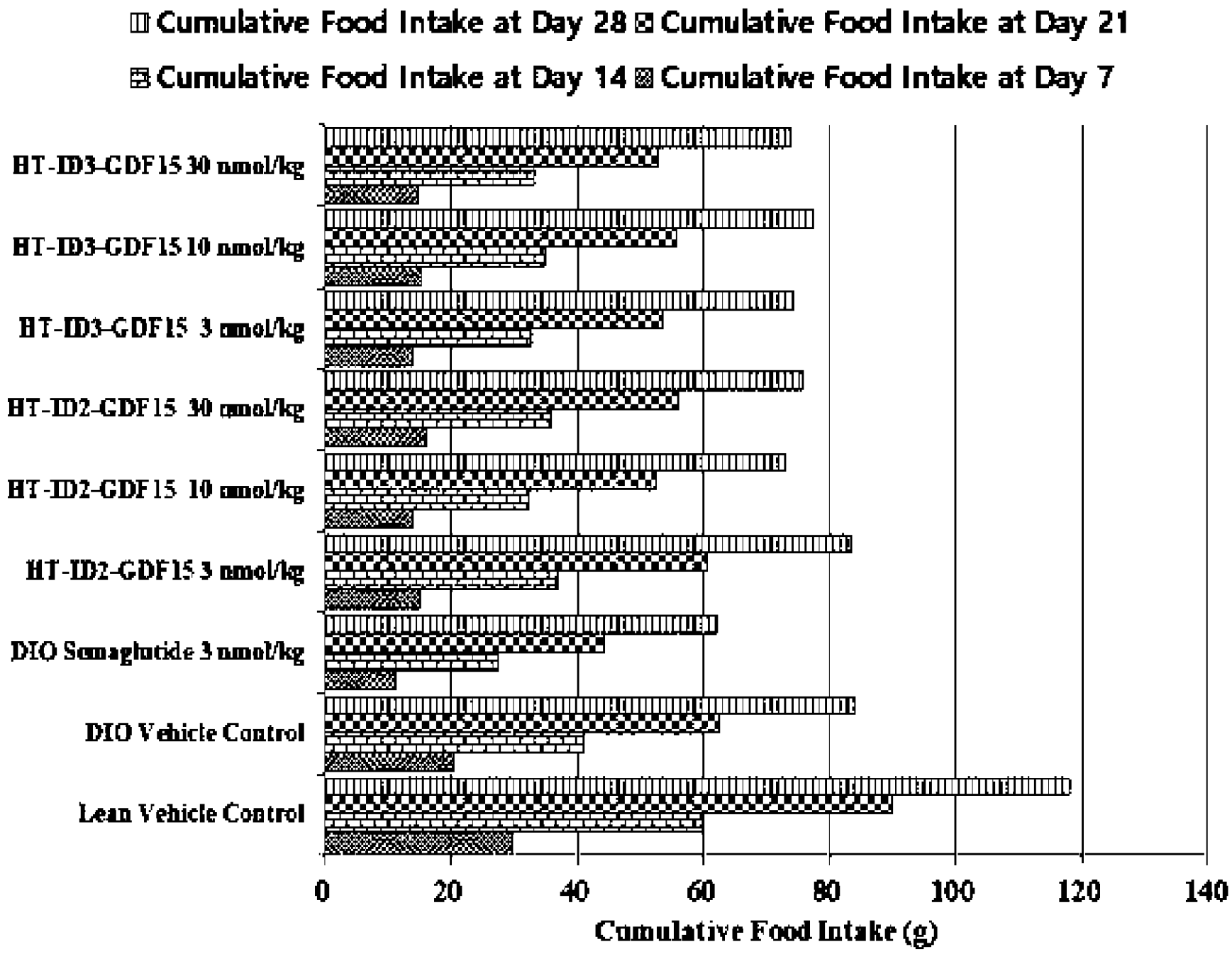
【FIG. 10a】


Body Weight (% of Initial)
60
65
70
75
80
85
90
95
100
105
Body Weight at Day 14
Body Weight at Day 7
HT-ID3-GDF15 30 nmol/kg
HT-ID3-GDF15 10 nmol/kg
HT-ID3-GDF15 3 nmol/kg
HT-ID2-GDF15 30 nmol/kg
HT-ID2-GDF15 10 nmol/kg
HT-ID?-GDF15 3 nmol/kg
DIO Semaglutide 3 nmol/kg
DIO Vehicle Control
Lean Vehicle Control

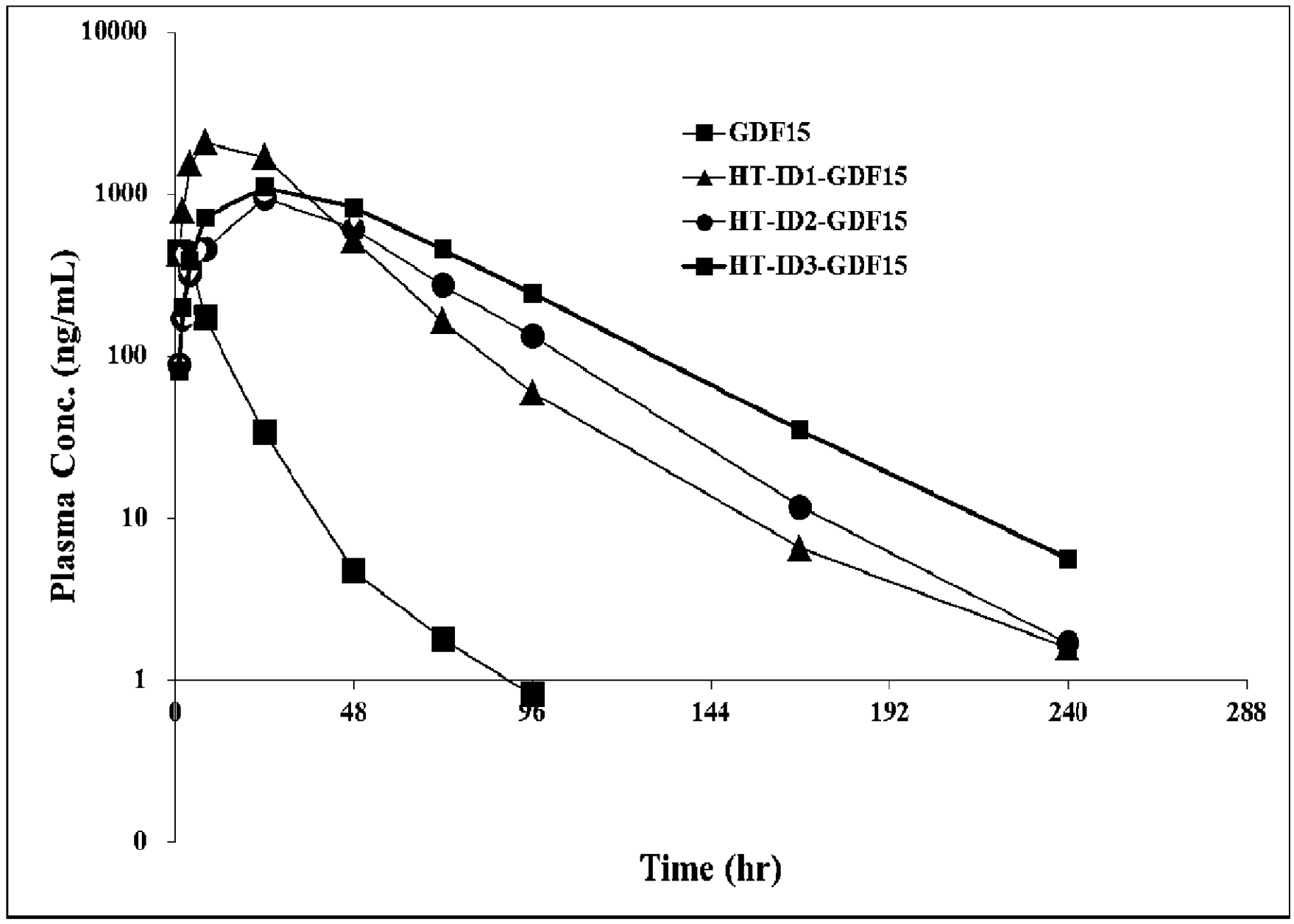
【FIG. 10b】


Body Weight at Day 28
Body Weight at Day 21
HT-ID3-GDF15 30 nmol/kg
HT-ID3-GDF15 10 nmol/kg
HT-ID3-GDF15 3 nmol/kg
HT-ID2-GDF15 30 nmol/kg
HT-ID2-GDF15 10 nmol/kg
HT-ID2-GDF15 3 nmol/kg
DIO Semaglutide 3 nmol/kg
DIO Vehicle Control
Lean Vehicle Control
60
65
70
75
80
85
90
95
100
105
Body Weight (% of Initial)

【FIG. 11】


Cumulative Food Intake at Day 28
Cumulative Food Intake at Day 21
Cumulative Food Intake at Day 14
Cumulative Food Intake at Day 7
HT-ID3-GDF15 30 nmol/kg
HT-ID3-GDF15 10 nmol/kg
HT-ID3-GDF15 3 nmol/kg
HT-ID2-GDF15 30 nmol/kg
HT-ID2-GDF15 10 nmol/kg
HT-ID2-GDF15 3 nmol/kg
DIO Semaglutide 3 nmol/kg
DIO Vehicle Control
Lean Vehicle Control
0
20
40
60
80
100
120
140
Cumulative Food Intake (g)

【FIG. 12】

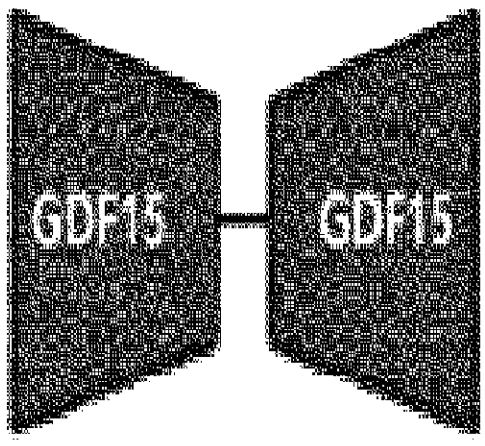

10000
1000
100
10
1
0
Plasma Conc. (ng/mL)
0
48
96
144
192
240
288
Time (hr)
GDF15
HT-ID1-GDF15
HT-ID2-GDF15
HT-ID3-GDF15

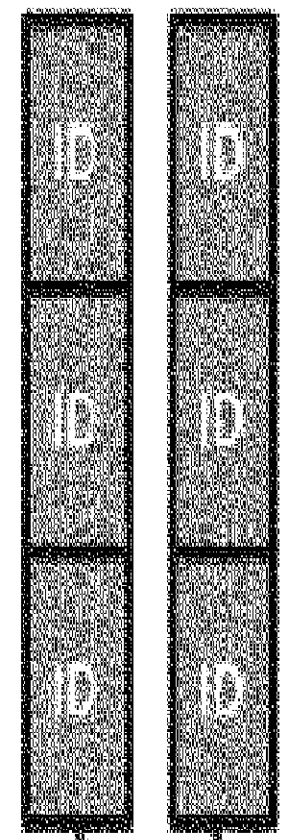
[Fig. 2b]

GDF15

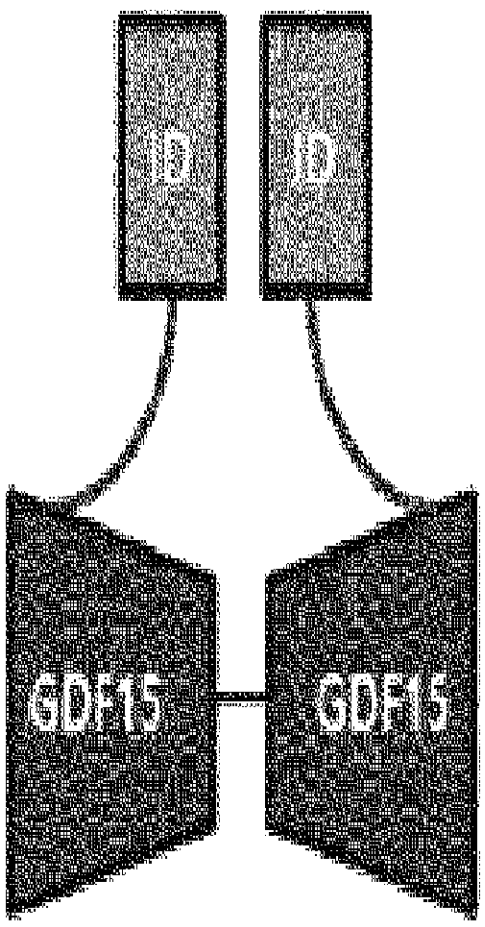
ID2-GDF15

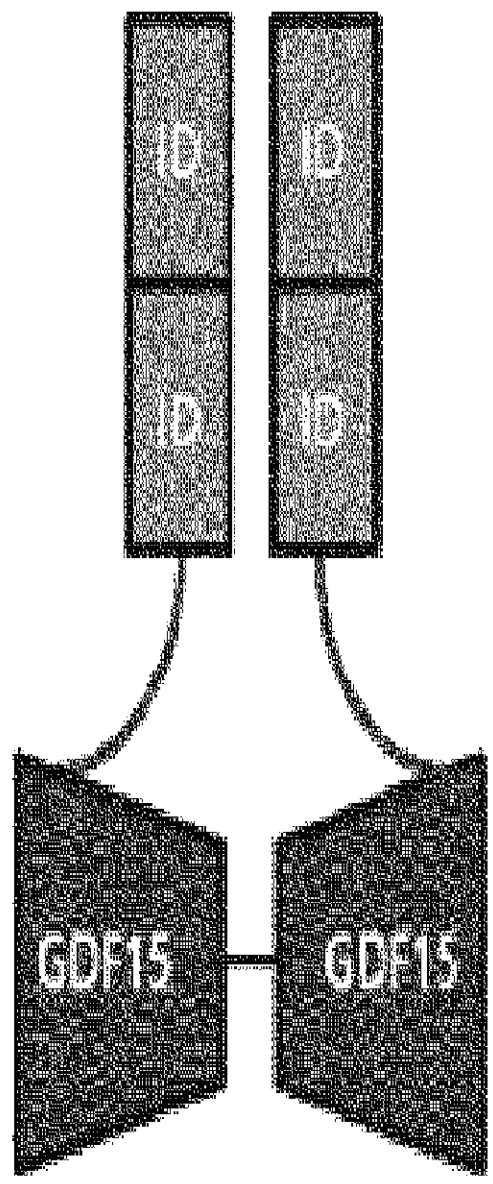
ID3-GDF15

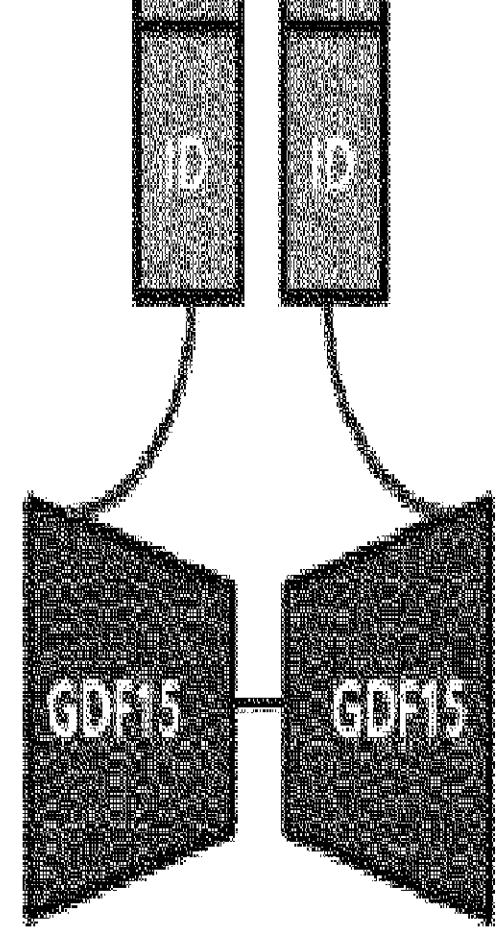




ID1-GDF15







– Cysteine Bond GS Linker

ID: ESPKAQASSVPTAQPQAEGSLAKATTAPATT RNT (SEQ ID NO: 1)

GS Linker: GGGGSGGGGS GGGGSGGGGS (SEQ ID NO: 17)

GDF15: SEQ ID NO: 3