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(54) Title: GFRAL RECEPTOR THERAPIES

(57) Abstract: The present invention relates to the field of medicine. More particularly, the invention relates to a method of treating diabetes or obesity by agonizing the GFRAL receptor, a compound for use in such treatment and pharmaceutical compositions containing such a compound. The invention also relates to a method of treating cachexia by antagonizing, inhibiting, neutralizing or blocking the GFRAL receptor, a compound for use in such treatment and pharmaceutical compositions containing such a compound.

GFRAL RECEPTOR THERAPIES

The present invention relates to the field of medicine. More particularly, the invention relates to a method of treating diabetes or obesity by agonizing the GFRAL receptor, a compound for use in such treatment and pharmaceutical compositions containing such a compound. The invention also relates to a method of treating cachexia by antagonizing, inhibiting, neutralizing or blocking the GFRAL receptor, a compound for use in such treatment and pharmaceutical compositions containing such a compound.

GDF15 belongs to the transforming growth factor-beta (TGFβ) superfamily. Among many reported biological functions of GDF15, the regulation of energy homeostasis may be important due to its potential in the treatment of obesity and metabolic diseases. The connection between GDF15 and body weight was initially based on the observation of a correlation between elevated serum GDF15 levels and weight loss in individuals with advanced prostate cancer. Furthermore, overexpression of GDF15 in animal models leads to lean phenotype, hypophagia and improvement of metabolic parameters. It has been reported that GDF15 modulates food intake and body weight by acting on the hypothalamus and brainstem. However, the mechanism of the action of GDF15 is not understood on the molecular level and no receptor of GDF15 has so far been identified.

Applicant has discovered that GDF15 binds with high affinity towards a remote member of the GFRα receptor family, GFRAL (GDNF receptor alpha like), which is a membrane protein with a single transmembrane domain with no known ligand. GFRAL is a member of the Glial cell line-derived neurotrophic factor (GDNF) family of receptors. The GDNF family ligands belong to the cysteine-knot protein family and function as homodimers. Unlike canonical TGF-β superfamily members, all GDNF family ligands signal through a complex between the RET receptor and GDNF family receptor-α. GFRα receptors are plasma membrane proteins with a glycosyl phosphatidylinositol (GPI) anchor. Four different GFRα receptors have been characterized (GFRα1–4) and their ligand specificity has been determined. GDNF binds to GFRα1, NRTN binds to GFRα2, ARTN to GFRα3, and PSPN to GFRα4. Interestingly, two distant homologs of GFRα

receptors, GFRAL and Gas1 (growth arrest-specific 1), have been identified based on sequence homology. Their ligands have been heretofore unknown.

GFRAL is a distant homolog of the GFRα family, and it is more closely related to GFRα-3 (30% identity) than it is to GFRα-1, -2, or -4. One distinction between GFRAL and other members of the GFRα family is GFRAL lacks a C-terminal GPI anchor motif. Instead, GFRAL is predicted to be a membrane protein with a single transmembrane domain.

Applicant discloses herein that the effects of GDF15 on body weight, food intake and glucose parameters in an animal model require the GFRAL receptor. The efficacy of recombinant GDF15 to reduce blood glucose and body weight is absent in homozygous GFRAL-deficient mice. These data provide unequivocal evidence to support GFRAL as the GDF15 receptor. This discovery is the basis for developing new compounds that interact with the GFRAL receptor to treat one or more of diabetes, obesity and cachexia.

The inventions described herein provide methods of modulating GDF15 and GFRAL activity with a compound comprising agonizing, antagonizing, inhibiting, neutralizing or blocking the GFRAL receptor. The compound may comprise an antibody which binds to the GFRAL receptor with a greater affinity than GDF15 and blocks GDF15 from binding to the GFRAL receptor. The inventions also comprise methods of reducing blood glucose and reducing body weight in a mammal comprising administering a compound or pharmaceutical composition to agonize the GFRAL receptor. The inventions further comprise methods of increasing blood glucose and increasing body weight in a mammal comprising administering a compound or pharmaceutical composition to antagonize, inhibit, neutralize or block the GFRAL receptor.

The present invention comprises a method of reducing blood glucose in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which agonizes the GFRAL receptor. The invention further comprises a method of increasing blood glucose in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which antagonizes, inhibits, neutralizes or blocks the GFRAL receptor. Also, the invention comprises a method of reducing body weight in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which agonizes the GFRAL receptor. The

present invention further comprises a method of increasing body weight in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which antagonizes, inhibits, neutralizes or blocks the GFRAL receptor.

The invention comprises the foregoing methods wherein the compound blocks the GFRAL receptor by inhibiting the binding of GDF15 and wherein the compound comprises an antibody or fragment thereof. The invention further comprises the foregoing methods wherein the mammal is a human and the GFRAL receptor has the amino acid sequence of SEQ ID NO: 4 and the GDF15 has the amino acid sequence of SEQ ID NO: 2. The present invention further comprises the foregoing methods wherein the mammal is a mouse and wherein the GFRAL receptor has the amino acid sequence of SEQ ID NO: 9 and the GDF15 has the amino acid sequence of SEQ ID NO: 7.

The present invention comprises the foregoing methods wherein the compound is administered with one or more pharmaceutically acceptable excipients and wherein the compound is administered with one or more of GLP-1, insulin, an insulin analog, a DPP-4 inhibitor, an SGLT2 inhibitor and glucagon.

The present invention comprises a pharmaceutical composition comprising a compound which agonizes the GFRAL receptor and one or more pharmaceutically acceptable excipients. The invention further comprises a pharmaceutical composition comprising a compound which antagonizes, inhibits, neutralizes or blocks the GFRAL receptor and one or more pharmaceutically acceptable excipients. The invention also comprises the foregoing composition further comprising one or more of GLP-1, insulin, an insulin analog, a DPP-4 inhibitor, an SGLT2 inhibitor and glucagon. The invention further comprises the foregoing composition wherein the compound and one or more of GLP-1, insulin, an insulin analog, a DPP-4 inhibitor, an SGLT2 inhibitor and glucagon are fused.

The present invention comprises an isolated compound which agonizes the GFRAL receptor. The invention further comprises an isolated compound which antagonizes, inhibits, neutralizes or blocks the GFRAL receptor. The invention also comprises the foregoing compound wherein the compound is a peptide, protein, an antibody or fragment thereof. The invention further comprises the foregoing compound

wherein the antibody or fragment thereof binds to the same epitope or amino acid region on GFRAL as GDF15.

The present invention comprises a method of treating diabetes in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which agonizes the GFRAL receptor. The invention further comprises a method of treating obesity in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which agonizes the GFRAL receptor. The invention also comprises a method of treating cachexia in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which antagonizes, inhibits, neutralizes or blocks the GFRAL receptor.

The invention comprises a protein comprising (a) a GDF15-binding soluble fragment of an insoluble human GFRAL receptor, wherein the insoluble human GFRAL receptor specifically binds to human GDF15 and (b) all of the domains of the constant region of a human immunoglobulin IgG heavy chain other than the first domain of said constant region; wherein said protein specifically binds human GDF15. The invention further comprises the foregoing protein wherein the insoluble human GFRAL receptor comprises SEQ ID NO: 4. The invention also comprises the foregoing protein wherein the protein consists essentially of the extracellular region of the insoluble human GFRAL receptor and all the domains of the constant region of a human IgG1 immunoglobulin heavy chain other than the first domain of the constant region.

For the present invention, “an antibody” refers to an intact antibody (comprising a complete or full length Fc region), a substantially intact antibody, or a portion or fragment of an antibody comprising an antigen-binding region, e.g., a Fab fragment, Fab’ fragment or $F(ab')_2$ fragment of an animal, humanized or human antibody. The term “monoclonal antibody” as used herein refers to an antibody from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope(s), except for possible variants that may arise during production of the monoclonal antibody, such as variants generally being present in minor amounts. Such monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding

polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool if hybridoma clones, phage clones or recombinant DNA clones. It should be understood that the selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity in vivo, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, the monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques known in the art, including one or more of the hybridoma method, recombinant DNA methods, and phage display technologies.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while portions of the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity. Methods of making chimeric antibodies are known in the art.

It is well known in the art that agents for the treatment of diabetes and/or obesity may be combined with other agents for the treatment of diabetes and/or obesity. The compound of the invention, or a pharmaceutically acceptable salt thereof, may be co-administered, simultaneously or sequentially, with other effective treatment(s) for

diabetes or obesity including, but not limited to GLP-1, insulin, insulin analogs, DPP-4 inhibitors, SGLT2 inhibitors and glucagon. The compound of the invention, or a pharmaceutically acceptable salt thereof, alone or in combination with other effective treatement(s) may be administered, simultaneously or sequentially, following approved medical procedures such as bariatric surgeries, for example, gastric bypass surgery or adjustable gastric banding procedures.

The interaction of GDF15 and a group of GFRα receptor related proteins is tested through a co-immunoprecipitation assay. Three receptors, GFRα4, GFRAL (SEQ ID. NO: 4) and Gas1, are FLAG™ -tagged and are transiently overexpressed in HEK293 cells. FLAG™, a registered trademark of Sigma-Aldrich, is a tag that can be added to a protein, having the sequence motif Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys. Cell lysates are harvested and incubated with GDF15-tandem Fc fusion recombinant protein before immunoprecipitation with anti-FLAG™ antibody. Although all three proteins are expressed and immunoprecipitated, GDF15 (SEQ ID. NO: 2)-tandem Fc is only co-immunoprecipitated in the presence of GFRAL (Figure 1), demonstrating direct interaction between GFRAL and GDF15.

To provide more evidence for the interactions between the GFRAL and GDF15 and to gain a better understanding of the specificity of the interaction, the recombinant protein of the extra cellular domain ("ECD") of GFRAL (SEQ ID. NO: 4) is expressed and purified to develop a solid-phase assay to generate a more quantitative measurement of the relative binding affinities. Recombinant native GDNF protein is utilized as a positive control in this assay and it binds to the canonical GFRα receptors, with the strongest interaction with GFRα1 (Figure 2A). No interaction is observed between GDNF and GFRAL or Gas1. Conversely, native GDF15 (SEQ ID. NO: 2) binds strongly to the ECD of GFRAL, but does not recognize any of the other receptors (Figure 2B). These results demonstrate the interaction between GFRAL and GDF15 is selective and specific.

SPR spectroscopy is used to assess the affinity between GFRAL (SEQ ID. NO:4) and GDF15 (SEQ ID. NO: 2). Purified recombinant ECD of GFRAL is immobilized on a GE Life Sciences CM5 chip via ammine coupling. Various concentrations of recombinant GDF15 protein are delivered in the mobile phase (Fig 3). Six datasets are fit

to a bivalent analyze model and the affinity of the protein (KD) is calculated. The affinity between native recombinant GDF15 and the ECD of GFRAL is ~0.7 nM.

An animal model is used to confirm that the biological function of GDF15 requires GFRAL. Mice with whole body deletion of GFRAL are acquired from Taconic Biosciences, Inc. for this testing. Homozygous GFRAL knockout mice are viable and with no gross abnormalities. Male homozygous knockout mice weigh less than wild-type gender matched littermates, while no significant body weight difference is observed between female KO and wild-type littermates.

Treatment of mice with GDF15 suppresses food intake and weight gain with improvement of glucose parameters. To determine whether GFRAL is required for these effects, we inject both wild-type and homozygous knockout mice with recombinant tandem Fc GDF15 (SEQ ID. NO: 2) protein for 13 days at 0.1 mg/kg dose once every two days. Wild type ("WT") animals exhibited sustained weight loss and significantly attenuated food intake (Figure 4). We also examine the role of GDF15 in glucose homeostasis. GDF15 led to a significant decrease of baseline glucose level in the WT mice (Figure 4). During the oral glucose tolerance test (OGTT) at the end of the study, WT animals treated with GDF15 had reduced glucose excursion compared to the vehicle treatment group (Figure 4).

However, the effects of GDF15 on body weight, food intake and glucose parameters are absent in the GFRAL knockout cohort (Figure 4). GDF15 treatment having no effects on GFRAL knockout mice confirmed our hypothesis that GFRAL is the endogenous GDF15 receptor that mediates the biological activities of GDF15. This approach may also be used to identify potential therapeutically effective GFRAL agonist antibodies or proteins by measuring the effects on food intake, weight gain and glucose compared in WT animals with a GFRAL receptor to the same type of animals treated with GDF15.

GFRAL antibodies are generated. Six mouse monoclonal antibodies against mouse GFRAL are generated with mouse hybridomas created from mice exposed to a recombinant protein antigen of SEQ ID NO: 11. The six resultant antibodies are selected to test their ability to interfere with GDF15 binding. Two antibodies, 8A2 and 8G2, dose dependently inhibit the binding between GDF15 and GFRAL (Figure 5). These two

antibodies bind to both human and mouse GFRAL receptors. The other four antibodies, 7F10, 5E1, 6F10 and 2H8, bind only to mouse GFRAL receptors.

Human and mouse GDF15 amino acid sequences are given below as SEQ ID NOs: 1 and 6, respectively. The pro-domain of these sequences must be cleaved off the full length protein in order to generate the active GDF15 molecule. The active GDF15 molecule amino acid sequences are given below as SEQ ID NO: 2 for human GDF15 and SEQ ID NO: 7 for mouse GDF15.

Description of Figures:

Figure 1. A co-immunoprecipitation assay shows that GFRAL interacts with GDF15. Cell lysates prepared from HEK293 cells are transiently transfected with FLAG™ tagged GFRα4, GFRAL or GAS1 and are incubated with recombinant tandem Fc-GDF15 protein. Immunoprecipitation is performed using anti-FLAG™ antibody followed by immunoblotting with anti-FLAG™ and anti-Fc antibodies.

Figure 2. A solid-phase binding assay shows that (A) Recombinant GDNF protein binds most strongly to GFRα1 and (B) recombinant GDF15 interacts specifically with the ECD of GFRAL.

Figure 3. A Surface Plasmon Resonance ("SPR") sensorgram shows binding between GDF15 (SEQ ID. NO: 2) and the ECD of GFRAL (SEQ ID. NO: 4). The binding between recombinant ECD of GFRAL with (A) native GDF15 and (B) tandem Fc-GDF15 is studied at 25°C. The biosensor chip response is plotted as a function of time.

Figure 4. The effects of GDF15 (SEQ ID NO. 7), in mice with and without GFRAL receptors (SEQ ID NO. 9) are shown. Effects are shown on (A) body weight, (B) food intake and (C) fed blood glucose level during the treatment with GDF15 in normal chow-fed, 10 week old wild-type or Gfral–/– (KO) mice (n = 9 for wild-type; n = 8 for KO), (D) Oral glucose tolerance test (OGTT) after 13 day GDF15 treatment in male mice and (E-H) are the same respective tests in female mice.

Figure 5. Antibodies are tested to determine whether they block the interaction between GDF15 and GFRAL. Interactions between GDF15 (SEQ ID. NO: 2) and GFRAL (SEQ ID. NO: 9) are assessed by solid-phase binding assay in the presence of the

different GFRAL antibodies that are generated. 8A2 and 8G2 are able to inhibit the binding in a dose dependent fashion.

Methods:

Recombinant native GDF15, GDNF, GFRα1, GFRα2, GFRα3, Gas1 proteins, anti-GDF15 and anti-GDNF are purchased from R&D Systems. Tandem Fc GDF15 comprises a first Fc region and a second Fc region and native GDF15 (SEQ ID. NO: 2) at the C-terminus. The first and the second Fc regions are linked through a flexible linker to form a contiguous polypeptide and dimerize to form an Fc dimer. Recombinant tandem Fc and ECD of GFRAL are expressed and purified by Novoprotein. GFRAL knockout mice are acquired from Taconic, the exon 2 and 3 from GFRAL gene are replaced by LacZ/Neo cassette through homologous recombination.

Immunoprecipitation and immunoblotting analysis. HEK293 cells are maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cells are transfected with expression vectors using the Lipofectamine 2000 transfection reagent (Invitrogen). Cell lysate is harvested in PBS and 1 % NP40 plus protease inhibitor cocktail. Immunoprecipitation is performed with anti-FLAG™ antibody-conjugated beads in the presence of 1 μg/ml tandem Fc GDF15 recombinant protein. Immunoblotting is performed with anti-FLAG™ and anti-human Fc antibodies.

Solid-phase Binding Assay. Nunc 96-well plates are coated overnight at 4 °C with 50 μl of 0.2 μg/ml recombinant GFRα receptors. After washing with PBS with 0.05% Tween 20, 50 μl of 1 μg/ml recombinant GDNF or GDF15 (SEQ ID. NO: 2) proteins are added to each well at room temperature. After 2 h, anti-GDNF or anti GDF15 antibody is added, and the plates are incubated for another 1 h at room temperature. The plates are washed and HRP conjugated secondary antibody is used for detection.

SPR spectroscopy. A Biacore® 3000 instrument is used to measure the binding kinetics of GDF15 (SEQ ID. NO: 2) to GFRAL (SEQ ID. NO: 4) receptor. Measurements are performed at 25°C. Samples are dissolved in PBS + 0.05% Tween 20, pH7.4. GFRAL protein is immobilized on flow cells of a CM5 sensor chip at a level of 243 response units (RUs) using amine coupling chemistry. Binding is evaluated using

multiple cycles. Each cycle consists of the following steps: 1) injection of about 45 µl of GDF15 at a flow rate of 30 µl/min followed by 5 minutes for dissociation; and 2) regeneration using about 15 µl of 10 mM glycine hydrochloride, pH 1.5. Association and dissociation rates for each cycle are evaluated using BIA Evaluation Software.

Animals and Treatments. Wild-type and GFRAL homozygous knockout littermates are used in the study. Recombinant tandem Fc GDF15 protein or vehicle (PBS) is injected intraperitoneally ("i.p") at 0.1 mg/kg once every two days. After the indicated times, body weight and food intake are measured. Tail vein blood is collected and blood glucose is measured by a glucose meter.

Oral Glucose Tolerance Test. Mice are fasted 6 hours before oral administration of glucose (2 g/kg), and glucose levels will be measured immediately pre-dose, 15, 30, 60 and 120 minutes after glucose challenge.

Sequences

Human GDF15 AA Sequence (SEQ ID NO: 1)

MPGQELRTVNGSQMLLVLLVLSWLPHGGALSLAEASRASFPGPSELHSEDSRFRELRKRY
EDLLTRLRANQSWEDSNTDLVPAPAVRILTPEVRLGSGGHLHLRISRAALPEGLPEASRL
HRALFRLSPTASRSWDVTRPLRRQLSLARPQAPALHLRLSPPPSQSDQLLAESSSARPQL
ELHLRPQAARGRRRARARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMC
IGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDL
LAKDCHCI

Human Active GDF15 AA Sequence (SEQ ID NO: 2)

ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQ
IKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

Human GDF15 DNA Sequence (SEQ ID NO: 3)

ATGCCCGGGCAAGAACTCAGGACGGTGAATGGCTCTCAGATGCTCCTGGTGTTGCTGGTG
CTCTCGTGGCTGCCGCATGGGGGCGCCCTGTCTCTGGCCGAGGCGAGCCGCGCAAGTTTC
CCGGGACCCTCAGAGTTGCACTCCGAAGACTCCAGATTCCGAGAGTTGCGGAAACGCTAC
GAGGACCTGCTAACCAGGCTGCGGGCCAACCAGAGCTGGGAAGATTCGAACACCGACCTC
GTCCCGGCCCCTGCAGTCCGGATACTCACGCCAGAAGTGCGGCTGGGATCCGGCGGCCAC
CTGCACCTGCGTATCTCTCGGGCCGCCCTTCCCGAGGGGCTCCCCGAGGCCTCCCGCCTT
CACCGGGCTCTGTTCCGGCTGTCCCCGACGGCGTCAAGGTCGTGGGACGTGACACGACCG
CTGCGGCGTCAGCTCAGCCTTGCAAGACCCCAGGCGCCCGCGCTGCACCTGCGACTGTCG
CCGCCGCCGTCGCAGTCGGACCAACTGCTGGCAGAATCTTCGTCCGCACGGCCCCAGCTG
GAGTTGCACTTGCGGCCGCAAGCCGCCAGGGGGCGCCGCAGAGCGCGTGCGCGCAACGGG
GACCACTGTCCGCTCGGGCCCGGGCGTTGCTGCCGTCTGCACACGGTCCGCGCGTCGCTG
GAAGACCTGGGCTGGGCCGATTGGGTGCTGTCGCCACGGGAGGTGCAAGTGACCATGTGC
ATCGGCGCGTGCCCGAGCCAGTTCCGGGCGGCAAACATGCACGCGCAGATCAAGACGAGC
CTGCACCGCCTGAAGCCCGACACGGTGCCAGCGCCCTGCTGCGTGCCCGCCAGCTACAAT
CCCATGGTGCTCATTCAAAAGACCGACACCGGGGTGTCGCTCCAGACCTATGATGACTTG
TTAGCCAAAGACTGCCACTGCATATGA

Human GFRAL Receptor AA Sequence (SEQ ID NO: 4)

MIVFIFLAMGLSLENEYTSQTNNCTYLREQCLRDANGCKHAWRVMEDACNDSDPGDPCKM
RNSSYCNLSIQYLVESNFQFKECLCTDDFYCTVNKLLGKKCINKSDNVKEDKFKWNLTTR
SHHGFKGMWSCLEVAEACVGDVVCNAQLASYLKACSANGNPCDLKQCQAAIRFFYQNIPF
NIAQMLAFCDCAQSDIPCQQSKEALHSKTCAVNMVPPPTCLSVIRSCQNDELCRRHYRTF
QSKCWQRVTRKCHEDENCISTLSKQDLTCSGSDDCKAAYIDILGTVLQVQCTCRTITQSE
ESLCKIFQHMLHRKSCFNYPTLSNVKGMALYTRKHANKITLTGFHSPFNGEVIYAAMCMT
VTCGILLLVMVKLRTSRISSKARDPSSIQIPGEL

Human GFRAL Receptor DNA Sequence (SEQ ID NO: 5)

ATGATAGTGTTTATTTTCTTGGCTATGGGGTTAAGCTTGGAAAATGAATACACTTCCCAA
ACCAATAATTGCACATATTTAAGAGAGCAATGCTTACGTGATGCAAATGGATGTAAACAT
GCTTGGAGAGTAATGGAAGATGCCTGCAATGATTCAGATCCAGGTGACCCCTGCAAGATG
AGGAATTCATCATACTGTAACCTGAGTATCCAGTACTTAGTGGAAAGCAATTTCCAATTT
AAAGAGTGTCTTTGCACTGATGACTTCTATTGTACTGTGAACAAACTGCTTGGAAAAAAA
TGTATCAATAAATCAGATAACGTGAAAGAGGATAAATTCAAATGGAATCTAACTACACGT
TCCCATCATGGATTCAAAGGGATGTGGTCCTGTTTGGAAGTGGCAGAGGCATGTGTAGGG
GATGTGGTCTGTAATGCACAGTTGGCCTCTTACCTTAAAGCTTGCTCAGCAAATGGAAAT
CCGTGTGATCTGAAACAGTGCCAAGCAGCCATACGGTTCTTCTATCAAAATATACCTTTT
AACATTGCCCAGATGTTGGCTTTTTGTGACTGTGCTCAATCTGATATACCTTGTCAGCAG
TCCAAAGAAGCTCTTCACAGCAAGACATGTGCAGTGAACATGGTTCCACCCCCTACTTGC
CTCAGTGTAATTCGCAGCTGCCAAAATGATGAATTATGCAGGAGGCACTATAGAACATTT
CAGTCAAAATGCTGGCAGCGTGTGACTAGAAAGTGCCATGAAGATGAGAATTGCATTAGC
ACCTTAAGCAAACAGGACCTCACTTGTTCAGGAAGTGATGACTGCAAAGCTGCTTACATA
GATATCCTTGGGACGGTCCTTCAAGTGCAATGTACCTGTAGGACCATTACACAAAGTGAG
GAATCTTTGTGTAAGATTTTCCAGCACATGCTTCATAGAAAATCATGTTTCAATTATCCA
ACCCTGTCTAATGTCAAAGGCATGGCATTGTATACAAGAAAACATGCAAACAAAATCACT
TTAACTGGATTTCATTCCCCCTTCAATGGAGAAGTAATCTATGCTGCCATGTGCATGACA
GTCACCTGTGGAATCCTTCTGTTGGTTATGGTCAAGCTTAGAACTTCCAGAATATCAAGT
AAAGCAAGAGATCCTTCATCGATCCAAATACCTGGAGAACTCTGA

Mouse GDF15 AA Sequence (SEQ ID NO: 6)

MAPPALQAQPPGGSQLRFLLFLLLLLLLLSWPSQGDALAMPEQRPSGPESQLNADELRGR
FQDLLSRLHANQSREDSNSEPSPDPAVRILSPEVRLGSHGQLLLRVNRASLSQGLPEAYR
VHRALLLLTPTARPWDITRPLKRALSLRGPRAPALRLRLTPPPDLAMLPSGGTQLELRLR
VAAGRGRRSAHAHPRDSCPLGPGRCCHLETVQATLEDLGWSDWVLSPRQLQLSMCVGECP
HLYRSANTHAQIKARLHGLQPDKVPAPCCVPSSYTPVVLMHRTDSGVSLQTYDDLVARGC
HCA

Mouse Active GDF15 AA Sequence (SEQ ID NO: 7)

AHPRDSCPLGPGRCCHLETVQATLEDLGWSDWVLSPRQLQLSMCVGECPHLYRSANTHAQ
IKARLHGLQPDKVPAPCCVPSSYTPVVLMHRTDSGVSLQTYDDLVARGCHCA

Mouse GDF15 DNA Sequence (SEQ ID NO: 8)

ATGGCCCCGCCCGCGCTCCAGGCCCAGCCTCCAGGCGGCTCTCAACTGAGGTTCCTGCTG
TTCCTGCTGCTGTTGCTGCTGCTGCTGTCATGGCCATCGCAGGGGGACGCCCTGGCAATG
CCTGAACAGCGACCCTCCGGCCCTGAGTCCCAACTCAACGCCGACGAGCTACGGGGTCGC
TTCCAGGACCTGCTGAGCCGGCTGCATGCCAACCAGAGCCGAGAGGACTCGAACTCAGAA
CCAAGTCCTGACCCAGCTGTCCGGATACTCAGTCCAGAGGTGAGATTGGGGTCCCACGGC
CAGCTGCTACTCCGCGTCAACCGGGCGTCGCTGAGTCAGGGTCTCCCCGAAGCCTACCGC
GTGCACCGAGCGCTGCTCCTGCTGACGCCGACGGCCCGCCCCTGGGACATCACTAGGCCC
CTGAAGCGTGCGCTCAGCCTCCGGGGACCCCGTGCTCCCGCATTACGCCTGCGCCTGACG

CCGCCTCCGGACCTGGCTATGCTGCCCTCTGGCGGCACGCAGCTGGAACTGCGCTTACGG
GTAGCCGCCGGCAGGGGGCGCCGAAGCGCGCATGCGCACCCAAGAGACTCGTGCCCACTG
GGTCCGGGGCGCTGCTGTCACTTGGAGACTGTGCAGGCAACTCTTGAAGACTTGGGCTGG
AGCGACTGGGTGCTGTCCCCGCGCCAGCTGCAGCTGAGCATGTGCGTGGGCGAGTGTCCC
CACCTGTATCGCTCCGCGAACACGCATGCGCAGATCAAAGCACGCCTGCATGGCCTGCAG
CCTGACAAGGTGCCTGCCCCGTGCTGTGTCCCCTCCAGCTACACCCCGGTGGTTCTTATG
CACAGGACAGACAGTGGTGTGTCACTGCAGACTTATGATGACCTGGTGGCCCGGGGCTGC
CACTGCGCTTGA

Mouse GFRAL Receptor AA Sequence (SEQ ID NO: 9)

MLVFIFLAVTLSSENESSSQTNDCAHLIQKCLIDANGCEQSWRSMEDTCLTPGDSCKINN
SLHCNLSIQALVEKNFQFKECLCMDDLHCTVNKLFGKKCTNKTDNMEKDNKDKWNLTTTP
FYHGFKQMQSCLEVTEACVGDVVCNAQLALYLKACSANGNLCDVKHCQAAIRFFYQNMPF
NTAQMLAFCDCAQSDIPCQQSKETLHSKPCALNIVPPPTCLSVIHTCRNDELCRTHYRTF
QTECWPHITGKCHEDETCISMLGKQDLTCSGSESCRAAFLGTFGTVLQVPCACRGVTQAE
EHVCMIFQHMLHSKSCFNYPTPNVKDISSYEKKNSKEITLTGFNSFFNGELLYVVVCMAV
TCGILFLVMLKLRIQSEKRDPSSIEIAGGVIIQ

Mouse GFRAL Receptor DNA Sequence (SEQ ID NO: 10)

ATGCTAGTGTTCATTTTCCTGGCTGTTACGTTAAGCTCAGAAAATGAATCCTCTTCCCAA
ACAAATGATTGTGCACATTTAATACAGAAATGCTTGATTGATGCAAATGGCTGTGAGCAG
TCATGGAGATCAATGGAAGACACCTGCCTTACTCCAGGTGACTCCTGCAAGATAAATAAT
TCACTACATTGTAACCTGAGTATCCAGGCTTTGGTGGAAAAAAATTTCCAATTTAAAGAG
TGTCTTTGTATGGATGACCTCCACTGTACAGTAAACAAACTTTTTGGAAAAAAGTGCACC
AATAAGACAGATAACATGGAAAAGGACAATAAAGATAAATGGAATCTAACTACTACTCCT
TTCTATCATGGATTCAAACAGATGCAGTCTTGTTTGGAGGTGACAGAGGCGTGTGTAGGG
GATGTGGTTTGTAATGCACAGTTGGCCCTTTACCTTAAAGCATGCTCAGCAAATGGAAAT
CTGTGTGATGTGAAACACTGCCAAGCAGCCATACGGTTCTTCTATCAAAATATGCCTTTT
AACACTGCCCAGATGTTGGCTTTTTGTGACTGTGCTCAATCTGATATACCCTGTCAGCAA
TCCAAAGAAACTCTTCACAGCAAGCCATGTGCACTGAATATAGTTCCACCCCCCACTTGC
CTCAGTGTAATTCACACTTGCCGAAATGATGAATTATGCAGGACACACTACCGAACATTC
CAGACAGAATGCTGGCCCCACATAACTGGGAAGTGCCATGAAGATGAGACCTGCATTAGC
ATGTTAGGCAAGCAAGACCTTACTTGTTCTGGGAGTGAGAGCTGCAGGGCTGCCTTCCTA
GGAACCTTTGGGACAGTCCTGCAAGTACCCTGTGCTTGCAGGGGCGTTACACAGGCTGAA
GAACACGTGTGCATGATTTTCCAGCACATGCTTCATAGCAAATCGTGTTTCAATTACCCA
ACTCCTAATGTCAAAGACATTTCCTCATATGAAAAAAAGAATTCAAAAGAAATTACTCTG
ACTGGATTCAATTCTTTCTTCAATGGAGAACTACTCTATGTTGTTGTGTGCATGGCAGTT
ACCTGTGGAATTCTTTTCTTGGTGATGCTCAAGTTAAGGATACAAAGTGAAAAAAGAGAT
CCCTCATCCATCGAAATAGCTGGAGGTGTCATCATTCAGTGA

Recombinant Protein Antigen to generate Mouse antibodies to GFRAL (SEQ ID NO: 11)

DHHHHHHAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP

REPQVYTKPPSRDELTKNQVSLSCLVKGFYPSDIAVEWESNGQPENNYKTTVPVLDSDGS
FRLASYLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGRAQTNDCAHL
IQKCLIDANGCEQSWRSMEDTCLTPGDSCKINNSLHCNLSIQALVEKNFQFKECLCMDDL
HCTVNKLFGKKCTNKTDNMEKDNKDKWNLTTTPFYHGFKQMQSCLEVTEACVGDVVCNAQ
LALYLKACSANGNLCDVKHCQAAIRFFYQNMPFNTAQMLAFCDCAQSDIPCQQSKETLHS
KPCALNIVPPPTCLSVIHTCRNDELCRTHYRTFQTECWPHITGKCHEDETCISMLGKQDL
TCSGSESCRAAFLGTFGTVLQVPCACRGVTQAEEHVCMIFQHMLHSKSCFNYPTPNVKDI
SSYEKKNSKE

I (WE) CLAIM:

1. A method of reducing blood glucose in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which agonizes the GFRAL receptor.
2. A method of increasing blood glucose in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which antagonizes, inhibits, neutralizes or blocks the GFRAL receptor.
3. A method of reducing body weight in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which agonizes the GFRAL receptor.
4. A method of increasing body weight in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which antagonizes, inhibits, neutralizes or blocks the GFRAL receptor.
5. The method of either of claim 2 or 4 wherein the compound blocks the GFRAL receptor by inhibiting the binding of GDF15.
6. The method of any of claims 1-5 wherein the compound comprises an antibody or fragment thereof.
7. The method of any of claims 1-6 wherein the mammal is a human.
8. The method of claim 7 wherein the GFRAL receptor has the amino acid sequence of SEQ ID NO: 4 and the GDF15 has the amino acid sequence of SEQ ID NO: 2.
9. The method of any of claims 1-6 wherein the mammal is a mouse.
10. The method of claim 9 wherein the GFRAL receptor has the amino acid sequence of SEQ ID NO: 9 and the GDF15 has the amino acid sequence of SEQ ID NO: 7.
11. The method of any of claims 1-10 wherein the compound is administered with one or more pharmaceutically acceptable excipients.

12. The method of any of claims 1-11 wherein the compound is administered with one or more of GLP-1, insulin, an insulin analog, a DPP-4 inhibitor, an SGLT2 inhibitor and glucagon.

13. A pharmaceutical composition comprising a compound which agonizes the GFRAL receptor and one or more pharmaceutically acceptable excipients.

14. A pharmaceutical composition comprising a compound which antagonizes, inhibits, neutralizes or blocks the GFRAL receptor and one or more pharmaceutically acceptable excipients.

15. The composition of claim 13 or 14, further comprising one or more of GLP-1, insulin, an insulin analog, a DPP-4 inhibitor, an SGLT2 inhibitor and glucagon.

16. The composition of claim 15 wherein the compound and one or more of GLP-1, insulin, an insulin analog, a DPP-4 inhibitor, an SGLT2 inhibitor and glucagon are fused.

17. An isolated compound which agonizes the GFRAL receptor.

18. An isolated compound which antagonizes, inhibits, neutralizes or blocks the GFRAL receptor.

19. The compound of claim 16 or 17, wherein the compound is an antibody or fragment thereof.

20. The compound of claim 19, wherein the antibody or fragment thereof binds to the same epitope or amino acid region on GFRAL as GDF15.

21. The compound of claim 17 or 18, wherein the compound is a peptide or protein which binds to the same epitope or amino acid region on GFRAL as GDF15.

22. A method of treating diabetes in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which agonizes the GFRAL receptor.

23. A method of treating obesity in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which agonizes the GFRAL receptor.

24. A method of treating cachexia in a mammal comprising administering to a mammal in need thereof an effective amount of a compound which antagonizes, inhibits, neutralizes or blocks the GFRAL receptor.

25. A protein comprising (a) a GDF15-binding soluble fragment of an insoluble human GFRAL receptor, wherein the insoluble human GFRAL receptor specifically binds to human GDF15 and (b) all of the domains of the constant region of a human immunoglobulin IgG heavy chain other than the first domain of said constant region; wherein said protein specifically binds human GDF15.

26. The protein of claim 25 wherein the insoluble human GFRAL receptor comprises SEQ ID NO: 4.

27. The protein of claim 25 wherein the protein consists essentially of the extracellular region of the insoluble human GFRAL receptor and all the domains of the constant region of a human IgG1 immunoglobulin heavy chain other than the first domain of the constant region.

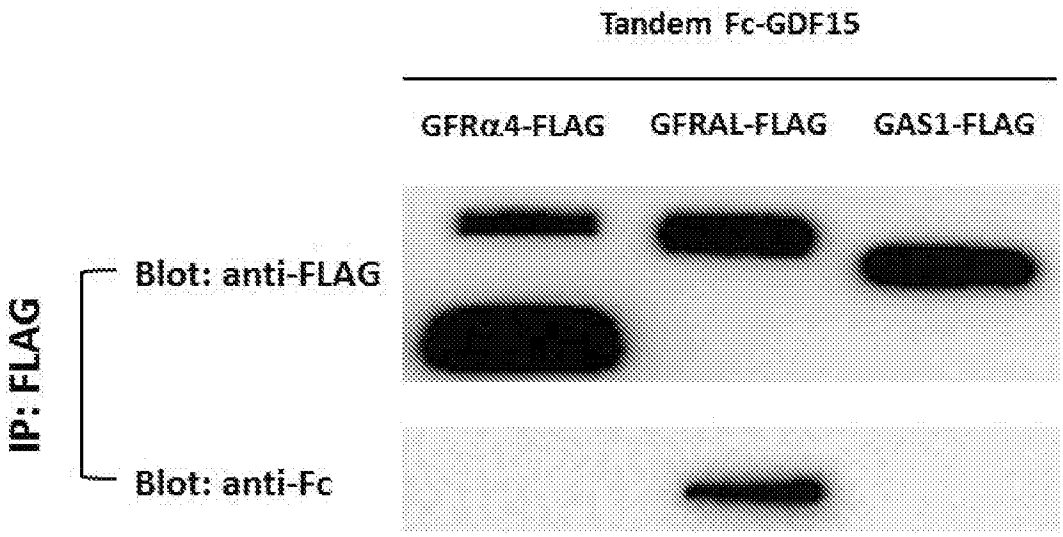
Tandem Fc-GDF15
GFRα4-FLAG
GFRAL-FLAG
GAS1-FLAG
IP: FLAG
Blot: anti-FLAG
Blot: anti-Fc

Fig. 1

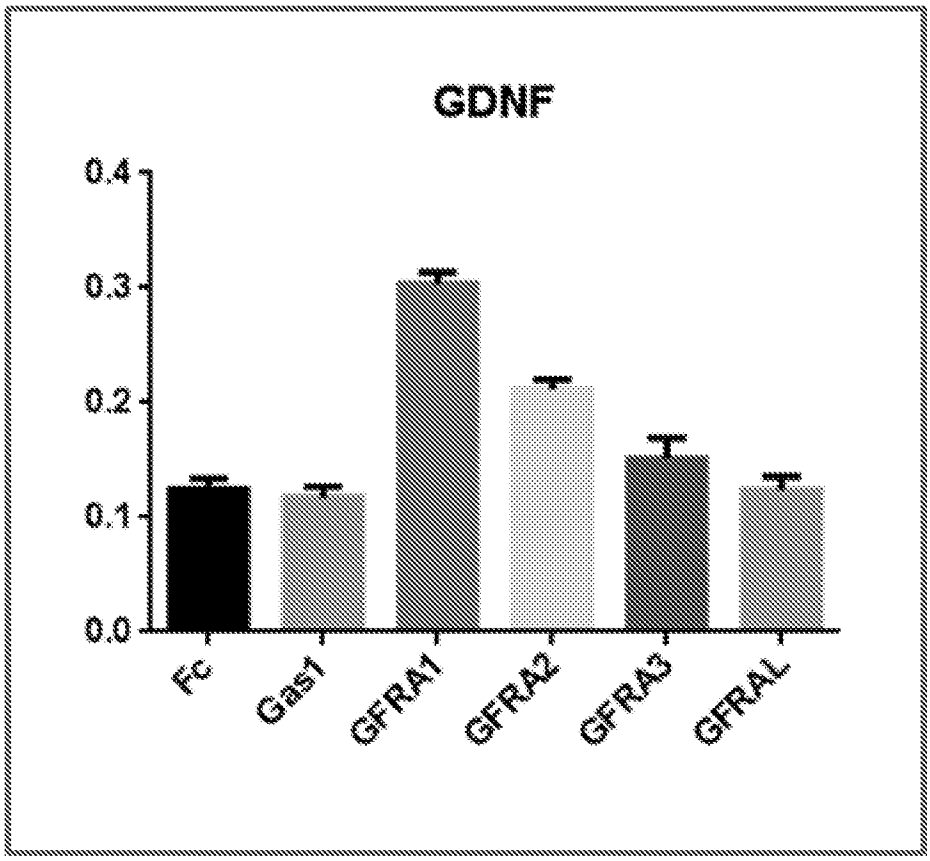

Fig. 2A

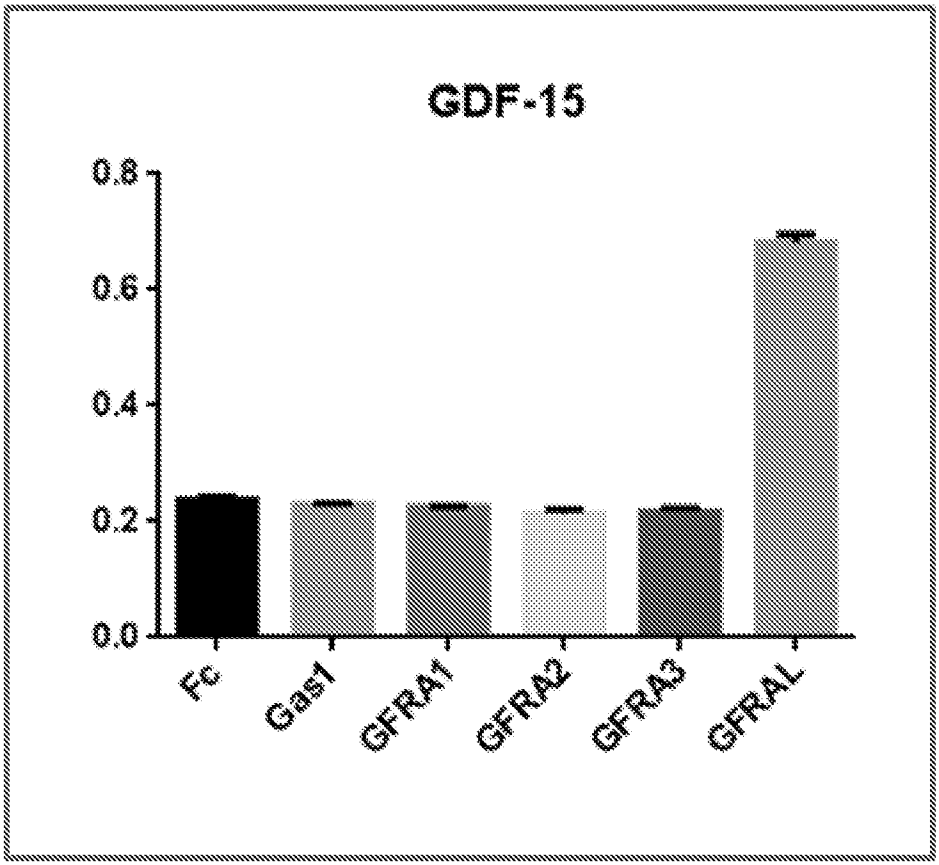

Fig. 2B

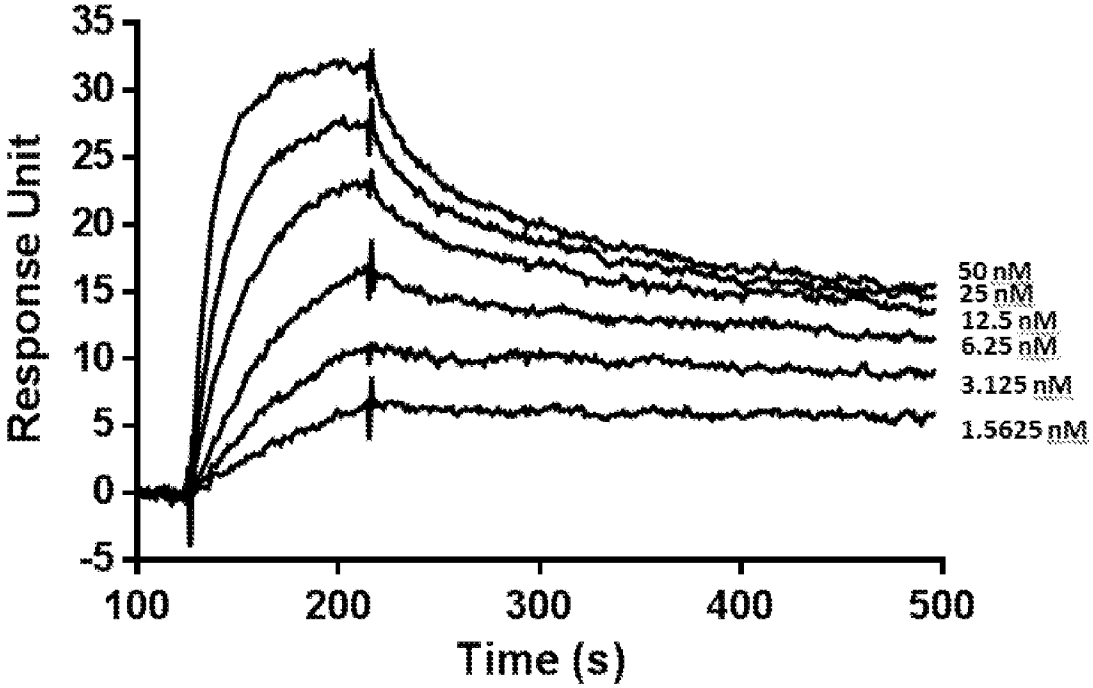
Response Unit
35
30
25
20
15
10
5
0
-5
100
200
300
400
500
Time (s)
50 nM
25 nM
12.5 nM
6.25 nM
3.125 nM
1.5625 nM

Fig. 3

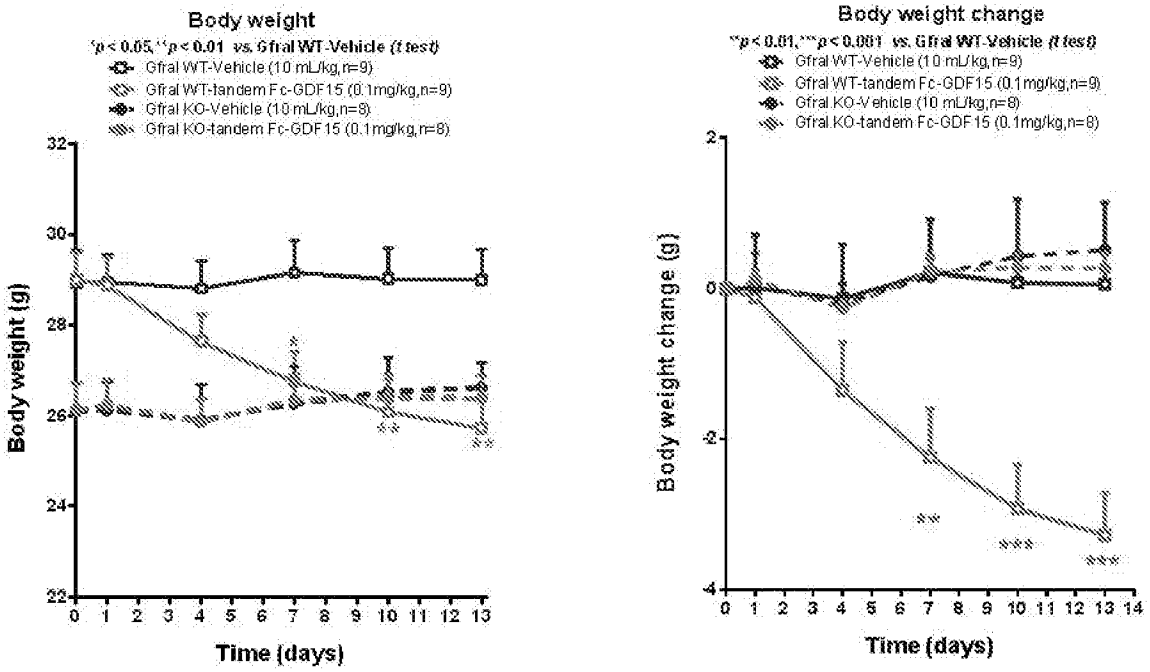
Body weight
*p < 0.05, **p < 0.01 vs. Gfral WT-Vehicle (t test)
Gfral WT-Vehicle (10 mL/kg,n=9)
Gfral WT-tandem Fc-GDF15 (0.1mg/kg,n=9)
Gfral KO-Vehicle (10 mL/kg,n=8)
Gfral KO-tandem Fc-GDF15 (0.1mg/kg,n=8)
Body weight (g)
Time (days)
Body weight change
**p < 0.01, ***p < 0.001 vs. Gfral WT-Vehicle (t test)
Gfral WT-Vehicle (10 mL/kg,n=9)
Gfral WT-tandem Fc-GDF15 (0.1mg/kg,n=9)
Gfral KO-Vehicle (10 mL/kg,n=8)
Gfral KO-tandem Fc-GDF15 (0.1mg/kg,n=8)
Body weight change (g)
Time (days)

Fig. 4A

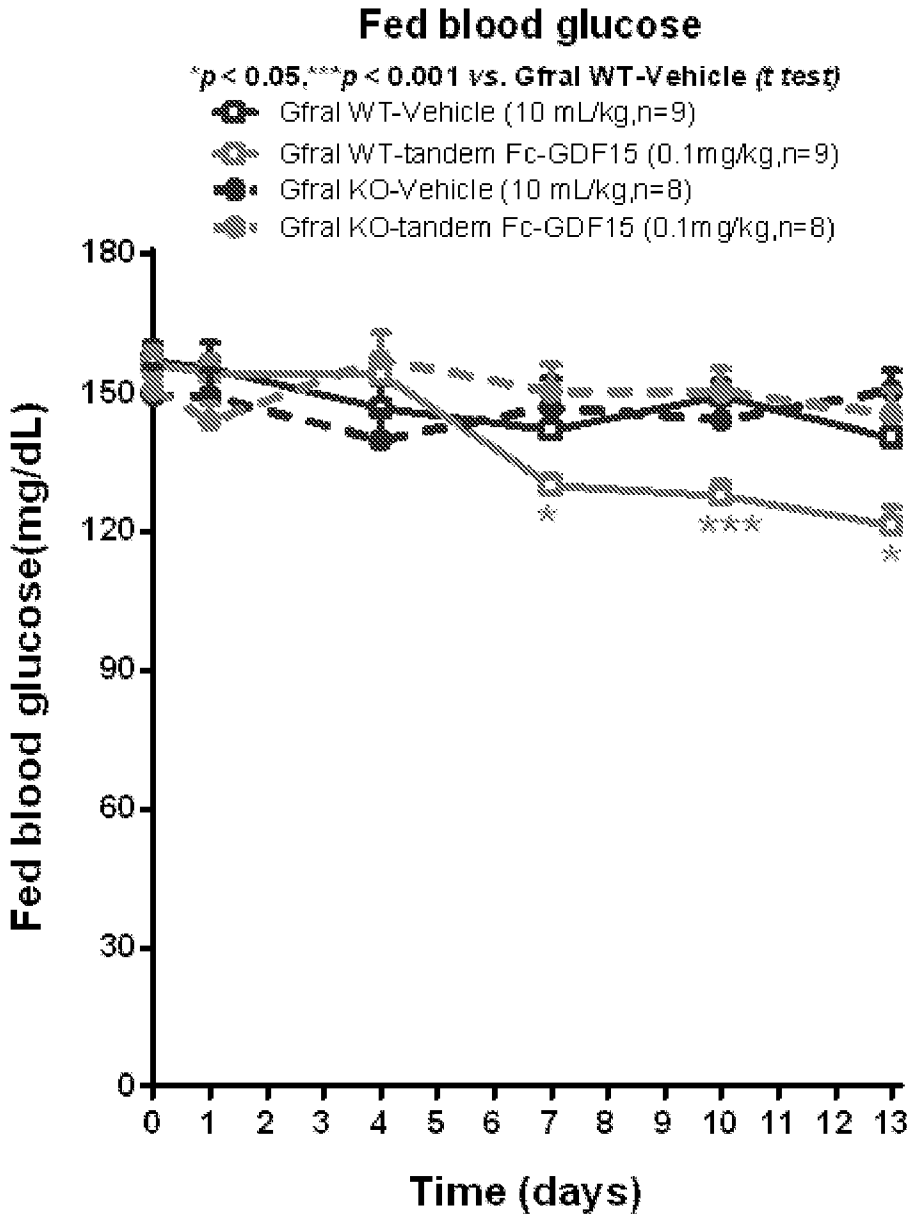
Fed blood glucose
*p < 0.05,***p < 0.001 vs. Gfral WT-Vehicle (t test)
Gfral WT-Vehicle (10 mL/kg,n=9)
Gfral WT-tandem Fc-GDF15 (0.1mg/kg,n=9)
Gfral KO-Vehicle (10 mL/kg,n=8)
Gfral KO-tandem Fc-GDF15 (0.1mg/kg,n=8)
Fed blood glucose(mg/dL)
180
150
120
90
60
30
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Time (days)

Fig. 4B

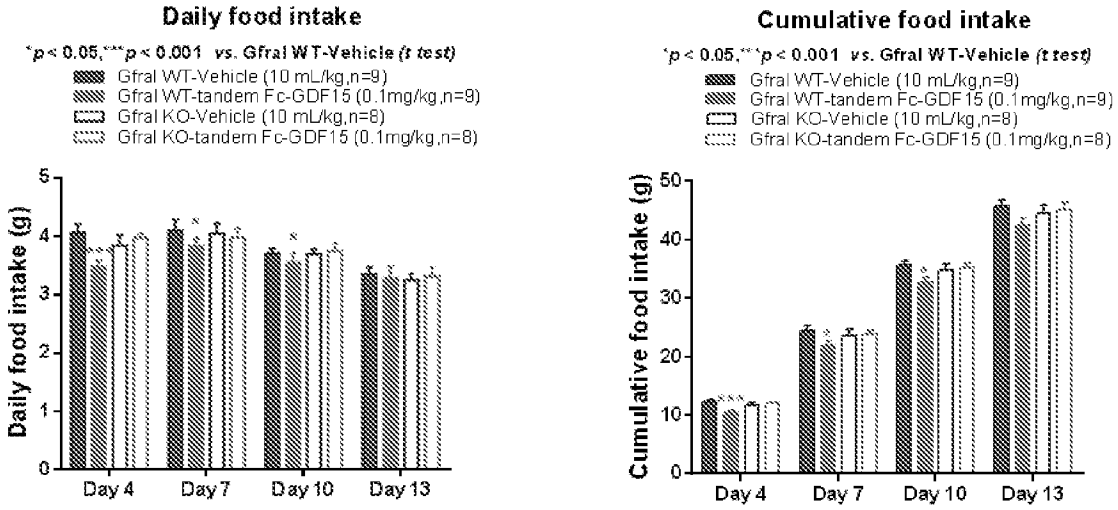
Daily food intake
*p < 0.05,***p < 0.001 vs. Gfral WT-Vehicle (t test)
Gfral WT-Vehicle (10 mL/kg,n=9)
Gfral WT-tandem Fc-GDF15 (0.1mg/kg,n=9)
Gfral KO-Vehicle (10 mL/kg,n=8)
Gfral KO-tandem Fc-GDF15 (0.1mg/kg,n=8)
Daily food intake (g)
0
1
2
3
4
5
Day 4
Day 7
Day 10
Day 13
Cumulative food intake
*p < 0.05,** *p < 0.001 vs. Gfral WT-Vehicle (t test)
Gfral WT-Vehicle (10 mL/kg,n=9)
Gfral WT-tandem Fc-GDF15 (0.1mg/kg,n=9)
Gfral KO-Vehicle (10 mL/kg,n=8)
Gfral KO-tandem Fc-GDF15 (0.1mg/kg,n=8)
Cumulative food intake (g)
0
10
20
30
40
50
Day 4
Day 7
Day 10
Day 13

Fig. 4C

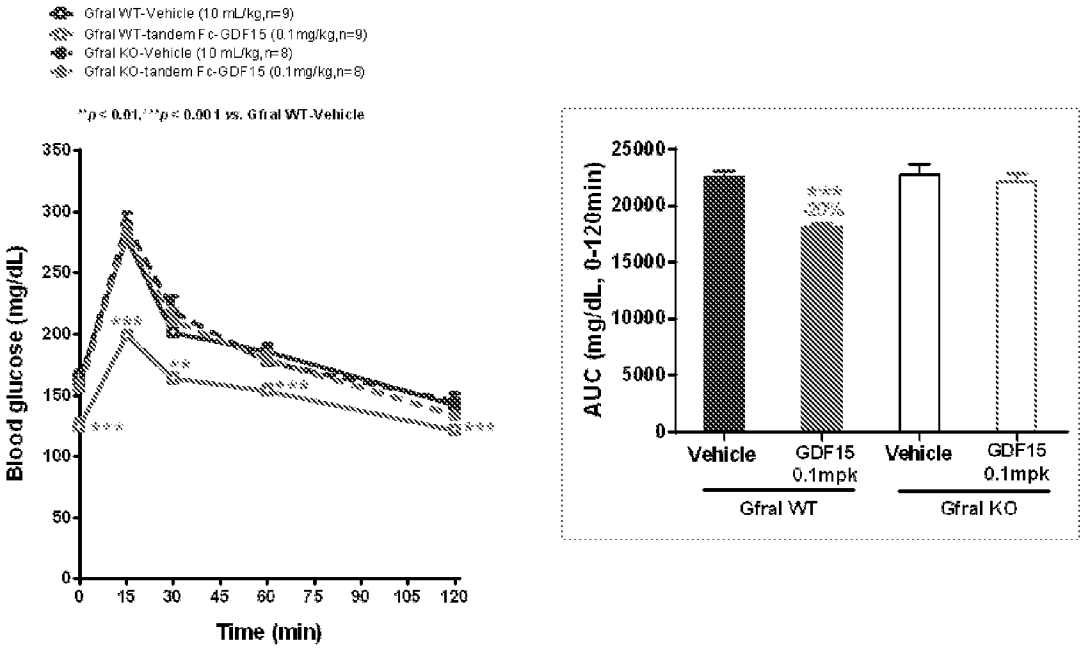
Gfral WT-Vehicle (10 mL/kg,n=9)
Gfral WT-tandem Fc-GDF15 (0.1mg/kg,n=9)
Gfral KO-Vehicle (10 mL/kg,n=8)
Gfral KO-tandem Fc-GDF15 (0.1mg/kg,n=8)
**p < 0.01, ***p < 0.001 vs. Gfral WT-Vehicle
Blood glucose (mg/dL)
Time (min)
AUC (mg/dL, 0-120min)
Vehicle
GDF15 0.1mpk
Vehicle
GDF15 0.1mpk
Gfral WT
Gfral KO

Fig. 4D

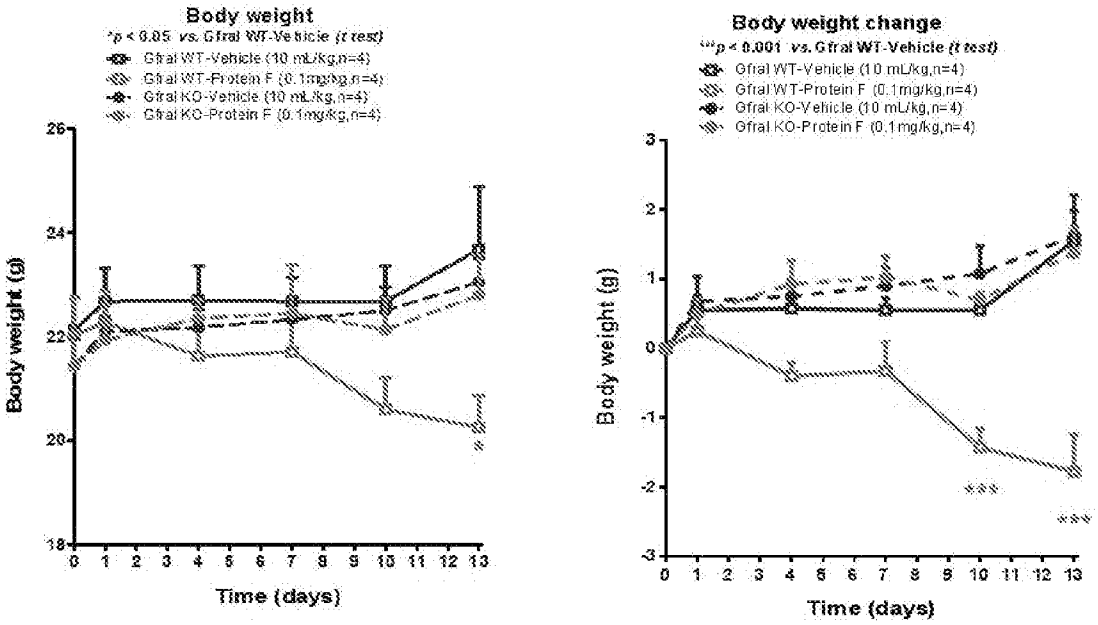
Body weight
*p < 0.05 vs. Gfral WT-Vehicle (t test)
Gfral WT-Vehicle (10 mL/kg,n=4)
Gfral WT-Protein F (0.1mg/kg,n=4)
Gfral KO-Vehicle (10 mL/kg,n=4)
Gfral KO-Protein F (0.1mg/kg,n=4)
Body weight (g)
Time (days)
Body weight change
***p < 0.001 vs. Gfral WT-Vehicle (t test)
Gfral WT-Vehicle (10 mL/kg,n=4)
Gfral WT-Protein F (0.1mg/kg,n=4)
Gfral KO-Vehicle (10 mL/kg,n=4)
Gfral KO-Protein F (0.1mg/kg,n=4)
Body weight (g)
Time (days)

Fig. 4E

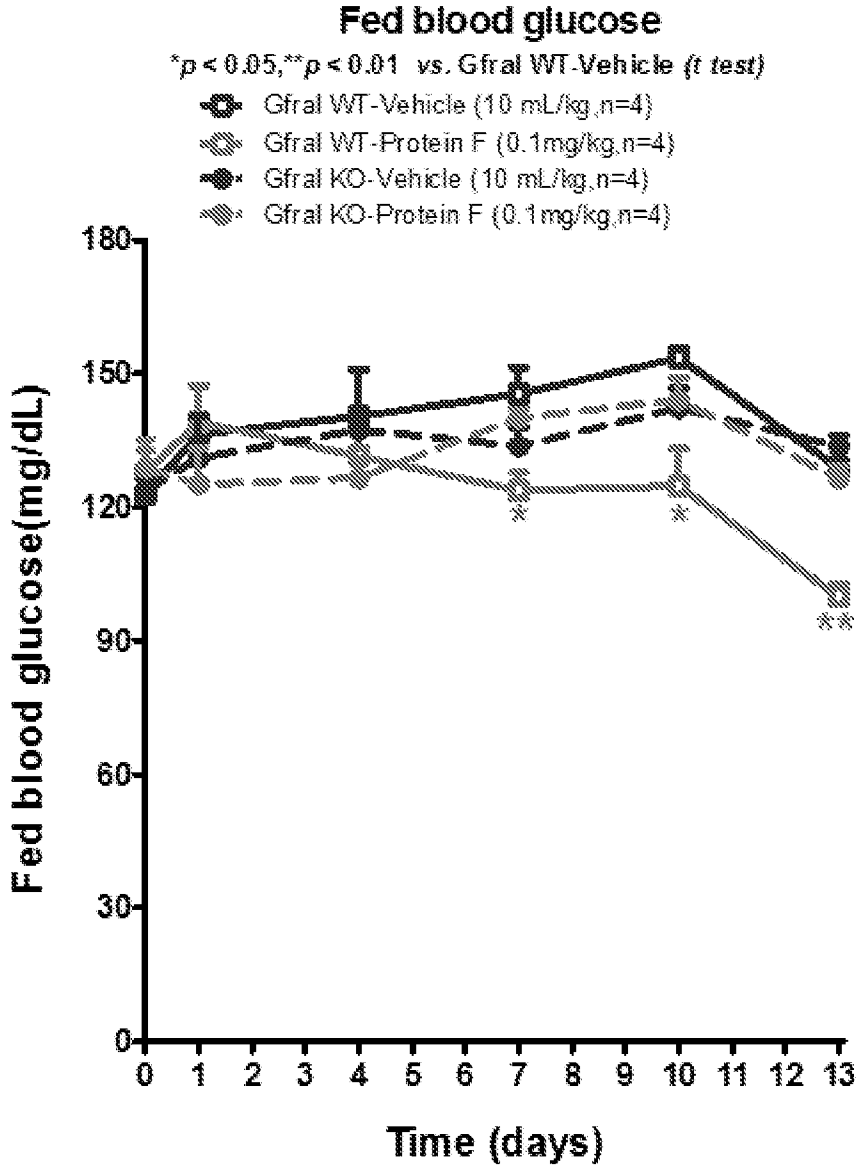
Fed blood glucose
*p < 0.05,**p < 0.01 vs. Gfral WT-Vehicle (t test)
Gfral WT-Vehicle (10 mL/kg,n=4)
Gfral WT-Protein F (0.1mg/kg,n=4)
Gfral KO-Vehicle (10 mL/kg,n=4)
Gfral KO-Protein F (0.1mg/kg,n=4)
Fed blood glucose(mg/dL)
180
150
120
90
60
30
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Time (days)

Fig. 4F

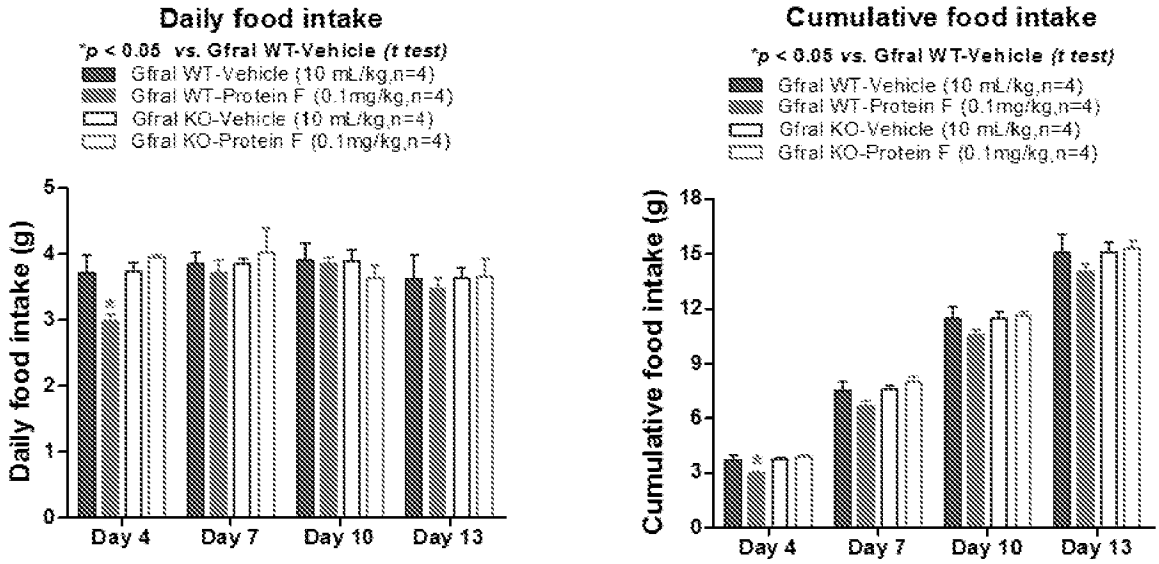
Daily food intake
*p < 0.05 vs. Gfral WT-Vehicle (t test)
Gfral WT-Vehicle (10 mL/kg,n=4)
Gfral WT-Protein F (0.1mg/kg,n=4)
Gfral KO-Vehicle (10 mL/kg,n=4)
Gfral KO-Protein F (0.1mg/kg,n=4)
Daily food intake (g)
0
1
2
3
4
5
Day 4
Day 7
Day 10
Day 13
Cumulative food intake
*p < 0.05 vs. Gfral WT-Vehicle (t test)
Gfral WT-Vehicle (10 mL/kg,n=4)
Gfral WT-Protein F (0.1mg/kg,n=4)
Gfral KO-Vehicle (10 mL/kg,n=4)
Gfral KO-Protein F (0.1mg/kg,n=4)
Cumulative food intake (g)
0
3
6
9
12
15
18
Day 4
Day 7
Day 10
Day 13

Fig. 4G

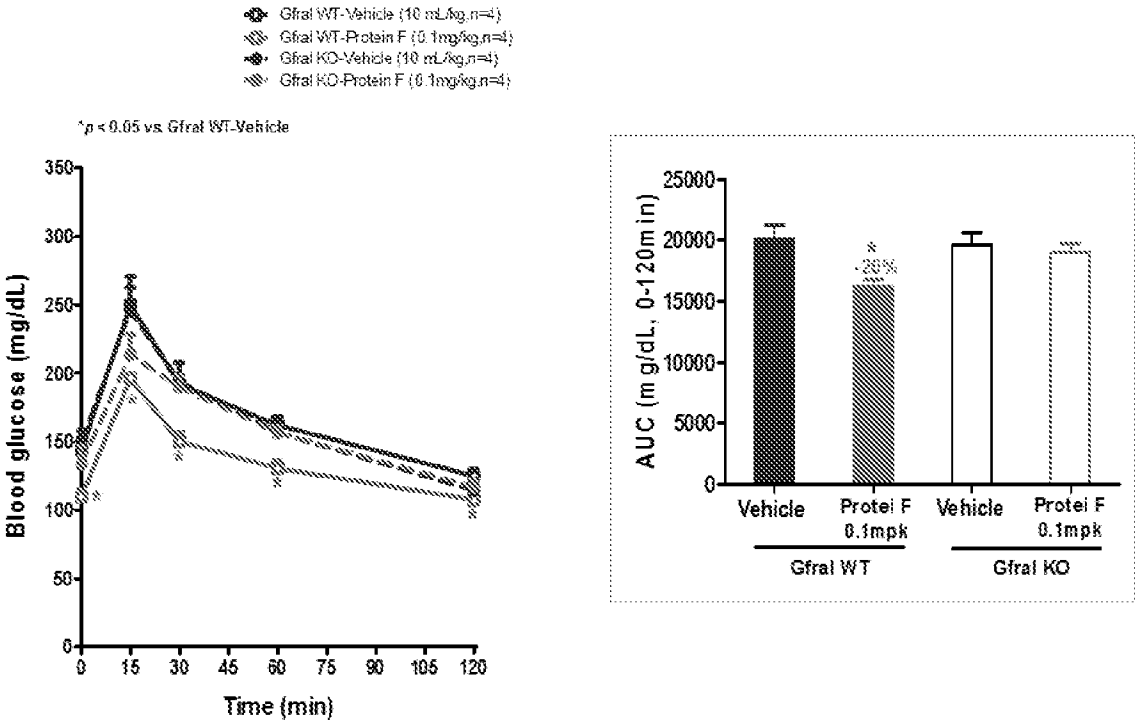
Gfral WT-Vehicle (10 mL/kg,n=4)
Gfral WT-Protein F (0.1mg/kg,n=4)
Gfral KO-Vehicle (10 mL/kg,n=4)
Gfral KO-Protein F (0.1mg/kg,n=4)
*p < 0.05 vs. Gfral WT-Vehicle
Blood glucose (mg/dL)
Time (min)
AUC (mg/dL, 0-120min)
-20%
Vehicle
Protei F 0.1mpk
Vehicle
Protei F 0.1mpk
Gfral WT
Gfral KO

Fig. 4H

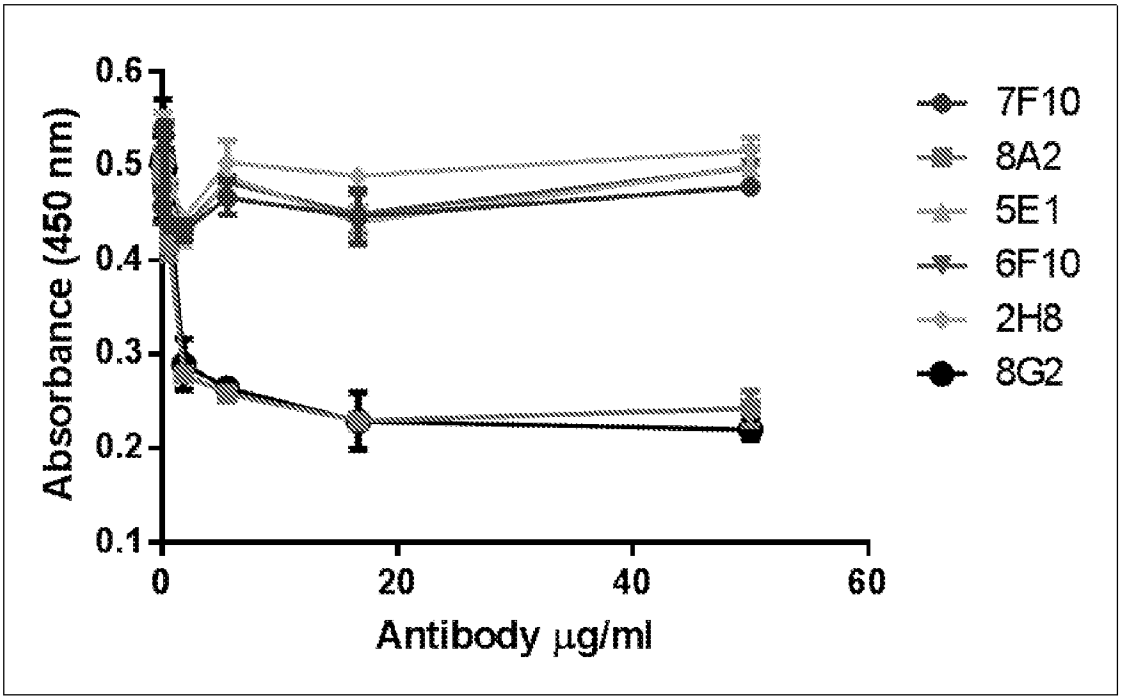
0.6
0.5
0.4
0.3
0.2
0.1
Absorbance (450 nm)
0
20
40
60
Antibody μg/ml
7F10
8A2
5E1
6F10
2H8
8G2

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/074809

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395(2006.01)i; A61P 3/04(2006.01)i; A61P 3/08(2006.01)i; A61P 3/10(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K; A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNMED;CPRSABS;CNABS;DWPI;SIPOABS;CNTXT;WOTXT;EPTXT;JPTXT;CNKI;Pubmed;Google scholar: GFR alpha receptor like, GFRAL, GFR alpha, GDNF, GDF15, glucose, diabetes, T2DM, obesity, metabolism, body weight, agoni+, antagoni +, antibody, Ig, immunoglobulin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Liu H et al. "GDNF Jia Zu Shou Ti α (GFRα) Yang Dan Bai GFRAL Zai Bu Ru Dong Wu Shen Jing Xi Bao De Biao Da He Gong Neng Yan Jiu" *Proceedings of The 7th Biennial Meeting and the 5th Congress of the Chinese Society for Neuroscience,* 10 December 2013 (2013-12-10), Page 269 See the whole document	1-27
A	Hong JH et al. "GDF15 Is a Novel Biomarker for Impaired Fasting Glucose" *DIABETES & METABOLISM JOURNAL,* Vol. 38, No. 6, 15 December 2014 (2014-12-15), Pages 472-479 See the whole document	1-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 November 2016	**05 December 2016**

Name and mailing address of the ISA/CN	Authorized officer
STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China**	**LI,Kangqi**
Facsimile No. **(86-10)62019451**	Telephone No. **(86-10)62411034**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/074809

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☑ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☑ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/074809

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **1-12,22-24**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 1-12, 22-24 relate to methods for treating various kinds of diseases or pathologic conditions in a mammal. The subject matter of claims 1-12, 22-24 relates to a treatment method of the human or animal body, and therefore, according to the criteria set out in Rule 39.1(iv), relates to subject matter for which an international search is not required.

[2] Claims 1-12, 22-24 might be reasonably expected to be direct to the alleged uses of a compound which angonizes or antagonizes the GFRAL receptor for the manufacture of pharmaceutical compositions for treating or preventing the diseases or pathologic conditions in a mammal. The international search is carried out on the basis of said condition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).