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(54) Titre : PROCEDES DE TRAITEMENT DE LA RESISTANCE A L'INSULINE GRAVE PAR INTERFERENCE AVEC LA SIGNALISATION DU RECEPTEUR DU GLUCAGON

(54) Title: METHODS OF TREATING SEVERE INSULIN RESISTANCE BY INTERFERING WITH GLUCAGON RECEPTOR SIGNALING

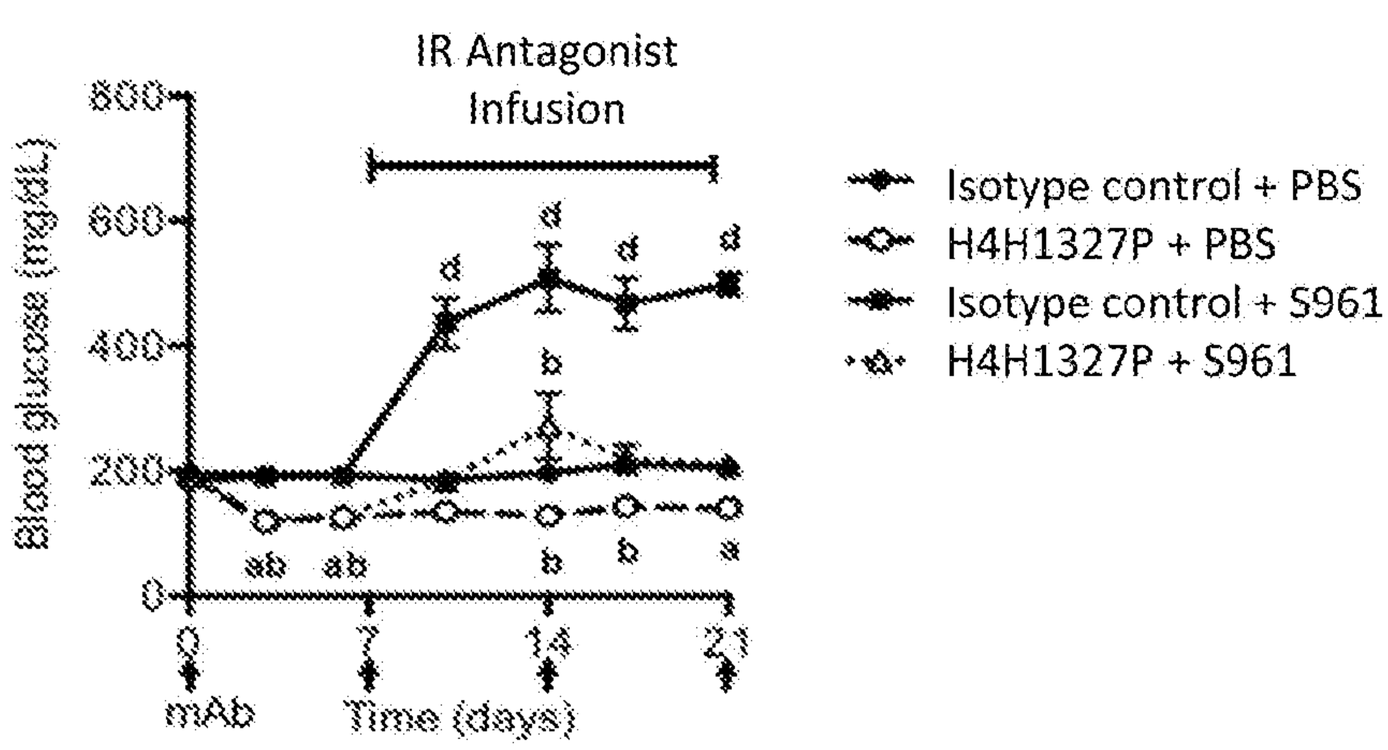

FIG. 1A

(57) Abrégé/Abstract:
Provided herein are methods of treating a patient with severe insulin resistance. The methods comprise administering to a patient in need thereof a therapeutic amount of a GCG/GCGR signaling pathway inhibitor, such that blood glucose or beta-hydroxybutyrate levels are lowered or that the severe insulin resistance is mediated, or a condition or disease characterized by severe insulin resistance is mediated, or at least one symptom or complication associated with the condition or disease is alleviated or reduced in severity. The GCG/GCGR signaling pathway inhibitor can be a small molecule inhibitor of the signaling pathway, an antisense inhibitor of the signaling pathway, a GCG neutralizing monoclonal antibody, a GCGR antagonist, a peptide inhibitor of the signaling pathway, a DARPin, a Spiegelmer, an aptamer, engineered Fn type-III domains, etc. The therapeutic methods are useful for treating a human suffering from severe insulin resistance.

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(54) Title: METHODS OF TREATING SEVERE INSULIN RESISTANCE BY INTERFERING WITH GLUCAGON RECEPTOR SIGNALING

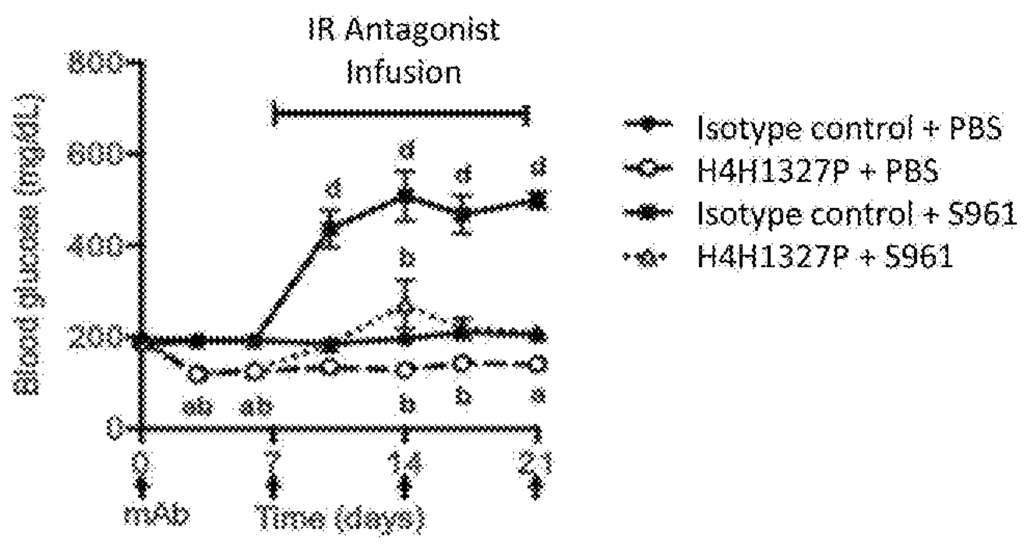

FIG. 1A

(57) Abstract: Provided herein are methods of treating a patient with severe insulin resistance. The methods comprise administering to a patient in need thereof a therapeutic amount of a GCG/GCGR signaling pathway inhibitor, such that blood glucose or beta-hydroxybutyrate levels are lowered or that the severe insulin resistance is mediated, or a condition or disease characterized by severe insulin resistance is mediated, or at least one symptom or complication associated with the condition or disease is alleviated or reduced in severity. The GCG/GCGR signaling pathway inhibitor can be a small molecule inhibitor of the signaling pathway, an antisense inhibitor of the signaling pathway, a GCG neutralizing monoclonal antibody, a GCGR antagonist, a peptide inhibitor of the signaling pathway, a DARPin, a Spiegelmer, an aptamer, engineered Fn type-III domains, etc. The therapeutic methods are useful for treating a human suffering from severe insulin resistance.

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WO 2018/044903 A1

WO 2018/044903 A1

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METHODS OF TREATING SEVERE INSULIN RESISTANCE BY INTERFERING WITH GLUCAGON RECEPTOR SIGNALING

TECHNICAL FIELD

[0001] The invention relates to methods of using a glucagon (GCG) inhibitor or a glucagon receptor (GCGR) antagonist to treat or to slow the progression of severe insulin resistance, and/or reducing the therapeutic insulin dose in a patient in need thereof.

SEQUENCE LISTING

[0002] An official copy of the sequence listing is submitted concurrently with the specification electronically via EFS-Web as an ASCII formatted sequence listing with a file name of 10282WO01_SEQ_LIST_ST25, a creation date of August 25, 2017, and a size of about 116 kilobytes. The sequence listing contained in this ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND

[0003] Glucagon is a 29 residue polypeptide hormone, which in cooperation with insulin, mediates homeostatic regulation of the amount of glucose in the blood. Glucagon primarily acts by stimulating certain cells, for example, liver cells, to release glucose when blood glucose levels fall to maintain normal blood glucose levels. The action of glucagon is opposite to that of insulin, which stimulates cells to take up and store glucose whenever blood glucose levels rise. Glucagon is produced in the alpha cells of the pancreas, whereas insulin is secreted from the neighboring beta cells.

[0004] It is an imbalance of glucagon and insulin that may play an important role in several diseases, such as diabetes mellitus and diabetic ketoacidosis. In particular, studies have shown that higher basal glucagon levels and lack of suppression of postprandial glucagon secretion contribute to diabetic conditions in humans (Muller et al. (1970), N Eng J Med, 283: 109-115).

[0005] It is believed that glucagon's effects on elevating blood glucose levels are mediated in part by the activation of certain cellular pathways following the binding of glucagon (GCG) to its receptor (designated GCGR). GCGR is a member of the secretin subfamily (family B) of G-protein-coupled receptors and is predominantly expressed in the liver. The binding of glucagon to its receptor triggers a G-protein signal transduction cascade, activating intracellular cyclic AMP and leading to an increase in glucose output through de

novo synthesis (gluconeogenesis) and glycogen breakdown (glycogenolysis) (Wakelam et al., (1986) Nature, 323:68-71; Unson et al., (1989) Peptides, 10:1171-1177; and Pittner and Fain, (1991) Biochem. J., 277:371-378).

[0006] The action of glucagon can be suppressed by providing an antagonist, such as a small molecule inhibitor, a GCG antibody, or a GCGR antibody, as described herein. Anti-GCG antibodies are mentioned, e.g., in U.S. Pat. Nos. 4,206,199; 4,221,777; 4,423,034; 4,272,433; 4,407,965; 5,712,105; and in PCT publications WO2007/124463 and WO2013/081993. Anti-GCGR antibodies are described in U.S. Pat. Nos. 5,770,445, 7,947,809, and 8,545,847; European patent application EP2074149A2; EP patent EP0658200B1; US patent publications 2009/0041784; 2009/0252727; and 2011/0223160; and PCT publication WO2008/036341. Small molecule inhibitors of GCG or GCGR are mentioned, e.g. in WO 07/47676; WO 06/86488; WO 05/123688; WO 05/121097; WO 06/14618; WO 08/42223; WO 08/98244; WO 2010/98948; US 20110306624; WO 2010/98994; WO 2010/88061; WO 2010/71750; WO 2010/30722; WO 06/104826; WO 05/65680; WO 06/102067; WO 06/17055; WO 2011/07722; or WO 09/140342.

[0007] Severe insulin resistance syndromes are rare metabolic disorders in which patients do not respond well to insulin. Current treatments available for severe insulin resistance syndromes include regular feedings and very high doses of insulin in attempt to provide adequate glycemic control. Administration of IGF-I, while effective in the short term, failed to provide long-term glycemic control in patients with severe insulin resistance. Vestergaard et al., (1997) European Journal of Endocrinology, 136:475-482. Administration of recombinant leptin has shown some success in patients with Rabson-Mendenhall syndrome (RMS) by reducing blood glucose levels over several months. Cochran et al., (2004) Journal of Clinical Endocrinology and Metabolism, 89:1548-1554.

[0008] Given the absence of effective therapies to treat, or to slow the progression of severe insulin resistance disease, i.e., to extend the life and/or improve the quality of life of a patient having severe insulin resistance, there is a need to identify and explore the use of other agents for treating these diseases, such as the GCG/GCGR signaling pathway inhibitors and antagonists as described herein.

BRIEF SUMMARY

[0009] Provided herein are methods for treating a patient with a condition or disease characterized by severe insulin resistance by administering a GCG inhibitor or a GCGR

antagonist, e.g. a pharmaceutical composition comprising a GCG inhibitor or GCGR antagonist. A GCG inhibitor or GCGR antagonist is a compound capable of blocking or inhibiting the glucagon receptor signaling pathway. The antagonist may take the form of a small molecule inhibitor, peptide inhibitor, CRISPR technology (Clustered regularly interspaced short palindromic repeats; CRISPR technology can generate GCGR knock-down or deletion of regulatory sequences affecting GCGR activity), an antisense inhibitor, DARPin, and a GCG or GCGR neutralizing monoclonal antibody. The GCG inhibitor or GCGR antagonist can be administered alone, in a pharmaceutical composition, or in conjunction with one or more therapeutic agents useful in treating a condition or disease associated with severe insulin resistance, or in treating one or more symptoms associated with the condition or disease, or in lowering blood glucose and/or ketones in a patient having a condition or disease associated with severe insulin resistance.

[0010] In some embodiments, methods are provided for lowering blood glucose levels and/or beta-hydroxybutyrate levels, or for decreasing ketonemia and/or ketoacidosis, or for treating a condition or disease associated with, or characterized in part by high blood glucose and/or ketonemia and/or ketoacidosis, or at least one symptom or complication associated with the condition or disease. In some aspects, the method comprises administering to a patient having severe insulin resistance a therapeutically effective amount of a composition comprising an inhibitor of GCG/GCGR signaling, such that blood glucose or beta-hydroxybutyrate levels are lowered or that the condition or disease is mediated, or at least one symptom or complication associated with the condition or disease is alleviated or reduced in severity. In some embodiments, the inhibitor of GCGR signaling is a GCGR antagonist, such as an anti-GCGR antibody. In some embodiments, the anti-GCGR antibody has a HCVR/LCVR sequence pair of SEQ ID NOs: 86/88. In some embodiments, the inhibitor of GCGR signaling is a GCG inhibitor, such as an anti-GCG antibody. In some embodiments, the anti-GCG antibody has a HCVR/LCVR sequence pair of SEQ ID NOs: 182/190. In some embodiments, the anti-GCG antibody has a HCVR/LCVR sequence pair of SEQ ID NOs: 166/174.

[0011] In some aspects, methods are provided for treating a patient with severe insulin resistance, wherein the patient exhibits elevated levels of blood glucose. The method comprises administering to the patient a therapeutically effective amount of a composition comprising a GCG inhibitor or a GCGR antagonist.

[0012] In some aspects, methods are provided for treating a patient with severe insulin resistance, wherein the patient does not exhibit elevated levels of blood glucose. The

method comprises administering to the patient a therapeutically effective amount of a composition comprising a GCG inhibitor or a GCGR antagonist.

[0013] In some embodiments, methods are provided for reducing the amount and/or dosage of insulin necessary to treat a patient with severe insulin resistance, wherein the patient exhibits severe insulin resistance and/or elevated levels of blood glucose. In some aspects, the method comprises administering to the patient a therapeutically effective amount of a composition comprising a GCG inhibitor or a GCGR antagonist. In some aspects, the GCG inhibitor or GCGR antagonist is administered concomitantly with insulin. The amount and/or dosage of insulin may be reduced by about 30% to about 95%, or by about 90%, when administered concomitantly with an isolated human monoclonal antibody that binds specifically to the GCGR.

[0014] In some aspects, the GCGR antagonist can be an anti-GCGR antibody. The anti-GCGR antibody can inhibit or antagonize the GCGR. The anti-GCGR antibody can inhibit or block the GCGR signaling pathway. In some aspects, the GCG inhibitor can be an anti-GCG antibody. The anti-GCG antibody can inhibit binding of GCG to the GCGR.

[0015] In certain embodiments, the antibody or antigen-binding fragment specifically binds hGCGR, and comprises the heavy and light chain CDR domains contained within heavy and light chain sequence pairs selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/68, 70/78, 86/88, 90/98, 106/108, 110/118, 126/128, 130/138 and 146/148.

[0016] In certain embodiments, the antibody or antigen-binding fragment comprises the heavy and light chain CDR domains contained within the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 86/88.

[0017] In certain embodiments, the antibody or antigen-binding fragment comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 86/88.

[0018] In one embodiment, the human antibody or antigen-binding fragment of a human antibody that binds hGCGR, comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0019] In one embodiment, the human antibody or antigen-binding fragment of a human antibody that binds hGCGR comprises a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68,

78, 88, 98, 108, 118, 128, 138 and 148, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0020] In certain embodiments, the human antibody or fragment thereof that binds hGCGR comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/68, 70/78, 86/88, 90/98, 106/108, 110/118, 126/128, 130/138, and 146/148. In certain embodiments, the HCVR/LCVR amino acid sequence pair is selected from the group consisting of SEQ ID NO: 34/42, 70/78, 86/88, 110/118 and 126/128.

[0021] In certain embodiments, the isolated human antibody or an antigen-binding fragment thereof that binds specifically to hGCGR comprises a HCVR comprising the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within the HCVR sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and/or a LCVR comprising the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within the LCVR sequences selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148.

[0022] In certain embodiments, the methods provided herein contemplate the use of an isolated human antibody or antigen-binding fragment thereof that binds hGCGR comprising a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 76, 96, 116 and 136, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and/or a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 84, 104, 124 and 144, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0023] In one embodiment, the methods provided herein contemplate use of an antibody or fragment thereof that further comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 72, 92, 112 and 132, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 74, 94, 114 and 134, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 80, 100, 120 and 140, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99%

sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 82, 102, 122 and 142, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0024] In one embodiment, the antibody or antigen-binding fragment of an antibody comprises:

(a) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 76, 96, 116 and 136; and

(b) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 84, 104, 124 and 144.

[0025] In a related embodiment, the antibody or antigen-binding fragment of the antibody further comprises:

(c) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 72, 92, 112 and 132;

(d) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 74, 94, 114 and 134;

(e) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 80, 100, 120 and 140; and

(f) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 82, 102, 122 and 142.

[0026] In one embodiment, the antibody or antigen-binding fragment thereof comprises a HCVR comprising a HCDR1 domain having an amino acid sequence selected from one of SEQ ID NO: 4, 20, 36, 52, 72, 92, 112 and 132; a HCDR2 domain having an amino acid sequence selected from one of SEQ ID NO: 6, 22, 38, 54, 74, 94, 114 and 134; a HCDR3 domain having an amino acid sequence selected from one of SEQ ID NOs: 8, 24, 40, 56, 76, 96, 116 and 136; and a LCVR comprising a LCDR1 domain having an amino acid sequence selected from one of SEQ ID NO: 12, 28, 44, 60, 80, 100, 120 and 140; a LCDR2 domain having an amino acid sequence selected from one of SEQ ID NO: 14, 30, 46, 62, 82, 102, 122 and 142; and a LCDR3 domain having an amino acid sequence selected from one of SEQ ID NO: 16, 32, 48, 64, 84, 104, 124 and 144.

[0027] In certain embodiments, the human antibody or antigen-binding fragment of a human antibody that binds to human GCGR comprises a HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NO: 8/16, 24/32, 40/48, 56/64, 76/84, 86/88, 96/104, 116/124 and 136/144. Non-limiting examples of anti-GCGR antibodies

having these HCDR3/LCDR3 pairs are the antibodies designated H4H1345N, H4H1617N, H4H1765N, H4H1321B and H4H1321P, H4H1327B and H4H1327P, H4H1328B and H4H1328P, H4H1331B and H4H1331P, H4H1339B and H4H1339P, respectively.

[0028] In one embodiment, the isolated antibody or antigen-binding fragment thereof useful according to the methods provided herein, that specifically binds to GCG and neutralizes at least one activity associated with GCG, comprises: (a) three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) amino acid sequence selected from the group consisting of SEQ ID NOs: 150, 166, 182, 198, 214, 230, 246, 262, 278 and 294; and (b) three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) amino acid sequence selected from the group consisting of SEQ ID NOs: 158, 174, 190, 206, 222, 238, 254, 270, 286 and 302.

[0029] In some embodiments, the isolated antibody or antigen-binding fragment thereof that specifically binds to GCG and neutralizes at least one activity associated with GCG, comprises an HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 150, 166, 182, 198, 214, 230, 246, 262, 278 and 294 and a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 158, 174, 190, 206, 222, 238, 254, 270, 286 and 302.

[0030] In some embodiments, the isolated antibody or antigen-binding fragment thereof that specifically binds to GCG and neutralizes at least one activity associated with GCG, comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 150/158; 166/174; 182/190; 198/206; 214/222; 230/238; 246/254; 262/270; 278/286 and 294/302.

[0031] In some embodiments, the HCVR/LCVR amino acid sequence pair comprises SEQ ID NOs: 166/174.

[0032] In some embodiments, the HCVR/LCVR amino acid sequence pair comprises SEQ ID NOs: 182/190.

[0033] In one embodiment, the isolated antibody or antigen-binding fragment thereof useful according to the methods provided herein, comprises:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 152, 168, 184, 200, 216, 232, 248, 264, 280, and 296;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 154, 170, 186, 202, 218, 234, 250, 266, 282, and 298;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 156, 172, 188, 204, 220, 236, 252, 268, 284, and 300;
(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 160, 176, 192, 208, 224, 240, 256, 272, 288, and 304;
(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 162, 178, 194, 210, 226, 242, 258, 274, 290, and 306; and
(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 164, 180, 196, 212, 228, 244, 260, 276, 292, and 308.

[0034] In one embodiment, the isolated antibody or antigen-binding fragment thereof useful according to the methods provided herein, comprises:
(a) a HCDR1 domain comprising the amino acid sequence of SEQ ID NO: 168;
(b) a HCDR2 domain comprising the amino acid sequence of SEQ ID NO: 170;
(c) a HCDR3 domain comprising the amino acid sequence of SEQ ID NO: 172;
(d) a LCDR1 domain comprising the amino acid sequence of SEQ ID NO: 176;
(e) a LCDR2 domain comprising the amino acid sequence of SEQ ID NO: 178; and
(f) a LCDR3 domain comprising the amino acid sequence of SEQ ID NO: 180.

[0035] In one embodiment, the isolated antibody or antigen-binding fragment thereof useful according to the methods provided herein, comprises:
(a) a HCDR1 domain comprising the amino acid sequence of SEQ ID NO: 184;
(b) a HCDR2 domain comprising the amino acid sequence of SEQ ID NO: 186;
(c) a HCDR3 domain comprising the amino acid sequence of SEQ ID NO: 188;
(d) a LCDR1 domain comprising the amino acid sequence of SEQ ID NO: 192;
(e) a LCDR2 domain comprising the amino acid sequence of SEQ ID NO: 194; and
(f) a LCDR3 domain comprising the amino acid sequence of SEQ ID NO: 196.

[0036] Also useful according to the methods provided herein are antibodies or antigen-binding fragments thereof that specifically bind GCG, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences provided herein or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0037] Also useful according to the methods provided herein are antibodies or antigen-binding fragments thereof that specifically bind GCG, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences provided herein or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0038] Also useful according to the methods provided herein are antibodies or antigen-binding fragments thereof that specifically bind GCG, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences provided herein or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0039] Also useful according to the methods provided herein are antibodies or antigen-binding fragments thereof that specifically bind GCG, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences provided herein or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0040] Also useful according to the methods provided herein are antibodies or antigen-binding fragments thereof that specifically bind GCG, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences provided herein or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0041] Also useful according to the methods provided herein are antibodies or antigen-binding fragments thereof that specifically bind GCG, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed herein or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0042] Also useful according to the methods provided herein are antibodies or antigen-binding fragments thereof that specifically bind GCG, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid provided herein paired with any of the LCDR3 amino acid sequences provided herein. According to certain embodiments, the antibodies, or antigen-binding fragments thereof, comprise an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary anti-GCG antibodies provided herein. In certain embodiments, the HCDR3/LCDR3 amino acid sequence pair comprises SEQ ID NOs: 172/180.

[0043] Also useful according to the methods provided herein are antibodies or antigen-binding fragments thereof that specifically bind GCG, comprising a set of six CDRs (i.e., HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary anti-GCG antibodies provided herein. In certain embodiments, the HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 amino acid sequence set comprises SEQ ID NOs: 168/170/172/176/178/180. In certain embodiments, the

HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 amino acid sequence set comprises SEQ ID NOs: 184/186/188/192/194/196.

[0044] In a related embodiment, the antibodies, or antigen-binding fragments thereof that specifically bind GCG, comprise a set of six CDRs (i.e., HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-GCG antibodies provided herein. For example, the antibodies or antigen-binding fragments thereof that specifically bind GCG, comprise the HCDR1/HCDR2/HCDR3/LCDR1/LCDR2/LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of: 166/174; 182/190; 198/206; 214/222; 230/238; 246/254; 262/270; 278/286 and 294/302.

[0045] Non-limiting examples of antibodies that specifically bind GCG and comprise the CDR sequences provided above, include HIH059P, H4H10223P, H4H10231P, H4H10232P, H4H10236P, H4H10237P, H4H10238P, H4H10250P, H4H10256P, and H4H10270P.

[0046] Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, e.g., the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. See, e.g., Kabat, (1991) "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md.; Al-Lazikani et al., (1997) J. Mol. Biol. 273:927-948; and Martin et al., (1989) Proc. Natl. Acad. Sci. USA 86:9268-9272. Public databases are also available for identifying CDR sequences within an antibody.

[0047] In some embodiments, a patient having severe insulin resistance may suffer from one of the conditions or diseases selected from the following: Donohue syndrome, Rabson-Mendenhall syndrome, Type A insulin resistance, Type B insulin resistance, HAIR-AN (hyperandrogenism, insulin resistance, and acanthosis nigricans) syndrome, pseudoacromegaly, Alstrom syndrome, myotonic dystrophy, Werner's syndrome, lipodystrophy, cirrhosis, monogenic morbid obesity, hyperproinsulinemia, carboxypeptidase E deficiency, defective arginine metabolism, Bardet-Biedl syndrome, and a condition or disease associated with the presence of one or more gene variants reported to cause severe

insulin resistance. In some embodiments, insulin degrading protease activity is detected in the patient sera. In some embodiments, neutralizing anti-insulin antibodies or anti-insulin receptor antibodies are detected in the patient sera. In some patients, severe insulin resistance arises in the context of autoimmune destruction of adipocytes leading to lipodystrophy.

[0048] In some aspects, the gene variant associated with severe insulin resistance is selected from the following: *INSR, PSMD6, ADRA2A, AGPAT2* (associated with lipodystrophy and insulin resistance), *AKT2, APPL1, BBS1* (associated with Bardet-Beidl Syndrome 1), *BSCL2, CIDEC, GRB10, IRS2, KLF14, LEP, LEPR, LMNA* (associated with lipodystrophy), *MC4R, PCNT, PIK2CA, POLD1* (associated with lipodystrophy), *PPARG, PTPRD, PTRF* (associated with lipodystrophy), *RASGRP1, TBC1D4,* and *TCF7L2*.

[0049] In some aspects, the composition comprising the glucagon/GCGR antagonist is administered to a patient in combination with at least one additional therapeutic agent. The additional therapeutic agent can be any agent that alleviates or reduces the symptoms and signs associated with severe insulin resistance. In some embodiments, at least one additional therapeutic agent is selected from the following: insulin, a biguanide, hIGF1, leptin, metraleptin, pioglitazone, vildagliptin, acarbose, alpha-glycosidase inhibitors, L-arginine, dipeptidyl-peptidase-4 inhibitors, insulin secretagogues, amylin receptor agonists, insulin sensitizers, FGF21, SGLT2 inhibitors, SGLT1 inhibitors, GLP-1 receptor agonists, GLP-1 receptor activators, a second GCG inhibitor, and a second GCGR antagonist. In some aspects, the insulin secretagogue is selected from sulfonylureas, ATP-sensitive K channel antagonists, and meglitinides. In some aspects, the insulin sensitizer is selected from thiazolidinedione and rosiglitazone. In some aspects, the additional therapeutic agent can be an agent that increases energy expenditure and/or brown fat activity, such as, for example, β3 adrenergic agonists (such as miglitol), NPR1 agonists, NPR3 antagonists, triiodothyronine, thiazolidinediones,VEGF, Irisin, meteorin-like, natriuretic peptides, orexin, norepinephrine, T4, bile acids, FGF-21, menthol, slit2-C BMP7, BMP8β, and FnIII domain-like/Tn3 scaffolds (binding molecules based on the third fibronectin type III domain of human tenascin C).

[0050] Other objects and advantages will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0051] Figures 1A-1E show blood glucose levels, insulin levels, glucagon levels, and B-hydroxybutyrate levels, as well as body weights, in a mouse model of severe insulin resistance. In Fig. 1A, mice treated with an insulin receptor antagonist, S961, and an antibody to the GCGR, H4H1327P, (open triangles) exhibited a rise in blood glucose levels relative to blood glucose levels in mice treated with the insulin receptor antagonist and an isotype control antibody (closed squares). In Fig. 1B, treatment of mice with S961 demonstrated an increase in insulin levels over time (closed squares), even in the presence of H4H1327P (open triangles). In Fig. 1C, mice treated with H4H1327P, in the absence (open circles) or presence of S961 (open triangles), exhibited higher levels of glucagon than the isotype control treated (closed circles) or S961 treated (closed squares) mice. In Fig. 1D, mice treated with S961 and H4H1327P (open triangles) maintained beta-hydroxybutyrate levels like those of the isotype control treated (closed circles) and antibody alone control (open circles). Mice treated with the insulin receptor antagonist in the absence of the GCGR antibody exhibited higher levels of beta-hydroxybutyrate (closed squares) relative to other treatment groups. Body weights among the four treatment groups were unchanged. See Fig. 1E.

[0052] Figures 2A-2F show blood glucose levels, insulin levels, glucagon levels, B-hydroxybutyrate levels, and amino acid levels, as well as body weights, in a mouse model of severe insulin resistance. The insulin receptor antagonist (S961) treatment preceded the antibody treatment, H4H1327P, causing increased blood glucose levels, and the ability of the antibody to decrease blood glucose levels was demonstrated within days of initiating antibody treatment (open triangles). See Fig. 2A. In Fig. 2B, treatment with S961 caused insulin levels to rise (closed squares), and subsequent treatment with the GCGR antibody, H4H1327P, did not lower the insulin levels (open triangles). As shown in Fig. 2C, glucagon levels were higher in mice treated with H4H1327P (open circles), and still higher in mice treated with both the antibody and S961 (open triangles). Fig. 2D shows plasma beta-hydroxybutyrate levels were elevated in response to treatment with S961 (closed squares), but within days of treatment with H4H1327P, the levels dropped to those of the untreated control and antibody alone control (open triangles). Fig. 2E shows amino acids levels were higher in mice treated with H4H1327P (open circles), and still higher in mice treated with both the antibody and S961 (open triangles). No changes in body weight were observed. See Fig. 2F.

[0053] Figures 3A and 3B provide the results of Western blot analysis on mice liver samples obtained from mice treated with one or both of S961 and H4H1327P. Treatment with H4H1327P reduced phosphoenolpyruvate carboxykinase (Pepck) in mice livers by 70% relative to the isotype treated control group, and treatment with S961 caused a 2.3 fold increase in Pepck levels. Treatment with H4H1327P reversed the increased levels caused by S961 to 30% below baseline. See Figures 3A and 3B.

[0054] Figures 4A-4D show the effects of the four treatments on pancreatic tissue: pancreas weight, Fig. 4A; pancreas α-cell mass, Fig. 4B; pancreas β-cell mass, Fig. 4C; and islet numbers relative to total pancreas area, Fig. 4D. β-cell mass doubled in the presence of S961 and H4H1327P when compared to S961 alone and increased 5.8-fold over control mice. See Fig. 4C.

DESCRIPTION

[0055] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0056] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0057] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All patents, applications and non-patent publications mentioned in this specification are incorporated herein by reference in their entireties.

General Description

[0058] Severe insulin resistance occurs in association with a variety of physiological and pathophysiological states. Clinical findings include hyperinsulinemia, acanthosis

nigricans, ovarian hyperandrogenism, polycystic ovaries, and eventual hyperglycemia and, in rare instances, patients can develop ketoacidosis. Although there is no consensus definition for severe insulin resistance to distinguish it from the more common insulin resistance, syndromic insulin resistance has been classified as either primary insulin-signaling defects (insulin receptoropathies or partial disruption of the insulin signaling pathway) or insulin resistance secondary to adipose tissue abnormalities (severe obesity or lipodystrophy). See Semple et al., (2011), Genetic Syndromes of Severe Insulin Resistance, Endocrine Reviews, 32(4):498-514.

[0059] Evidence of severe insulin resistance is seen in patients who require exogenous insulin at doses of more than 100 to 200 units per day, or in patients with chronically elevated circulating levels of endogenous insulin. Moller and Flier, (1991) New England Journal of Medicine, 325:938-948. Fasting insulin levels above 50-70 μU/mL or peak (post-oral glucose tolerance testing) insulin levels above 350 μU/mL suggest severe insulin resistance. Insulin sensitivity index values below $2x10^4$ μU/mL·min typically occur in the presence of severe insulin resistance. Patients with severe insulin resistance also exhibit a glucose disposal rate below 2 mg/kg·min. See Tritos and Mantzoros, (1998) Journal of Clinical Endocrinology and Metabolism, 83:3025-3030.

[0060] Insulin interacts with insulin receptors on the plasma membrane of target cells. The insulin receptor is a transmembrane tyrosine kinase receptor, and functions to regulate glucose homeostasis. The insulin receptor consists of two α subunits containing the site for insulin binding, and two β subunits containing the tyrosine kinase domain; the subunits are connected by disulfide bridges to form a 350 kDa β-α-α-β tetramer. Two isoforms of the receptor exist, an isoform with exon 11 (IR-B) and an isoform without exon 11 (IR-A), and the levels of the isoforms are expressed differently in various tissues. The IR-B isoform exhibits higher more efficient signaling activity the IR-A isoform, and the IR-B isoform is predominantly expressed in the liver, adipose tissue, and muscle tissue. The IR-A isoform is expressed in CNS cells and hematopoietic cells, and has slightly higher insulin binding affinity.

[0061] The tyrosine kinase activity of the activated insulin receptor is responsible for transmembrane signaling of glucose transport and regulation of glucose homeostasis.

[0062] Severe insulin resistance is typically associated with insulin receptor mutations, resulting in diminished expression on the cell surface or in the signaling capacity of the receptor. Other mutations include defects in receptor binding affinity or mutations in

proteins involved in the insulin signal transduction pathway, e.g. the conserved regions of the tyrosine kinase domain of the insulin receptor.

[0063] Patients having severe insulin resistance may suffer from a condition or disease selected from the following: Donohue syndrome, Rabson-Mendenhall syndrome, Type A insulin resistance, Type B insulin resistance, HAIR-AN (hyperandrogenism, insulin resistance, and acanthosis nigricans) syndrome, pseudoacromegaly, Alstrom syndrome, myotonic dystrophy, Werner's syndrome, lipodystrophy, cirrhosis, monogenic morbid obesity, hyperproinsulinemia, carboxypeptidase E deficiency, defective arginine metabolism, or Bardet-Biedl syndrome.

[0064] Genetic and acquired states of severe insulin resistance are rare disorders in which the body's tissues and organs do not respond properly to insulin. Clinical findings associated with severe insulin resistance include growth retardation, organomegaly, impaired development of skeletal and adipose tissue, soft tissue overgrowth, diabetes, hepatic steatosis, acanthosis nigricans, ovarian hyperandrogenism, and hirsutism. Laboratory findings include hyperinsulinemia, reduced insulin clearance, hyperglycemia, dyslipidemia, and elevated androgens. Each of the various syndromes associated with severe insulin resistance have unique features, in addition to some or all of the general clinical and laboratory features.

[0065] Donohue syndrome (DS, also called Leprechaunism) and Rabson-Mendenhall syndrome (RMS) are rare autosomal recessive conditions in which both alleles for the insulin receptor are abnormal, and patients fail to respond to endogenous and exogenous insulin. Individuals with DS and RMS are underdeveloped before birth, then fail to thrive as infants. Patients present with extremely high levels of circulating insulin, up to 1000 times the normal level. The primary metabolic consequence of DS is fasting hypoglycemia, and secondarily, post-prandial hyperglycemia. Individuals diagnosed with DS usually die before one year of age and do not develop diabetic ketoacidosis. Individuals with RMS also experience fasting hypoglycemia and typically survive infancy, but over time, develop severe and intractable diabetic ketoacidosis and a decline in insulin levels.

[0066] Ketonemia occurs when ketone bodies are formed by the breakdown of fatty acids and the deamination of amino acids and accumulate in the blood. If this continues untreated, the patients can then continue on to diabetic ketoacidosis. Beta-hydroxybutyrate and acetoacetic acid are two of the more common ketones, and elevated levels can be used to gauge the severity of ketonemia and an indicator of ketoacidosis.

[0067] Type A insulin resistance syndrome is another rare disorder characterized by severe insulin resistance, and symptoms typically present in adolescence for females, or

adulthood for males. Females present with primary amenorrhea or oligomenorrhea, ovarian cysts, hirsutism, and acanthosis nigricans, but are typically not overweight. Affected males present when they develop diabetes mellitus. As with DS and RMS, insulin receptor gene mutations are responsible for Type A insulin resistance syndrome.

[0068] Lipodystrophy refers to a group of disorders characterized by abnormal adipose distribution, utilization, and metabolism, due to defects in the insulin receptor itself or downstream components of the insulin signaling cascade. Patients with lipodystrophy present with a generalized or partial absence of adipose tissue, insulin resistance (with or without diabetes), significant dyslipidaemia, and fatty liver. Some lipodystrophy syndromes, like Berardinelli-Seip syndrome, are inherited, while others, including Lawrence syndrome, are acquired, sometimes after an infectious prodrome. Additional lipodystrophy syndromes include Kobberling-Dunnigan syndrome, lipodystrophy with other dysmorphic features, and cephalothoracic lipodystrophy.

[0069] Type B insulin resistance syndrome is different from DS, RMS, and Type A insulin resistance syndrome in that the former is associated with the presence of serum auto-antibodies against the insulin receptor, and may occur in the context of an autoimmune disease. Symptoms are similar to other insulin resistance syndromes, and include non-ketotic and severely insulin-resistant diabetes, acanthosis nigricans, and hirsutism, in addition to occasional paradoxal hypoglycemia.

[0070] HAIR-AN (hyperandrogenism, insulin resistance, and acanthosis nigricans) syndrome presents in young women, typically obese, with insulin resistance taking different forms; some individuals have high concentrations of insulin but normal levels of glucose, while others present with diabetic symptoms. Unlike the rarity of other syndromes of severe insulin resistance, HAIR-AN syndrome is estimated to affect around 5% of adolescent girls worldwide. The syndrome is associated with mutations of the tyrosine kinase domain of the insulin receptor gene.

[0071] Pseudoacromegaly presents with severe insulin resistance in association with acromegaloidism, and is possibly caused by a defect in the insulin signaling pathway or from high insulin levels signaling through the IGF-1 receptor.

[0072] Other severe insulin resistance syndromes include Alstrom syndrome, myotonic dystrophy, and Werner's syndrome, to name a few.

[0073] In some patients, the condition or disease is associated with the presence of a gene variant reported to cause severe insulin resistance. Exemplary gene variants include *INSR, PSMD6, ADRA2A, AGPAT2* (associated with lipodystrophy and insulin resistance),

AKT2, APPL1, BBS1 (associated with Bardet-Beidl Syndrome 1), *BSCL2, CIDEC, GRB10, IRS2, KLF14, LEP, LEPR, LMNA* (associated with lipodystrophy), *MC4R, PCNT, PIK2CA, POLD1* (associated with lipodystrophy), *PPARG, PTPRD, PTRF* (associated with lipodystrophy), *RASGRP1, TBC1D4,* and *TCF7L2*.

[0074] In some patients, insulin degrading protease activity is detected in the patient sera. In some patients, neutralizing anti-insulin antibodies or anti-insulin receptor antibodies are detected in the patient sera. In some patients, severe insulin resistance arises in the context of autoimmune destruction of adipocytes leading to lipodystrophy.

[0075] Patients with severe insulin resistance eventually develop hyperglycemia and, in some syndromes, ketoacidosis. For example, in patients with RMS, insulin levels start out very high early in life, even during periods of paradoxical fasting hypoglycemia. As the disease progresses, insulin levels while still elevated, drop. In addition, partially oxidized fatty acid levels increase, indicating that insulin is unable to suppress the release of fatty acids from adipocytes, ultimately resulting in constant ketoacidosis. Likewise, constant hyperglycemia results as insulin levels are no longer capable of suppressing hepatic glucose production and release. However, continuous infusion of extremely high concentrations of insulin (9.5 U/kg·hr) can reverse increased fatty acid oxidation and block ketonuria. Longo et al., (1991) Journal of Clinical Endocrinology & Metabolism, 84:2623-2629. In addition, hypertriglyceridemia and low high-density lipoprotein cholesterol levels are associated with severe insulin resistance.

[0076] Patients with severe insulin resistance syndromes have normal or even slightly elevated plasma glucagon levels despite hyperglycemia. West et al., (1975) Arch. Dis. Child., 50(9):703-708; Desbois-Mouthon et al., (1997) Pediatr. Res., 42(1):72-77. The hyperglycemia results from enhanced hepatic glucose output due to lack of insulin suppression and abnormally high glucagon signaling.

[0077] To date, there have been no studies examining the effects of antagonizing the GCG/GCGR signaling pathway on severe insulin resistance conditions or diseases. The studies described in the Examples use an antagonist of GCGR, as an exemplary inhibitor of the GCG/GCGR signaling pathway, in a mouse model of severe insulin resistance to demonstrate the effects on blood glucose levels and ketonemia, as measured by plasma beta-hydroxybutyrate levels, over several weeks of treatment.

Definitions

[0078] The "glucagon receptor", also referred to herein as "GCGR", belongs to the G protein-coupled receptor class 2 family and consists of a long amino terminal extracellular

domain, seven transmembrane segments, and an intracellular C-terminal domain. Glucagon receptors are notably expressed on the surface of hepatocytes where they bind to glucagon and transduce the signal provided thereby into the cell. Accordingly, the term "glucagon receptor" also refers to one or more receptors that interact specifically with glucagon to result in a biological signal. DNA sequences encoding glucagon receptors of rat and human origin have been isolated and disclosed in the art (EP0658200B1). The murine and cynomolgus monkey homologues have also been isolated and sequenced (Burcelin, et al., (1995) Gene 164:305-310); McNally et al., (2004) Peptides 25:1171-1178). As used herein, "glucagon receptor" and "GCGR" are used interchangeably. The expression "GCGR", "hGCGR" or fragments thereof, as used herein, refers to the human GCGR protein or fragment thereof, unless specified as being from a non-human species, e.g. "mouse GCGR", "rat GCGR", or "monkey GCGR".

[0079] The phrase "GCGR antagonist" refers to an inhibitor, antagonist, or inverse agonist of the GCGR signaling pathway. A “GCG inhibitor” may prevent the binding of glucagon to the receptor. A GCGR inhibitor may also prevent the binding of glucagon to the receptor. However, both effectively block or attenuate activation of the receptor, or may interfere with the signaling cascade downstream of the GCGR activation.

[0080] A GCGR antagonist is able to bind to the glucagon receptor and thereby antagonize the activity of GCG mediated by the GCGR. Inhibiting the activity of GCG by antagonizing the binding and activity of GCG at the GCGR reduces the rate of gluconeogenesis and glycogenolysis, and the concentration of glucose in plasma. Methods by which to determine the binding of a supposed antagonist with the glucagon receptor are known in the art and means by which to determine the interference with glucagon activity at the glucagon receptor are publicly available; see, e.g., S. E. de Laszlo et al., (1999) Bioorg. Med. Chem. Lett. 9:641-646. Contemplated as useful herein are GCGR antagonists or GCG inhibitors having as a functional component thereof a small molecule compound, or in other words a low molecular weight organic compound. A small molecule is typically less than 800 Daltons. Additionally, CRISPR technology can be used to knock-down GCG or GCGR expression.

[0081] The terms "inhibitor" or "antagonist" include a substance that retards or prevents a chemical or physiological reaction or response. Common inhibitors or antagonists include but are not limited to antisense molecules, antibodies, small molecule inhibitors, peptide inhibitors, DARPins, Spiegelmers, aptamers, engineered Fn type-III domains, and their derivatives.

[0082] An example of a GCG inhibitor or a GCGR signaling pathway antagonist includes, but is not limited to, an antibody (human or humanized), or an antigen binding portion thereof, to GCG or GCGR, that blocks binding or inhibits the activity of the GCGR signaling pathway. Exemplary GCGR antagonists that may be used in the methods described herein include isolated human monoclonal antibody or antigen-binding fragment thereof comprising: (a) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and/or (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148. Exemplary GCG inhibitors that may be used in the methods described herein include isolated human monoclonal antibody or antigen-binding fragment thereof comprising: (a) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 150, 166, 182, 198, 214, 230, 246, 262, 278, and 294; and/or (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 158, 174, 190, 206, 222, 238, 254, 270, 286, and 302.

[0083] A "therapeutically effective dose" is a dose that produces the desired effect for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[0084] By the phrase "substantially identical" is meant a protein sequence having at least 95% identity to an HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and/or (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148, and capable of binding GCGR and inhibiting the biological activity of GCGR. The phrase “substantially identical” is also meant a protein sequence having at least 95% identify to an HCVR having an amino acid sequence selected from the group consisting of the amino acid sequences SEQ ID NO: 150, 166, 182, 198, 214, 230, 246, 262, 278, and 294; and/or (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 158, 174, 190, 206, 222, 238, 254, 270, 286, and 302, and capable of binding GCG and inhibiting the biological activity of GCG.

[0085] The terms "identity" or "homology" are construed to mean the percentage of amino acid residues in the candidate sequence that are identical with the residue of a corresponding sequence to which it is compared, after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent identity for the entire sequence, and not

considering any conservative substitutions as part of the sequence identity. Neither N- or C-terminal extensions nor insertions will be construed as reducing identity or homology. Methods and computer programs for the alignment are well known in the art. Sequence identity may be measured using sequence analysis software (e.g., Sequence Analysis Software Package, Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Ave., Madison, Wis. 53705). This software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

[0086] The term "treating" (or "treat" or "treatment") refers to processes involving a slowing, interrupting, inhibiting, arresting, controlling, stopping, reducing, ameliorating, or reversing the progression, duration, or severity of an existing symptom, disorder, condition, or disease, but does not necessarily involve a total elimination of all disease-related symptoms, conditions, or disorders through use of a GCG inhibitor or GCGR antagonist as described herein. Furthermore, "treating", "treatment" or "treat" refers to an approach for obtaining beneficial or desired results including clinical results, which include, but are not limited to, one or more of the following: inhibiting, delaying or preventing the progression of severe insulin resistance; inhibiting, delaying or preventing the progression of a disease associated with severe insulin resistance, or characterized by elevated plasma insulin levels, elevated blood glucose levels, and/or ketonemia or ketoacidosis (as measured by elevated beta-hydroxybutyrate levels), such as in Donohue syndrome, Rabson-Mendenhall syndrome, Type A insulin resistance, Type B insulin resistance, HAIR-AN (hyperandrogenism, insulin resistance, and acanthosis nigricans) syndrome, pseudoacromegaly, Alstrom syndrome, myotonic dystrophy, Werner's syndrome, lipodystrophy, cirrhosis, monogenic morbid obesity, hyperproinsulinemia, carboxypeptidase E deficiency, defective arginine metabolism, Bardet-Biedl syndrome, or a condition or disease associated with the presence of a gene variant reported to cause severe insulin resistance; or inhibiting, preventing, or ameliorating at least one symptom associated with a disease associated with severe insulin resistance; or lowering blood glucose levels and/or beta-hydroxybutyrate levels (as an indicator of ketoacidosis), such that the condition or disease associated with high blood glucose levels and ketonemia is mediated, or at least one symptom or complication associated with the condition or disease is alleviated or reduced in severity. "Treatment" or "treating", as used herein, also refers to increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease and/or prolonging survival of patients. For

example, "treatment" or "treating" can include reducing the amount and/or dosage of insulin necessary to treat a patient with severe insulin resistance.

[0087] The phrase "insulin resistance" is a state in which a greater than normal amount of insulin is required to elicit a quantitatively normal response. The phrase "severe insulin resistance" generally refers to a clinical entity that typically presents with near-normal or elevated blood glucose levels despite marked elevations in endogenous insulin secretion and/or plasma levels of insulin. Evidence of severe insulin resistance is seen in patients who require exogenous insulin at doses of more than 100 to 200 units per day, or in patients with chronically elevated circulating levels of endogenous insulin. Moller and Flier, (1991) New England Journal of Medicine, 325:938-948. Fasting insulin levels above 50-70 μU/mL or peak (post-oral glucose tolerance testing) insulin levels above 350 μU/mL suggest severe insulin resistance. Insulin sensitivity index values below $2x10^4$ μU/mL·min typically occur in the presence of severe insulin resistance. Patients with severe insulin resistance also exhibit a glucose disposal rate below 2 mg/kg·min. See Tritos and Mantzoros, (1998) Journal of Clinical Endocrinology and Metabolism, 83:3025-3030.

<u>GCG/GCGR Signaling Pathway Inhibitors</u>

[0088] Provided herein are GCG inhibitors and GCGR antagonists for the treatment of conditions or diseases characterized by severe insulin resistance. In some embodiments, the antagonist is an inhibitor of glucagon. In some embodiments, the antagonist is an inhibitor of GCGR. In some embodiments, the GCGR antagonist is MK-0893, PF-06291874, LGD-6972, or LY2409021.

[0089] In some embodiments, the antagonist comprises an antibody capable of binding GCG or GCGR, or a fragment thereof. In some embodiments, the signaling pathway is inhibited by the interruption of GCG or GCGR expression, by, for example, using CRISPR technology or antisense.

[0090] In some embodiments, the GCG inhibitor or GCGR antagonist is an antisense molecule, antibody, small molecule inhibitor, peptide inhibitor, DARPin, Spiegelmer, aptamer, engineered Fn type-III domains, or a derivative thereof.

<u>Anti-GCGR Antibodies, Anti-GCG Antibodies, and Antibody Fragments</u>

[0091] In some embodiments, the GCGR antagonist is an antibody or antibody fragment as disclosed in U.S. Patent No. 8,545,847, incorporated by reference herein in its entirety. Antibodies disclosed therein are provided in Table 1.

Table 1

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H4H1345N	2	4	6	8	10	12	14	16
H4H1617N	18	20	22	24	26	28	30	32
H4H1765N	34	36	38	40	42	44	46	48
H4H1321B	50	52	54	56	58	60	62	64
H4H1321P	66	52	54	56	68	60	62	64
H4H1327B	70	72	74	76	78	80	82	84
H4H1327P	86	72	74	76	88	80	82	84
H4H1328B	90	92	94	96	98	100	102	104
H4H1328P	106	92	94	96	108	100	102	104
H4H1331B	110	112	114	116	118	120	122	124
H4H1331P	126	112	114	116	128	120	122	124
H4H1339B	130	132	134	136	138	140	142	144
H4H1339P	146	132	134	136	148	140	142	144

[0092] Additional GCGR antibodies or antibody fragments contemplated as useful herein include those disclosed in U.S. Pat. Nos. 5,770,445 and 7,947,809; European patent application EP2074149A2; EP patent EP0658200B1; U.S. patent publications 2009/0041784; 2009/0252727; and 2011/0223160; and PCT publication WO2008/036341. The patents and publications are incorporated by reference herein in their entirety.

[0093] In some embodiments, the GCG inhibitor is an antibody or antibody fragment thereof as disclosed in U.S. 2016/0075778, incorporated by reference herein in its entirety. Antibodies disclosed therein are provided in Table 2.

Table 2

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H1H059P	150	152	154	156	158	160	162	164
H4H10223P	166	168	170	172	174	176	178	180
H4H10231P	182	184	186	188	190	192	194	196
H4H10232P	198	200	202	204	206	208	210	212
H4H10236P	214	216	218	220	222	224	226	228
H4H10237P	230	232	234	236	238	240	242	244
H4H10238P	246	248	250	252	254	256	258	260

H4H10250P	262	264	266	268	270	272	274	276
H4H10256P	278	280	282	284	286	288	290	292
H4H10270P	294	296	298	300	302	304	306	308

[0094] Additional GCG antibodies or antibody fragments contemplated as useful herein include those disclosed in U.S. Pat. Nos. 4,206,199; 4,221,777; 4,423,034; 4,272,433; 4,407,965; 5,712,105; and PCT publications WO2007/124463 and WO2013/081993.

[0095] Antibody fragments include any fragment having the required target specificity, e.g. antibody fragments either produced by the modification of whole antibodies (e.g. enzymatic digestion), or those synthesized de novo using recombinant DNA methodologies (scFv, single domain antibodies, DVD (dual variable domain immunoglobulins), or dAbs (single variable domain antibodies)) or those identified using human phage or yeast display libraries (see, for example, McCafferty et al. (1990) Nature 348:552-554). Alternatively, antibodies can be isolated from mice producing human, human-mouse, human-rat, and human-rabbit chimeric antibodies using standard immunization and antibody isolation methods, including but not limited to making hybridomas, or using B cell screening technologies, such as SLAM. Immunoglobulin binding domains also include, but are not limited to, the variable regions of the heavy (V_H) or the light (V_L) chains of immunoglobulins. Or by immunizing people and isolating antigen positive B cells and cloning the cDNAs encoding the heavy and light chain and coexpressing them in a cell, such as CHO.

[0096] The term "antibody" as used herein refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant regions, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. Within each IgG class, there are different isotypes (eg. IgG1, IgG2, IgG3, IgG4). Typically, the antigen-binding region of an antibody will be the most critical in determining specificity and affinity of binding.

[0097] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one light chain (about 25 kD) and one heavy chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100-110 or more amino acids primarily

responsible for antigen recognition. The terms "variable light chain" (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0098] Antibodies exist as intact immunoglobulins, or as a number of well-characterized fragments produced by digestion with various peptidases. For example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H -C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region. While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology.

[0099] Methods for preparing antibodies useful according to the methods herein are known to the art. See, for example, Kohler & Milstein (1975) Nature 256:495-497; Harlow & Lane (1988) Antibodies: a Laboratory Manual, Cold Spring Harbor Lab., Cold Spring Harbor, N.Y.). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Monoclonal antibodies can be humanized using standard cloning of the CDR regions into a human scaffold. Gene libraries encoding human heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity. Techniques for the production of single chain antibodies or recombinant antibodies (U.S. Pat. No. 4,946,778; U.S. Pat. No. 4,816,567) can be adapted to produce antibodies used in the methods disclosed herein. Also, transgenic mice, or other organisms such as other mammals, may be used to express human, human-mouse chimeric, human-rat chimeric, human-rabbit chimeric, or humanized antibodies. Alternatively, phage display or yeast display technology can be used to identify human antibodies and heteromeric Fab fragments that specifically bind to selected antigens.

Immunoconjugates

[0100] The disclosure encompasses treatment of severe insulin resistance with a human anti-GCGR monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as an agent that is capable of reducing blood glucose levels or addressing another symptom of severe insulin resistance. The type of therapeutic moiety that may be conjugated to the anti-GCGR antibody will take into account the condition to be

treated and the desired therapeutic effect to be achieved. For example, in an effort to lower blood glucose, and/or to maintain normal blood glucose levels, an agent such as biguanide (e.g. metformin), a sulfonylurea (e.g. glyburide, glipizide), a PPAR gamma agonist (e.g. pioglitazone, rosiglitazone); an alpha glucosidase inhibitor (e.g. acarbose, voglibose), an inhibitor of advanced glycation end-product formation (e.g. aminoguanidine), or a second GCGR inhibitor or GCG inhibitor may be conjugated to the GCGR antibody. Alternatively, if the desired therapeutic effect is to treat ketonemia or any other symptoms or conditions associated with severe insulin resistance, it may be advantageous to conjugate an appropriate agent to the anti-GCGR antibody. Examples of suitable agents for forming immunoconjugates are known in the art, see for example, WO 05/103081.

Multi-Specific Antibodies

[0101] The antibodies useful according to the methods provided herein may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, e.g., Tutt et al., (1991) J. Immunol. 147:60-69; Kufer et al., (2004) Trends Biotechnol. 22:238-244. The anti-GCGR antibodies can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multi-specific antibody with a second binding specificity. For example, bi-specific antibodies are contemplated where one arm of an immunoglobulin is specific for human GCGR or a fragment thereof, and the other arm of the immunoglobulin is specific for a second therapeutic target or is conjugated to a therapeutic moiety. In certain embodiments, one arm of an immunoglobulin is specific for an epitope on the N-terminal domain of hGCGR or a fragment thereof, and the other arm of the immunoglobulin is specific for an epitope on one of the EC loops of hGCGR, or a fragment thereof. In certain embodiments, one arm of an immunoglobulin is specific for one EC loop, or a fragment thereof, and the second arm is specific for a second EC loop, or a fragment thereof. In certain embodiments, one arm of an immunoglobulin is specific for one epitope on one EC loop of hGCGR and the other arm is specific for a second epitope on the same EC loop of hGCGR.

[0102] An exemplary bi-specific antibody format that can be used according to the methods described herein involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another

by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bi-specific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V821 (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bi-specific antibody format described above are contemplated within the scope of the present disclosure.

Antibody Screening and Selection

[0103] Screening and selection of preferred antibodies, useful according to the methods provided herein, can be conducted by a variety of methods known to the art. Initial screening for the presence of monoclonal antibodies specific to a target antigen may be conducted through the use of ELISA-based methods, for example. A secondary screen is preferably conducted to identify and select a desired monoclonal antibody for use in construction of antibody-drug conjugates. Secondary screening may be conducted with any suitable method known to the art. One preferred method, termed "Biosensor Modification-Assisted Profiling" ("BiaMAP") is described in U.S. Publication 2004/0101920, herein specifically incorporated by reference in its entirety. BiaMAP allows rapid identification of hybridoma clones producing monoclonal antibodies with desired characteristics. More specifically, monoclonal antibodies are sorted into distinct epitope-related groups based on evaluation of antibody:antigen interactions. Antibodies capable of blocking either a ligand or a receptor may be identified by a cell based assay, such as a luciferase assay utilizing a luciferase gene under the control of an NFκB driven promoter or cAMP response driven promoter. Stimulation of the GCGR by glucagon leads to a signal through NFκB/cAMP/CREB thus increasing luciferase levels in the cell. Blocking antibodies are identified as those antibodies that blocked glucagon induction of luciferase activity.

Treatment Population

[0104] The therapeutic methods provided herein are useful for treating individuals with severe insulin resistance or a condition or disease associated with severe insulin

resistance. Exemplary conditions or diseases include Donohue syndrome, Rabson-Mendenhall syndrome, Type A insulin resistance, Type B insulin resistance, HAIR-AN (hyperandrogenism, insulin resistance, and acanthosis nigricans) syndrome, pseudoacromegaly, Alstrom syndrome, myotonic dystrophy, Werner's syndrome, lipodystrophy, cirrhosis, monogenic morbid obesity, hyperproinsulinemia, carboxypeptidase E deficiency, defective arginine metabolism, Bardet-Biedl syndrome, and a condition or disease associated with the presence of a gene variant reported to cause severe insulin resistance. In some embodiments, insulin degrading protease activity is detected in the patient sera. In some embodiments, neutralizing anti-insulin antibodies are detected in the patient sera. In some patients, severe insulin resistance arises in the context of autoimmune destruction of adipocytes leading to lipodystrophy.

Therapeutic Administration and Formulations

[0105] Useful according to the methods provided herein are therapeutic compositions comprising a glucagon/GCGR antagonist, such as, for example, an anti-GCGR antibody. The administration of therapeutic compositions in accordance with the methods described herein will be administered via a suitable route including, but not limited to, intravenously, subcutaneously, intramuscularly, intrathecally, intracerebrally, intraventricularly, intranasally, or orally, with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN^{TM}), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0106] The dose of antibody may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When the antibody is used for lowering blood glucose levels and/or decreasing ketonemia (as measured by, for example, beta-hydroxybutyrate levels) associated with severe insulin resistance in various conditions and diseases, such as Type A insulin resistance syndrome, RMS, or DS, in a patient, it is advantageous to intravenously administer the antibody normally at a dose of about 0.01 to about 30 mg/kg body weight, more preferably about 0.02

to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition and response to treatment, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the antibody or antigen-binding fragment thereof can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 500 mg, about 5 to about 300 mg, or about 10 to about 200 mg, to about 100 mg, or to about 50 mg.

[0107] In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibody or antigen-binding fragment thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[0108] Various delivery systems are known and can be used to administer the pharmaceutical composition comprising the antibody, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu et al. (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, depot formulations, aerosol, intradermal, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, intrathecal, intraventricular, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0109] The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

[0110] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

[0111] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the

injectable preparations may be prepared, e.g., by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0112] A pharmaceutical composition useful herein can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition useful in the methods described herein. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0113] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition useful according to the methods described herein. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, Inn.), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, N.J.), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (Sanofi-Aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in

subcutaneous delivery of a pharmaceutical composition useful according to the methods described herein include, but certainly are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, Calif.), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.) and the HUMIRA™ Pen (Abbott Labs, Abbott Park, Ill.), to name only a few.

[0114] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 750 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Combination Therapies

[0115] In numerous embodiments, the GCG inhibitors or GCGR antagonists useful herein may be administered in combination with one or more additional compounds or therapies. Combination therapy may be simultaneous or sequential.

[0116] In some embodiments, the GCG inhibitor or GCGR antagonist is administered with at least one additional therapeutic agent selected from the following: insulin, a biguanide, hIGF1, leptin, pioglitazone, vildagliptin, acarbose, alpha-glycosidase inhibitors, L-arginine, dipeptidyl-peptidase-4 inhibitors, insulin secretagogues, amylin receptor agonists, insulin sensitizers, SGLT2 inhibitors, SGLT1 inhibitors, GLP-1 analogues, GLP-1 receptor activators, a second GCG inhibitor, and a second GCGR antagonist. In some embodiments, the GCG inhibitor or GCGR antagonist is administered with at least one additional therapeutic agent selected from the following: vanadate or vanadium salts, phenytoin, benzafibrate. In some embodiments, the GCG inhibitor or GCGR antagonist is administered with a dietary supplement such as ω-3 fatty acid rich fish oil.

[0117] In some embodiments, the insulin sensitizer is a thiazolidinedione, such as troglitazone. In some embodiments, the insulin sensitizer is rosiglitazone.

[0118] In some embodiments, the insulin secretagogue is a sulfonylurea, ATP-sensitive K channel antagonists, or a meglitinide.

[0119] The additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of the GCG inhibitor or the GCGR antagonist. For purposes of the present disclosure, such administration regimens are considered the

administration of a GCG inhibitor or a GCGR antagonist "in combination with" a second therapeutically active component.

Administration Regimens

[0120] According to certain embodiments described herein, multiple doses of the glucagon/GCGR antagonist may be administered to a subject over a defined time course. The methods comprise sequentially administering to a subject multiple doses of a glucagon/GCGR antagonist. As used herein, "sequentially administering" means that each dose of the antagonist is administered to the subject at a different point in time, e.g., on different days separated by a predetermined interval (e.g., hours, days, weeks or months). The methods described herein comprise sequentially administering to the patient a single initial dose of the glucagon/GCGR antagonist, followed by one or more secondary doses of the glucagon/GCGR antagonist, and optionally followed by one or more tertiary doses of the glucagon/GCGR antagonist.

[0121] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of an glucagon/GCGR antagonist useful herein. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of the glucagon/GCGR antagonist, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of the glucagon/GCGR antagonists contained in the initial, secondary and/or tertiary doses varies from one another (e.g., adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (e.g., 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (e.g., "maintenance doses").

Pharmaceutical Compositions

[0122] The methods disclosed herein contemplate the use of pharmaceutical compositions comprising at least a therapeutically effective amount of an active agent useful in treating severe insulin resistance, such as a glucagon/GCGR antagonist, and a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle

with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

[0123] In one embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0124] The active agents useful according to the methods described herein can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0125] The amount of the active agent which will be effective in the treatment of severe insulin resistance can be determined by standard clinical techniques based on the present description. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each subject's circumstances. However,

suitable dosage ranges for intravenous administration are generally about 20 micrograms to 2 grams of active compound per kilogram body weight. Suitable dosage ranges for intra-nasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0126] For systemic administration, a therapeutically effective dose can be estimated initially from in vitro assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Initial dosages can also be estimated from in vivo data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

[0127] Dosage amount and interval may be adjusted individually to provide plasma levels of the compounds that are sufficient to maintain therapeutic effect. In cases of local administration or selective uptake, the effective local concentration of the compounds may not be related to plasma concentration. One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

[0128] The amount of compound administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician. The therapy may be repeated intermittently while symptoms are detectable or even when they are not detectable. The therapy may be provided alone or in combination with other drugs.

Kits

[0129] Also provided herein is an article of manufacturing comprising packaging material and a pharmaceutical agent contained within the packaging material, wherein the pharmaceutical agent comprises at least one GCG/GCGR antagonist useful according to the methods disclosed herein, and wherein the packaging material comprises a label or package insert which indicates that the GCG/GCGR antagonist can be used for treating a condition or disease characterized by severe insulin resistance.

[0130] While the invention has been particularly shown and described with reference to a number of embodiments, it would be understood by those skilled in the art that changes in the form and details may be made to the various embodiments disclosed herein without departing from the spirit and scope of the invention and that the various embodiments disclosed herein are not intended to act as limitations on the scope of the claims.

EXAMPLES

[0131] The following examples are provided such that those of ordinary skill in the art have a complete disclosure and description of how to implement the methods disclosed herein. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Evaluation of a GCGR Antagonist in Preventing Hyperglycemia in a Mouse Model of Extreme Insulin Resistance

[0132] Administration of S961, an insulin receptor antagonist, by osmotic minipumps in mice causes severe insulin resistance and hyperglycemia (Gusarova V et al., (2014) Cell, 159:691-696; Yi P et al., (2013) Cell, 153:747-758; Schaffer L., (2008) Biochem. Biophys. Res. Commun., 376:380–383). This model of severe insulin resistance was used to determine the effect of an anti-GCGR antibody in preventing hyperglycemia, as well as the effects on blood glucose levels and plasma beta-hydroxybutyrate levels (as a measure of ketonemia), resulting from severe insulin resistance.

Materials:

- hIgG4 isotype control
- H4H1327P, anti-hGCGR hIgG4
- S961, insulin receptor antagonist (custom synthesized by Celtek Peptides using published sequence (Schaffer L., (2008) Biochem. Biophys. Res. Commun., 376:380–383))

Animals and Injections:

[0133] Twenty-nine mice were divided into four groups of six to eight mice. The first group was injected subcutaneously with 10 mg/kg of hIgG4 isotype control on Day 0, 6, and 14 and infused subcutaneously with PBS by osmotic minipumps (Alzet 2002) from Day 7. The second group was injected subcutaneously with 10 mg/kg of H4H1327P on Day 0, 6, and 14 and infused subcutaneously with PBS by osmotic minipumps (Alzet 2002) from Day 7. The third group was injected subcutaneously with 10 mg/kg of hIgG4 isotype control on Day 0, 6, and 14 and infused subcutaneously with S961 at 20 nmol/week by osmotic minipumps (Alzet 2002) from Day 7. The fourth group was injected subcutaneously with 10 mg/kg of H4H1327P on Day 0, 6, and 14 and infused subcutaneously with S961 at 20 nmol/week by osmotic minipumps (Alzet 2002) from Day 7. Mice were bled on Days 0, 3, 6, 10, 14, 17, and

21 for blood glucose measurements. Mean ± SEM of blood glucose levels at each time point was calculated for each group and shown in Table 3. Plasma was collected at baseline and Days 6, 14, and 21 to determine insulin and beta-hydroxybutyrate levels. Mean ± SEM of plasma beta-hydroxybutyrate and insulin levels at each time point was calculated for each group and shown in Tables 4 and 5.

Table 3: Blood glucose levels

	Time (days)	Isotype control + PBS	H4H1327P + PBS	Isotype control + S961	H4H1327P + S961
Blood Glucose (mg/dL)	0	196 ± 6	191 ± 4	186 ± 5	196 ± 3
	3	195 ± 7	119 ± 3	191 ± 6	124 ± 6
	6	194 ± 9	126 ± 4	192 ± 5	129 ± 12
	10	186 ± 4	135 ± 2	437 ± 40	185 ± 7
	14	197 ± 5	128 ± 4	508 ± 53	272 ± 53
	17	211± 6	144 ± 3	467 ± 41	219 ± 22
	21	206 ± 5	141 ± 5	499 ± 18	209 ± 6

Table 4: Plasma beta-hydroxybutyrate levels

	Time (days)	Isotype control + PBS	H4H1327P + PBS	Isotype control + S961	H4H1327P + S961
Beta-hydroxy butyrate (mg/dL)	0	0.20 ± 0.02	0.20 ± 0.02	0.21 ± 0.02	0.24 ± 0.02
	6	0.26 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.27 ± 0.01
	14	0.22 ± 0.02	0.23 ± 0.02	0.34 ± 0.04	0.26 ± 0.03
	21	0.23 ± 0.01	0.23 ± 0.02	0.34 ± 0.04	0.25 ± 0.03

Table 5: Plasma insulin levels

	Time (days)	Isotype control + PBS	H4H1327P + PBS	Isotype control + S961	H4H1327P + S961
Insulin (ng/mL)	0	0.80 ± 0.14	1.90 ± 0.69	1.15 ± 0.68	1.62 ± 0.67
	6	0.24 ± 0.04	0.24 ± 0.06	0.21 ± 0.10	0.24 ± 0.04
	14	0.37 ± 0.09	0.36 ± 0.05	22.83 ± 4.32	18.51 ± 2.30
	21	0.40 ± 0.13	0.46 ± 0.15	23.97 ± 4.36	25.11 ± 5.15

Results:

[0134] Statistical analysis was performed with Prism software (version 6). To assess the significance to the control group (Group 1), two-way ANOVA with Bonferroni multiple comparison test was used. a: $p<0.05$, b: $p<0.01$, c: $p<0.001$, d: $p<0.0001$.

[0135] H4H1327P-treated and PBS-infused animals (Group 2) showed reductions in blood glucose compared to isotype control-administered and PBS-infused animals (Group 1) post H4H1327P administration (between days 3 and 21), confirming glucose lowering efficacy of H4H1327P. Isotype control-administered and S961-infused animals (Group 3) showed increases in blood glucose compared to isotype control-administered and PBS-infused animals (Group 1) post infusion of S961 (between days 10 and 21), confirming hyperglycemic effect of S961. In H4H1327P-treated and S961-infused animals (Group 4), blood glucose levels were comparable to those of Group 1 mice between 10 and 21 days post S961 infusion. See Figure 1A.

[0136] Plasma insulin levels were elevated in isotype control-administered and S961-infused animals (Group 3) compared to isotype control-administered and PBS-infused animals (Group 1) on Days 14 and 21, confirming the action of S961 to inhibit insulin receptor during the duration of the study. The insulin levels were equally increased in H4H1327P-treated and S961-infused animals (Group 4) in comparison to isotype control-administered and S961-infused animals (Group 3). See Figure 1B.

[0137] Consistent with previous studies (Okamoto et al., (2015) Endocrinology, 156(8): 2781-2794), H4H1327P demonstrated increased plasma glucagon levels, an effect that was independent of S961 administration (See Figure 1C).

[0138] The levels of plasma beta-hydroxybutyrate were elevated in isotype control-administered and S961-infused animals (Group 3) compared to isotype control-administered and PBS-infused animals (Group 1) on Day 14 and 21, whereas they were not changed in H4H1327P-treated and S961-infused animals (Group 4). See Figure 1D. In addition, no differences in body weight were observed between the treatment groups (See Figure 1E).

[0139] These data indicate that H4H1327P prevents insulin receptor antagonist-induced hyperglycemia and ketonemia and lowers blood glucose even in the presence of severe hyperinsulinemia.

Example 2: Evaluation of a GCGR Antagonist in Reversing Hyperglycemia in a Mouse Model of Extreme Insulin Resistance

[0140] The effect of an anti-GCGR antibody in reversing established hyperglycemia induced by severe insulin resistance was determined using the same animal model and the same materials mentioned in Example 1, except that the insulin receptor antagonist was administered 4 days prior to injection of the anti-GCGR antibody. The effects on blood glucose and plasma beta-hydroxybutyrate levels were also determined.

Animals and Injections:

[0141] Thirty-two mice were divided into four groups of eight mice. The first group was infused subcutaneously with PBS by osmotic minipumps (Alzet 2002) from Day 0 and injected subcutaneously with 10 mg/kg of hIgG4 isotype control on Day 4, 11 and 18. The second group was infused subcutaneously with PBS from Day 0 and injected subcutaneously with 10 mg/kg of H4H1327P on Day 4, 11 and 18. The third group was infused subcutaneously with S961 at 20 nmol/week from Day 0 and injected subcutaneously with 10 mg/kg of hIgG4 isotype control on Day 4, 11 and 18. The fourth group was infused subcutaneously with S961 at 20 nmol/week from Day 0 and injected subcutaneously with 10 mg/kg of H4H1327P on Day 4, 11 and 18. Mice were bled on Days 0, 4, 7, 11, 14, 18 and 21 for blood glucose measurements. Mean ± SEM of blood glucose levels at each time point was calculated for each group and shown in Table 6. Plasma was collected at baseline and Days 4, 11, and 21 to determine insulin and beta-hydroxybutyrate levels. Mean ± SEM of plasma beta-hydroxybutyrate and insulin levels at each time point was calculated for each group and shown in Tables 7 and 8.

Table 6: Blood glucose levels (mg/dL)

Time (days)	PBS + isotype control	PBS + H4H1327P	S961 + isotype control	S961 + H4H1327P
0	186 ± 4	189 ± 4	192 ± 4	183 ± 4
4	196 ± 3	197 ± 3	491 ± 29	490 ± 21
7	216 ± 5	142 ± 6	523 ± 34	203 ± 6
11	206 ± 6	137 ± 4	533 ± 14	201 ± 6
14	210 ± 7	145 ± 5	595 ± 6	211 ± 9
18	202 ± 7	140 ± 4	550 ± 16	203 ± 5
21	168 ± 6	123 ± 4	526 ± 12	172 ± 5

Table 7: Plasma beta-hydroxybutyrate levels (mmol/L)

Time (days)	PBS + isotype control	PBS + H4H1327P	S961 + isotype control	S961 + H4H1327P
0	0.20 ± 0.01	0.22 ± 0.01	0.21 ± 0.02	0.18 ± 0.02
4	0.27 ± 0.01	0.25 ± 0.02	0.41 ± 0.02	0.37 ± 0.04
11	0.26 ± 0.02	0.24 ± 0.01	0.39 ± 0.03	0.26 ± 0.02
21	0.26 ± 0.01	0.25 ± 0.01	0.45 ± 0.06	0.26 ± 0.02

Table 8: Plasma insulin levels (ng/mL)

Time (days)	PBS + isotype control	PBS + H4H1327P	S961 + isotype control	S961 + H4H1327P
0	1.05 ± 0.31	0.77 ± 0.25	0.52 ± 0.08	0.42 ± 0.09
4	0.62 ± 0.32	0.50 ± 0.11	19.23 ± 3.18	21.68 ± 2.02
11	0.39 ± 0.09	0.35 ± 0.05	25.97 ± 3.48	64.25 ± 18.17
21	1.67 ± 0.47	0.37 ± 0.04	51.43 ± 15.03	64.78 ± 14.91

Results:

[0142] Statistical analysis was performed with Prism software (version 6). To assess the significance to the control group (Group1), two-way ANOVA with Bonferroni multiple comparison test was used. a: $p<0.05$, b: $p<0.01$, c: $p<0.001$, d: $p<0.0001$.

[0143] S961-infused and isotype control-administered animals (Group 3) showed increases in blood glucose compared to PBS-infused and isotype control-administered animals (Group 1) post S961 infusion (between days 4 and 21), confirming hyperglycemic effect of S961. S961-infused and H4H1327P-treated animals (Group 4) showed blood glucose levels that were nearly identical to those of PBS-infused and isotype control-administered and animals (Group 1) post H4H1327P administration. PBS-infused and H4H1327P-treated animals (Group 2) maintained reduced levels of blood glucose compared to isotype control-administered and PBS-infused animals (Group 1) post H4H1327P administration (between days 4 and 21), confirming glucose lowering efficacy of H4H1327P. See Figure 2A.

[0144] Plasma insulin levels were elevated in S961-infused and isotype control-administered animals (Group 3) compared to PBS-infused and isotype control-administered animals (Group 1) on Days 4, 11 and 21, confirming the action of S961 to inhibit insulin receptor during the duration of the study. See Figure 2B. The hyperinsulinemia (Table 8 and Figure 2B) and hyperglucagonemia (see Figure 2C) was more pronounced in mice that received both receptor antagonists.

[0145] The levels of plasma beta-hydroxybutyrate were elevated in S961-infused and isotype control-administered animals (Group 3) compared to PBS-infused and isotype control-administered animals (Group 1) on Days 11 and 21, whereas they were not changed in S961-infused and H4H1327P-treated animals (Group 4) at these same time points relative to Group 1 animals. See Figure 2D.

[0146] Consistent with previous findings (Okamoto et al., 2015), H4H1327P increased circulating amino acid levels, as did S961 but to a lesser extent than did the antibody (see Figure 2E). Inhibition of both insulin and glucagon receptors caused an additive increase in plasma amino acid levels (see Figure 2E). No changes in body weight were observed (see Figure 2F).

[0147] These data indicate that H4H1327P reverses insulin receptor antagonist-induced hyperglycemia and ketonemia and lowers blood glucose even in the presence of severe hyperinsulinemia.

Example 3: Evaluation of a GCGR Antagonist in Reversing Insulin Receptor Antagonist-Induced Liver Pepck Expression

[0148] Liver samples obtained from mice treated according to each of the four groups from Example 1 were lysed with ice-cold RIPA buffer (50 mM Tris, 150 mM NaCl, 1 mM of EDTA, 50 mM NaF, 10 mM β-glycerophosphate, 5 mM sodium pyrophosphate dibasic and 1% NP-40) in the presence of protease and phosphatase inhibitor cocktails (Thermo-Fisher), 1 mM DTT and 2 mM Na_3VO_4. Total sample lysates were mixed with 6x SDS loading buffer (Alfa-Aesar) and boiled for 5 min. Protein samples (10–100 μg) were loaded and separated on 4–20% gradient SDS-PAGE gels (Bio-Rad) and transferred to polyvinylidene difluoride membranes. The membranes were blocked for 1 h with 5% bovine serum albumin in 1x TBS supplemented with 0.1% Tween20 (Bio-Rad) and incubated with antibody against phosphoenolpyruvate carboxykinase (PEPCK) (1:250; Abcam). Bound antibodies were detected using horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies (1:10,000; Jackson ImmunoResearch) and enhanced chemiluminescence reagent (Thermo-Fisher). Band intensities were quantified in Image J software.

[0149] Western blot analysis revealed that levels of the rate limiting gluconeogenic enzyme phosphoenolpyruvate carboxykinase (Pepck) were reduced by 70% in livers of mice treated with H4H1327P (see Figures 3A and B). On the contrary, Pepck levels increased 2.3-fold in livers of mice infused with S961, an effect that was reversed to 30% below baseline by H4H1327P. Thus, the relative levels of glucagon and insulin signaling regulate Pepck expression, as demonstrated previously (Lynedjian et al., (1995); Rucktäschel et al., (2000); Chakravarty et al., (2005)). These data show that GCGR blockade with H4H1327P prevents severe insulin resistance-induced hyperglycemia in mice by suppressing hepatic glucose output.

Example 4: Evaluation of GCGR and Insulin Receptor Antagonism in α- and β-Cell Masses

[0150] The pancreas obtained from mice treated according to each of the four groups from Example 2 were fixed in 10% neutral buffered formalin solution for 48 h, embedded in paraffin, and sectioned onto slides. Pancreatic tissue and cells were permeabilized and hybridized with combinations of mRNA probes for mouse *Gcg* and *Ins2* according to the manufacturer's instructions (Advanced Cell Diagnostics). A chromagenic kit was used to amplify mRNA signal (Advanced Cell Diagnostics). Areas of glucagon and insulin positive cells were measured using Halo digital imaging analysis software (Indica Labs). The percent of glucagon and insulin positive areas in proportion to the whole pancreas area were calculated. α- and β-cell mass was calculated by multiplying the α- and β-cell area for each animal against their corresponding pancreas weight. Islet number was measured by counting the number of insulin positive islets on a section with the use of Halo digital imaging analysis software and normalized by the entire pancreas area of the section.

[0151] H4H1327P increased pancreas weight by 19%, an effect that was larger (33%) in the presence of both H4H1327P and S961 (see Figure 4A). RNA *in situ* hybridization (RNA ISH) using probes to *Gcg* and *Ins2* was used for morphometric analysis of pancreatic sections. H4H1327P increased α-cell mass 5.7-fold (see Figure 4B), and S961 administration increased β-cell mass 3-fold (see Figure 4C). H4H1327P alone did not affect β-cell mass, but unexpectedly, β-cell mass doubled in the simultaneous presence of S961 and H4H1327P when compared to S961 alone and increased 5.8-fold over control mice (see Figure 4C). It is important to note that the further expansion of the β-cell mass took place in settings of normal blood glucose levels (Table 3). α-cell mass was slightly increased by S961 treatment (1.6-fold) and in the simultaneous presence of H4H1327P (1.4-fold over H4H1327P alone) (see Figure 4B). S961 increased islet number per total pancreas area by 49%, whereas the combined treatment with S961 and H4H1327P increased islet number per area by 82% (see Figure 4D). In summary, compensatory increases in α- and β-cell masses were produced when glucagon and insulin signaling were inhibited. The novel finding is that β-cell mass doubled in insulin resistant mice when glucagon signaling was blocked and that this effect took place at normal blood glucose levels.

CLAIMS

What is claimed is:

1. A method for lowering blood glucose levels and/or levels of ketone bodies, or for treating a condition or disease associated with, or characterized in part by high blood glucose or elevated ketone bodies, or at least one symptom or complication associated with the condition or disease, the method comprising administering to a patient having severe insulin resistance a therapeutically effective amount of a composition comprising a glucagon (GCG) inhibitor or a glucagon receptor (GCGR) antagonist, such that blood glucose levels or levels of ketone bodies are lowered or that the condition or disease is mediated, or at least one symptom or complication associated with the condition or disease is alleviated or reduced in severity.

2. The method of claim 1, wherein the patient having severe insulin resistance suffers from a condition or disease selected from the group consisting of Donohue syndrome, Rabson-Mendenhall syndrome, Type A insulin resistance, Type B insulin resistance, HAIR-AN (hyperandrogenism, insulin resistance, and acanthosis nigricans) syndrome, pseudoacromegaly, Alstrom syndrome, myotonic dystrophy, Werner's syndrome, lipodystrophy, cirrhosis, monogenic morbid obesity, hyperproinsulinemia, carboxypeptidase E deficiency, defective arginine metabolism, Bardet-Biedl syndrome, and a condition or disease associated with the presence of a genetic variant reported to cause severe insulin resistance.

3. The method of claim 1, wherein insulin degrading protease activity is detected in the patient sera.

4. The method of claim 1, wherein neutralizing anti-insulin antibodies or anti-insulin receptor antibodies are detected in the patient sera.

5. The method of claim 1, wherein the insulin resistance is associated with a genetic variant of one or more genes selected from the group consisting of *INSR, PSMD6, ADRA2A, AGPAT2, AKT2, APPL1, BBS1, BSCL2, CIDEC, GRB10, IRS2, KLF14, LEP, LEPR, LMNA,*

MC4R, PCNT, PIK2CA, POLD1, PPARG, PTPRD, PTRF, RASGRP1, TBC1D4, and *TCF7L2*.

6. The method of claim 1, wherein the GCG inhibitor or GCGR antagonist is administered concomitantly with insulin.

7. The method of claim 1, wherein the composition is administered to the patient in combination with at least one additional therapeutic agent.

8. The method of claim 7, wherein the at least one additional therapeutic agent is selected from the group consisting of insulin, a biguanide, hIGF1, leptin, pioglitazone, vildagliptin, acarbose, alpha-glycosidase inhibitors, L-arginine, dipeptidyl-peptidase-4 inhibitors, insulin secretagogues, amylin receptor agonists, insulin sensitizers, FGF21, SGLT2 inhibitors, SGLT1 inhibitors, GLP-1 agonists, GLP-1 receptor activators, β3 adrenergic agonists, NPR1 agonists, NPR3 antagonists, tri-iodothyronine, a second GCG inhibitor, and a second GCGR antagonist.

9. The method of claim 1, wherein the GCG inhibitor or GCGR antagonist is an isolated human monoclonal antibody, or an antigen binding fragment thereof.

10. The method of claim 1, wherein the GCGR antagonist is an isolated human monoclonal antibody or antigen-binding fragment thereof comprising the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR), wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and the CDRs of a light chain variable region (LCVR), wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148.

11. The method of claim 10, wherein the isolated antibody or antigen-binding fragment thereof comprises: (a) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and/or (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148.

12. The method of claim 10, wherein the isolated antibody or antigen-binding fragment thereof comprises a HCVR/LCVR sequence pair selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/68, 70/78, 86/88, 90/98, 106/108, 110/118, 126/128, 130/138, and 146/148.

13. The method of claim 10, wherein the isolated antibody or antigen-binding fragment thereof comprises a HCVR/LCVR amino acid sequence pair as set forth in SEQ ID NOs: 86/88.

14. The method of claim 1, wherein the GCG inhibitor is an isolated human monoclonal antibody or antigen-binding fragment thereof comprising: (a) three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) amino acid sequence selected from the group consisting of SEQ ID NOs: 150, 166, 182, 198, 214, 230, 246, 262, 278, and 294; and (b) three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) amino acid sequence selected from the group consisting of SEQ ID NOs: 158, 174, 190, 206, 222, 238, 254, 270, 286, and 302.

15. The method of claim 14, wherein the isolated antibody or antigen binding fragment thereof comprises an HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 150, 166, 182, 198, 214, 230, 246, 262, 278, and 294 and/or a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 158, 174, 190, 206, 222, 238, 254, 270, 286, and 302.

16. The method of claim 14, wherein the isolated antibody or antigen-binding fragment thereof comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 150/158; 166/174; 182/190; 198/206; 214/222; 230/238; 246/254; 262/270; 278/286 and 294/302.

17. The method of claim 14, wherein the isolated antibody or antigen-binding fragment thereof comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 166/174 or SEQ ID NOs: 182/190.

18. The method of claim 8, wherein the insulin secretagogue is selected from the group consisting of sulfonylureas, ATP-sensitive K channel antagonists, and meglitinides.

19. The method of claim 8, wherein the insulin sensitizer is selected from the group consisting of thiazolidinedione and rosiglitazone.

20. The method of claim 1, wherein the ketone bodies are beta-hydroxybutyrate.

21. A method of treating a patient with severe insulin resistance, wherein the patient exhibits elevated levels of blood glucose, the method comprising administering to the patient a therapeutically effective amount of a composition comprising a GCG inhibitor or a GCGR antagonist.

22. The method of claim 21, wherein the patient having severe insulin resistance suffers from a condition or disease selected from the group consisting of Donohue syndrome, Rabson-Mendenhall syndrome, Type A insulin resistance, Type B insulin resistance, HAIR-AN (hyperandrogenism, insulin resistance, and acanthosis nigricans) syndrome, pseudoacromegaly, Alstrom syndrome, myotonic dystrophy, Werner's syndrome, lipodystrophy, cirrhosis, monogenic morbid obesity, hyperproinsulinemia, carboxypeptidase E deficiency, defective arginine metabolism, Bardet-Biedl syndrome, and a condition or disease associated with the presence of a genetic variant reported to cause severe insulin resistance.

23. The method of claim 21, wherein insulin degrading protease activity is detected in the patient sera.

24. The method of claim 21, wherein neutralizing anti-insulin antibodies or anti-insulin receptor antibodies are detected in the patient sera.

25. The method of claim 21, wherein the insulin resistance is associated with a genetic variant of one or more genes selected from the group consisting of *INSR, PSMD6, ADRA2A, AGPAT2, AKT2, APPL1, BBS1, BSCL2, CIDEC, GRB10, IRS2, KLF14, LEP, LEPR, LMNA, MC4R, PCNT, PIK2CA, POLD1, PPARG, PTPRD, PTRF, RASGRP1, TBC1D4*, and *TCF7L2*.

26. The method of claim 21, wherein the GCG inhibitor or GCGR antagonist is administered concomitantly with insulin.

27. The method of claim 21, wherein the composition is administered to the patient in combination with at least one additional therapeutic agent.

28. The method of claim 27, wherein the at least one additional therapeutic agent is selected from the group consisting of insulin, a biguanide, hIGF1, leptin, pioglitazone, vildagliptin, acarbose, alpha-glycosidase inhibitors, L-arginine, dipeptidyl-peptidase-4 inhibitors, insulin secretagogues, amylin receptor agonists, insulin sensitizers, FGF21, SGLT2 inhibitors, SGLT1 inhibitors, GLP-1 agonists, GLP-1 receptor activators, β3 adrenergic agonists, NPR1 agonists, NPR3 antagonists, tri-iodothyronine, a second GCG inhibitor, and a second GCGR antagonist.

29. The method of claim 21, wherein the GCG inhibitor or GCGR antagonist is an isolated human monoclonal antibody, or an antigen binding fragment thereof.

30. The method of claim 21, wherein the GCGR antagonist is an isolated human monoclonal antibody comprising the CDRs of a HCVR, wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and the CDRs of a LCVR, wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148.

31. The method of claim 30, wherein the isolated antibody or antigen-binding fragment thereof comprises: (a) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and/or (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148.

32. The method of claim 30, wherein the isolated antibody or antigen-binding fragment comprises a HCVR/LCVR sequence pair selected from the group consisting of SEQ ID NO:

2/10, 18/26, 34/42, 50/58, 66/68, 70/78, 86/88, 90/98, 106/108, 110/118, 126/128, 130/138, and 146/148.

33. The method of claim 30, wherein the isolated human monoclonal antibody comprises a HCVR/LCVR amino acid sequence pair as set forth in SEQ ID NOs: 86/88.

34. The method of claim 21, wherein the GCG inhibitor is an isolated human monoclonal antibody or antigen-binding fragment thereof comprising: (a) three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) amino acid sequence selected from the group consisting of SEQ ID NOs: 150, 166, 182, 198, 214, 230, 246, 262, 278, and 294; and (b) three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) amino acid sequence selected from the group consisting of SEQ ID NOs: 158, 174, 190, 206, 222, 238, 254, 270, 286, and 302.

35. The method of claim 34, wherein the isolated antibody or antigen binding fragment thereof comprises an HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 150, 166, 182, 198, 214, 230, 246, 262, 278, and 294 and/or a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 158, 174, 190, 206, 222, 238, 254, 270, 286, and 302.

36. The method of claim 34, wherein the isolated antibody or antigen-binding fragment thereof comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 150/158; 166/174; 182/190; 198/206; 214/222; 230/238; 246/254; 262/270; 278/286 and 294/302.

37. The method of claim 34, wherein the isolated antibody or antigen-binding fragment thereof comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 166/174 or SEQ ID NOs: 182/190.

38. The method of claim 28, wherein the insulin secretagogue is selected from the group consisting of sulfonylureas, ATP-sensitive K channel antagonists, and meglitinides.

39. The method of claim 28, wherein the insulin sensitizer is selected from the group consisting of thiazolidinedione and rosiglitazone.

40. A method of reducing the amount and/or dosage of insulin necessary to treat a patient with severe insulin resistance, wherein the patient exhibits severe insulin resistance and elevated levels of blood glucose, the method comprising administering to the patient a therapeutically effective amount of a composition comprising a GCG inhibitor or a GCGR antagonist.

41. The method of claim 40, wherein the GCG inhibitor or the GCGR antagonist is administered concomitantly with insulin.

42. The method of claim 40, wherein the amount and/or dosage of insulin may be reduced by about 30% to about 95% when administered concomitantly with a GCGR antagonist, and wherein the GCGR antagonist is an isolated human monoclonal antibody that binds specifically to the GCGR.

43. The method of claim 40, wherein the amount and/or dosage of insulin may be reduced by about 90% when administered concomitantly with a GCGR antagonist, wherein the antagonist is an isolated human monoclonal antibody that binds specifically to GCGR.

44. The method of claim 40, wherein the patient having severe insulin resistance suffers from a condition or disease selected from the group consisting of Donohue syndrome, Rabson-Mendenhall syndrome, Type A insulin resistance, Type B insulin resistance, HAIR-AN (hyperandrogenism, insulin resistance, and acanthosis nigricans) syndrome, pseudoacromegaly, Alstrom syndrome, myotonic dystrophy, Werner's syndrome, lipodystrophy, cirrhosis, monogenic morbid obesity, hyperproinsulinemia, carboxypeptidase E deficiency, defective arginine metabolism, Bardet-Biedl syndrome, and a condition or disease associated with the presence of a genetic variant reported to cause severe insulin resistance.

45. The method of claim 40, wherein insulin degrading protease activity is detected in the patient sera.

46. The method of claim 40, wherein neutralizing anti-insulin antibodies or anti-insulin receptor antibodies are detected in the patient sera.

47. The method of claim 40, wherein the insulin resistance is associated with a genetic variant of one or more genes selected from the group consisting of *INSR, PSMD6, ADRA2A, AGPAT2, AKT2, APPL1, BBS1, BSCL2, CIDEC, GRB10, IRS2, KLF14, LEP, LEPR, LMNA, MC4R, PCNT, PIK2CA, POLD1, PPARG, PTPRD, PTRF, RASGRP1, TBC1D4*, and *TCF7L2*.

48. The method of claim 40, wherein the composition is administered to the patient in combination with at least one additional therapeutic agent.

49. The method of claim 48, wherein the at least one additional therapeutic agent is selected from the group consisting of a biguanide, hIGF1, leptin, pioglitazone, vildagliptin, acarbose, alpha-glycosidase inhibitors, L-arginine, dipeptidyl-peptidase-4 inhibitors, insulin secretagogues, amylin receptor agonists, insulin sensitizers, FGF21, SGLT2 inhibitors, SGLT1 inhibitors, GLP-1 agonists, GLP-1 receptor activators, β3 adrenergic agonists, NPR1 agonists, NPR3 antagonists, tri-iodothyronine, a second GCG inhibitor, and a second GCGR antagonist.

50. The method of claim 40, wherein the GCG inhibitor or GCGR antagonist is an isolated human monoclonal antibody, or an antigen binding fragment thereof.

51. The method of claim 40, wherein the GCGR antagonist is an isolated human monoclonal antibody comprising the CDRs of a HCVR, wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and the CDRs of a LCVR, wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148.

52. The method of claim 51, wherein the isolated antibody or antigen-binding fragment thereof comprises: (a) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 70, 86, 90, 106, 110, 126, 130 and 146; and/or

(b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 68, 78, 88, 98, 108, 118, 128, 138 and 148.

53. The method of claim 51, wherein the isolated antibody or antigen-binding fragment comprises a HCVR/LCVR sequence pair selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/68, 70/78, 86/88, 90/98, 106/108, 110/118, 126/128, 130/138, and 146/148.

54. The method of claim 51, wherein the isolated antibody comprises a HCVR/LCVR amino acid sequence pair as set forth in SEQ ID NOs: 86/88.

55. The method of claim 40, wherein the GCG inhibitor is an isolated human monoclonal antibody or antigen-binding fragment thereof comprising: (a) three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) amino acid sequence selected from the group consisting of SEQ ID NOs: 150, 166, 182, 198, 214, 230, 246, 262, 278, and 294; and (b) three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) amino acid sequence selected from the group consisting of SEQ ID NOs: 158, 174, 190, 206, 222, 238, 254, 270, 286, and 302.

56. The method of claim 55, wherein the isolated antibody or antigen binding fragment thereof comprises an HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 150, 166, 182, 198, 214, 230, 246, 262, 278, and 294 and/or a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 158, 174, 190, 206, 222, 238, 254, 270, 286, and 302.

57. The method of claim 55, wherein the isolated antibody or antigen-binding fragment thereof comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 150/158; 166/174; 182/190; 198/206; 214/222; 230/238; 246/254; 262/270; 278/286 and 294/302.

58. The method of claim 55, wherein the isolated antibody or antigen-binding fragment thereof comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 166/174 or SEQ ID NOs: 182/190.

59. The method of claim 49, wherein the insulin secretagogue is selected from the group consisting of sulfonylureas, ATP-sensitive K channel antagonists, and meglitinides.

60. The method of claim 49, wherein the insulin sensitizer is selected from the group consisting of thiazolidinedione and rosiglitazone.

61. A method for lowering blood glucose levels and/or beta-hydroxybutyrate levels, or for treating a condition or disease associated with, or characterized in part by high blood glucose or high levels of ketone bodies, or at least one symptom or complication associated with the condition or disease, the method comprising administering to a patient having severe insulin resistance a therapeutically effective amount of a composition comprising an inhibitor of GCGR signaling, such that blood glucose levels or beta-hydroxybutyrate levels are lowered or that the condition or disease is mediated, or at least one symptom or complication associated with the condition or disease is alleviated or reduced in severity.

62. The method of claim 60, wherein the inhibitor of GCGR signaling is selected from the group consisting of antisense molecules, GCGR antibodies, small molecule inhibitors, peptide inhibitors, DARPins, Spiegelmers, aptamers, engineered Fn type-III domains, GCG antibodies, and derivatives thereof.

63. A method of suppressing hepatic glucose output, the method comprising administering to a patient having severe insulin resistance a therapeutically effective amount of a composition comprising an inhibitor of GCGR signaling, such that hepatic glucose output is suppressed.

64. A method of increasing β-cell mass in a patient having severe insulin resistance, the method comprising administering to the patient a therapeutically effective amount of a composition comprising an inhibitor of GCGR signaling, such that β-cell mass increases relative to the β-cell mass prior to treatment.

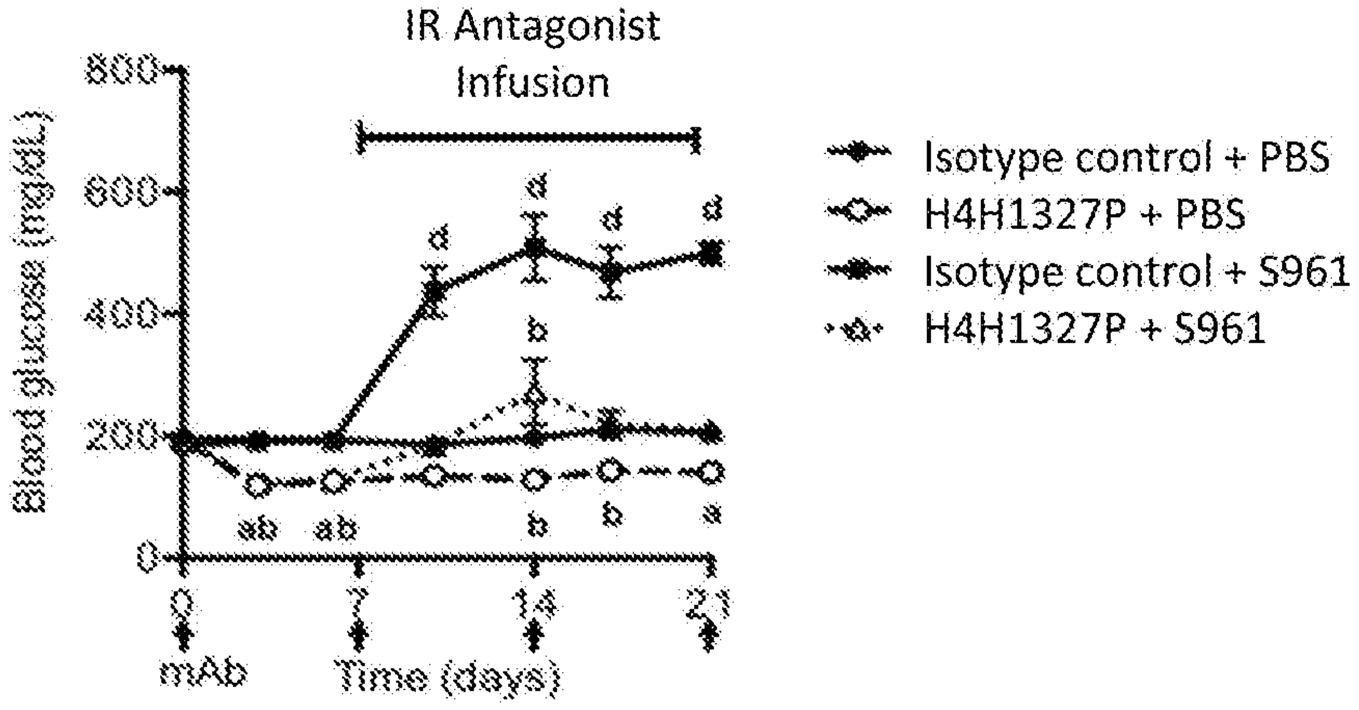
IR Antagonist
Infusion
Blood glucose (mg/dL)
800
600
400
200
0
a
a
a
a
b
ab
ab
b
b
a
0
7
14
21
mAb
Time (days)
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 1A

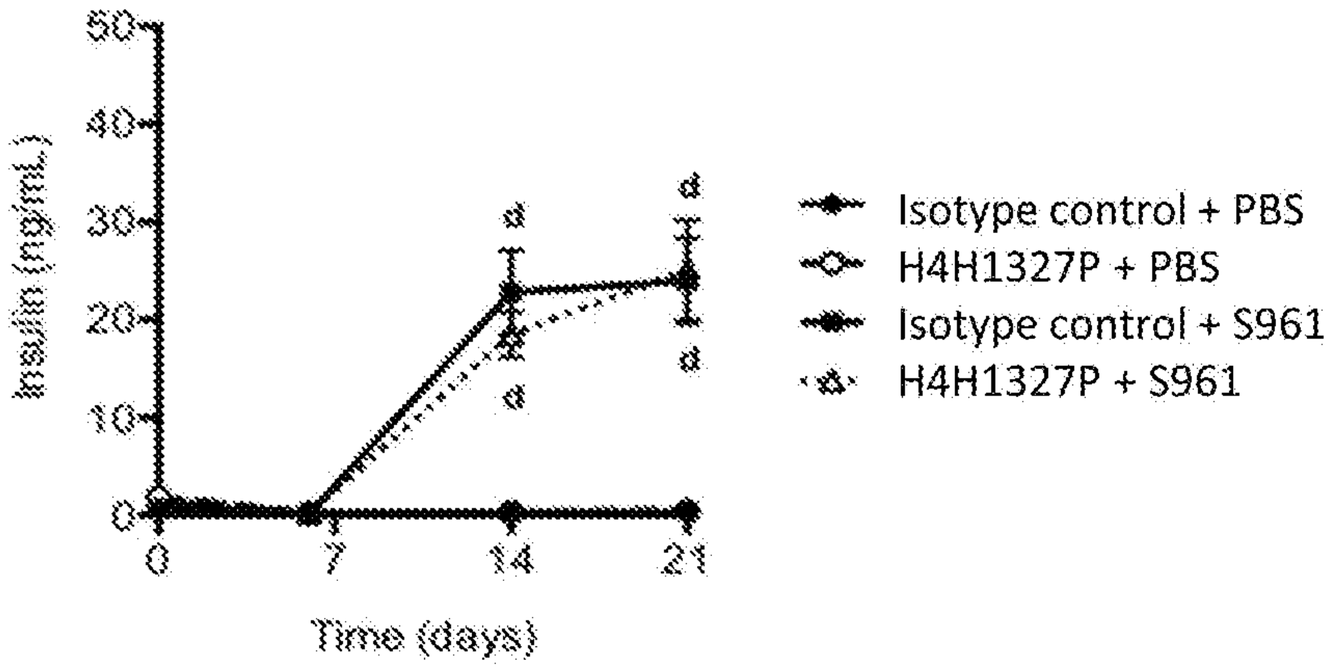
Insulin (ng/mL)
50
40
30
20
10
0
a
a
a
a
0
7
14
21
Time (days)
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 1B

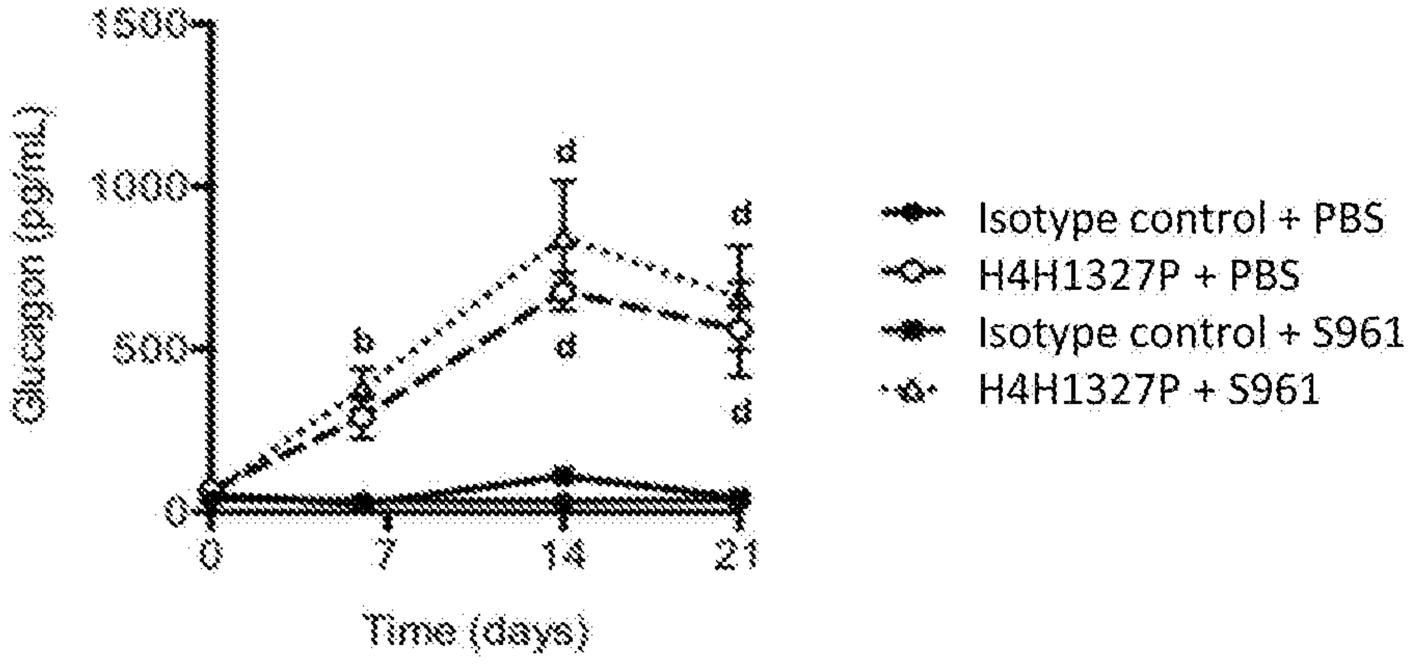
1500
1000
500
0
Glucagon (pg/mL)
a
b
a
a
a
0
7
14
21
Time (days)
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 1C

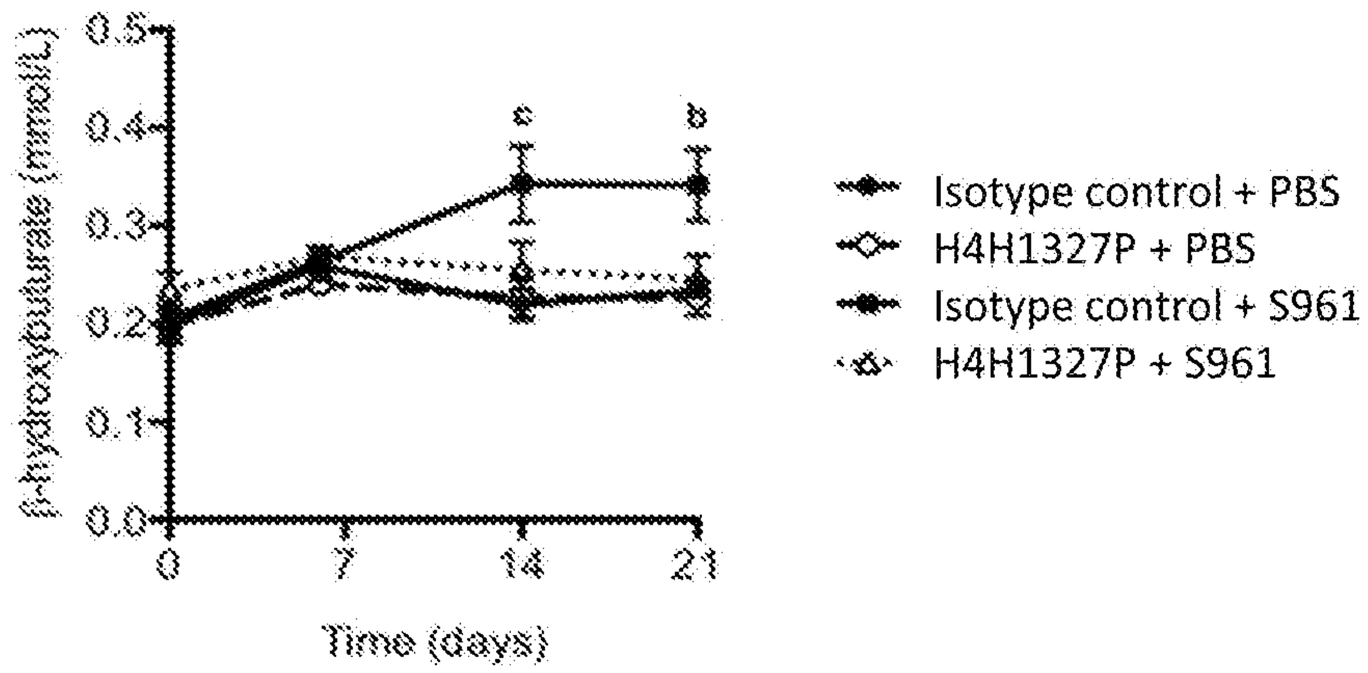
0.5
0.4
0.3
0.2
0.1
0.0
β-hydroxybutyrate (mmol/L)
c
a
0
7
14
21
Time (days)
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 1D

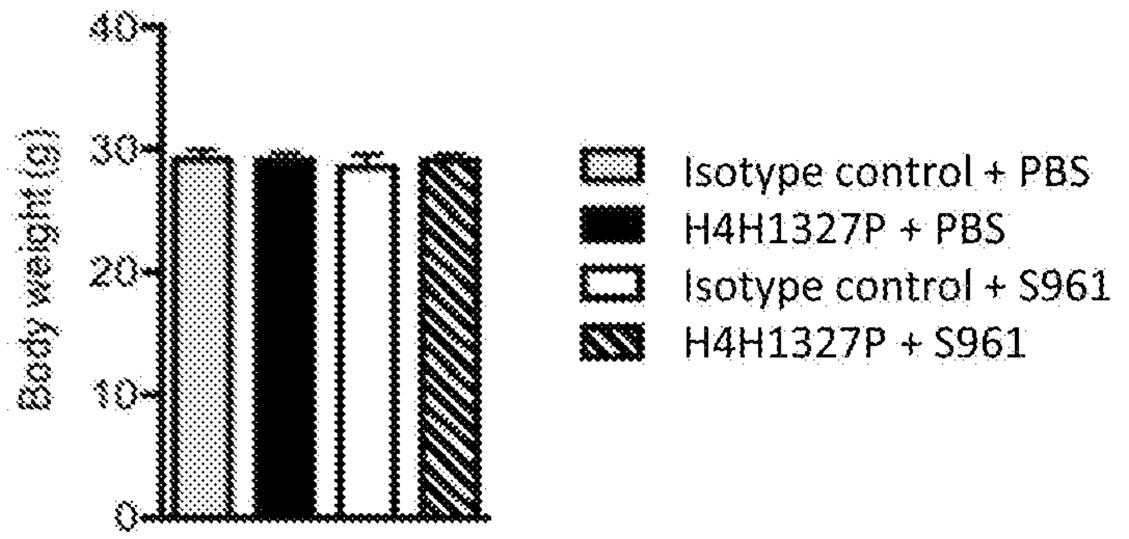
Body weight (g)
40
30
20
10
0
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 1E

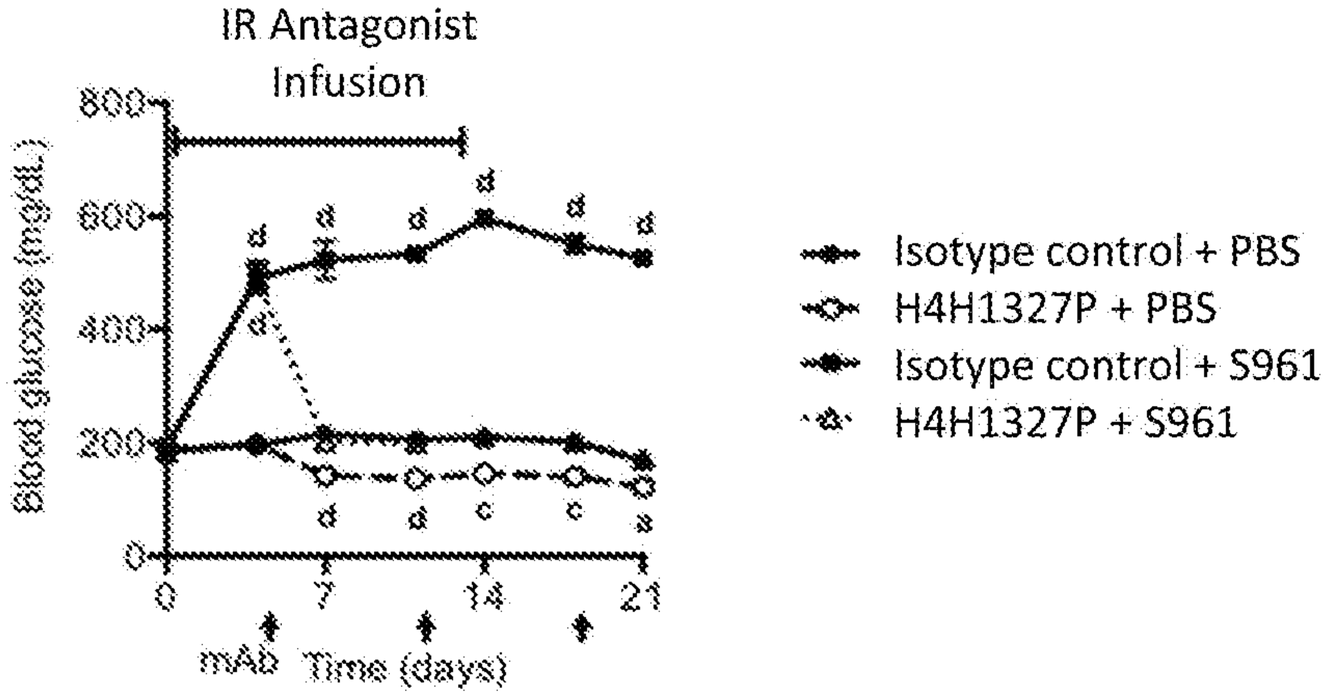
IR Antagonist
Infusion
Blood glucose (mg/dL)
800
600
400
200
0
0
7
14
21
mAb
Time (days)
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 2A

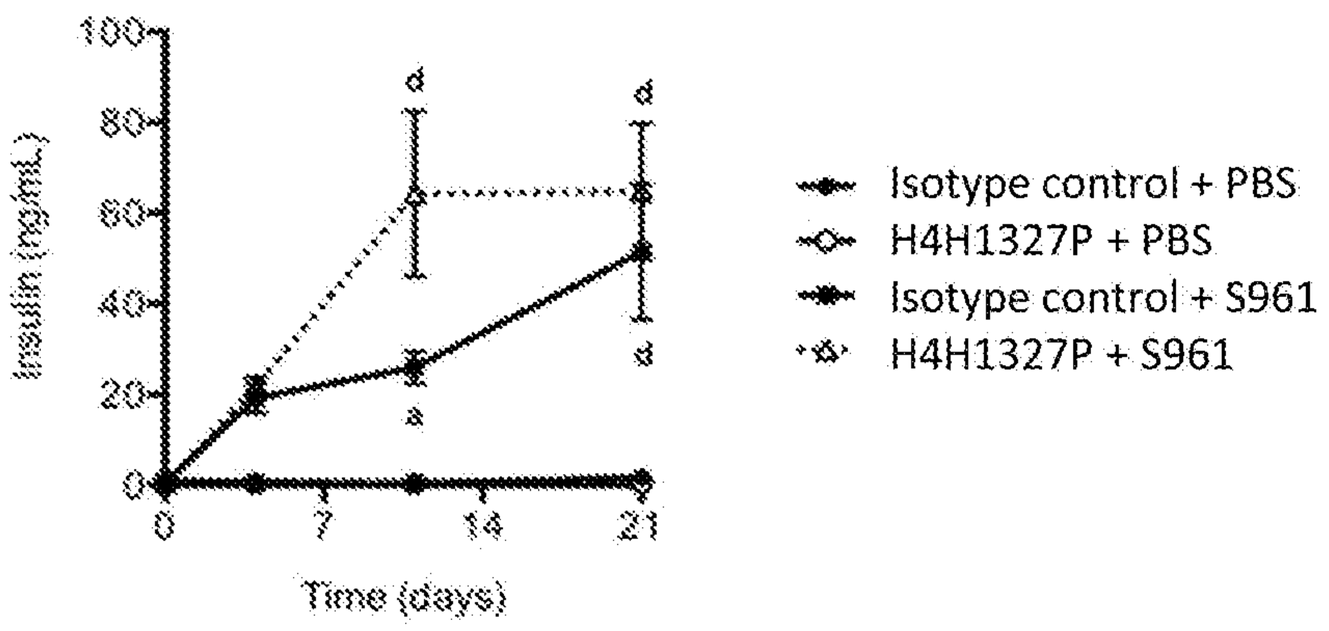
Insulin (ng/mL)
100
80
60
40
20
0
0
7
14
21
Time (days)
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 2B

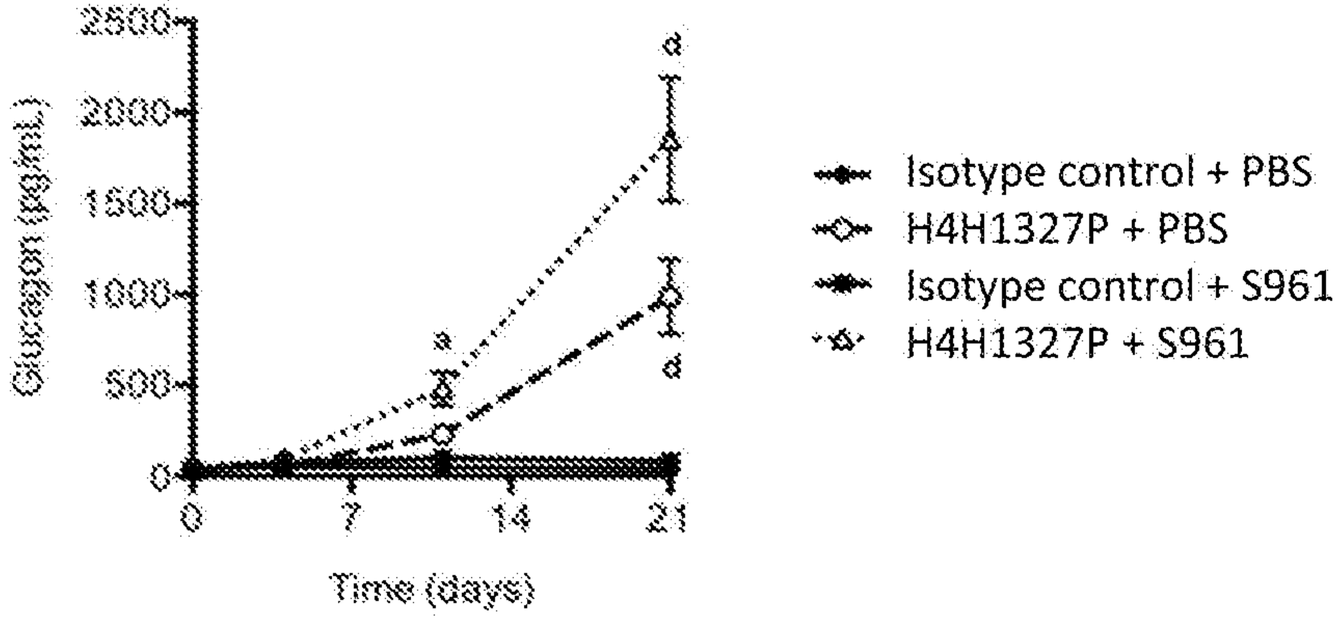
Glucagon (pg/mL)
2500
2000
1500
1000
500
0
0
7
14
21
Time (days)
a
a
a
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 2C

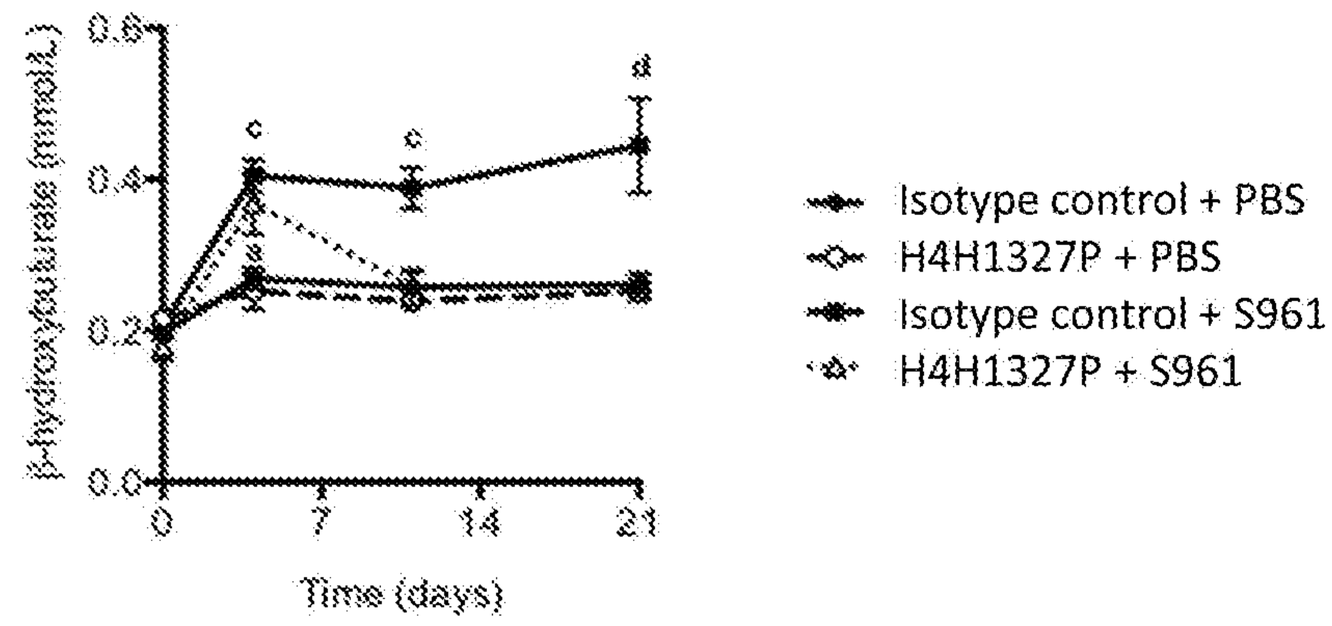
β-hydroxybutyrate (mmol/L)
0.6
0.4
0.2
0.0
0
7
14
21
Time (days)
c
c
a
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 2D

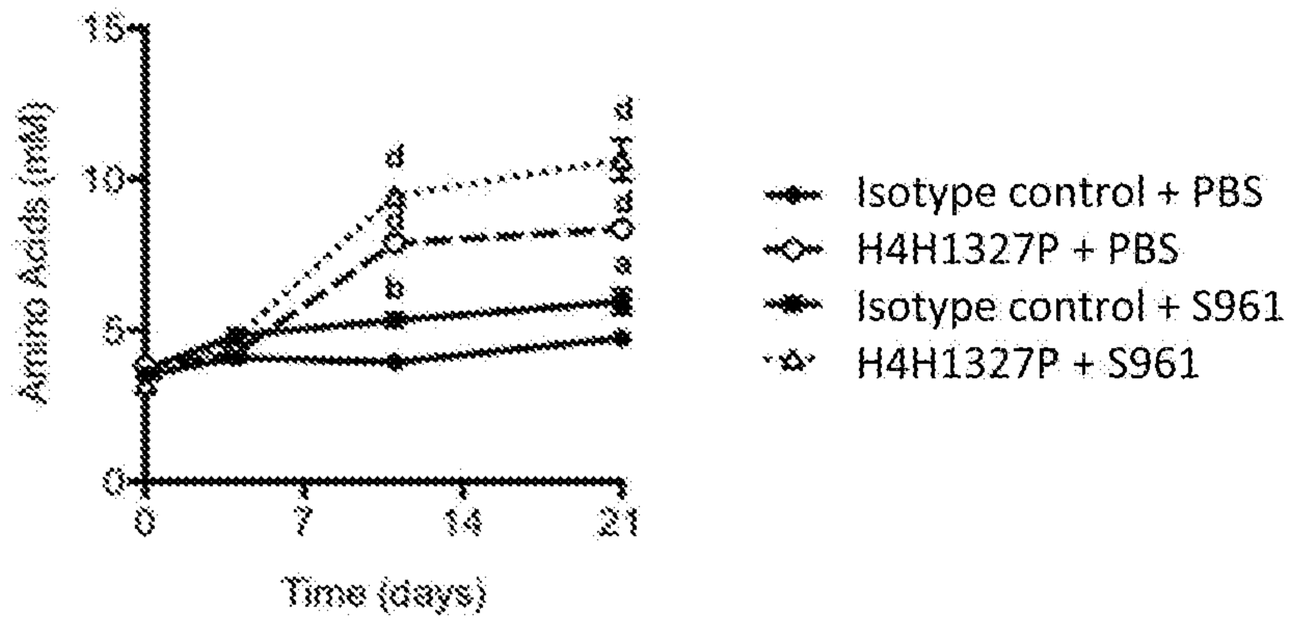
Amino Acids (mM)
15
10
5
0
0
7
14
21
Time (days)
a
a
a
b
b
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 2E

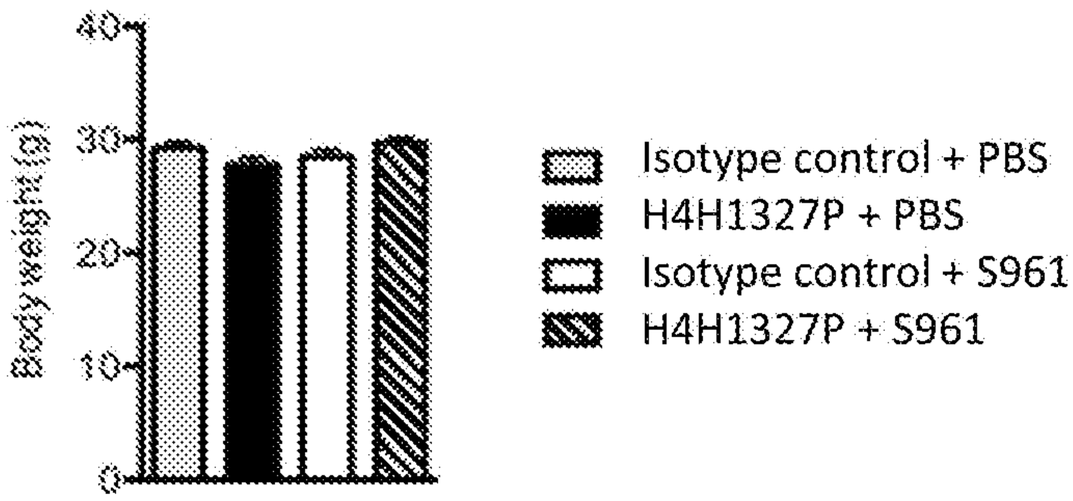
Body weight (g)
40
30
20
10
0
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 2F

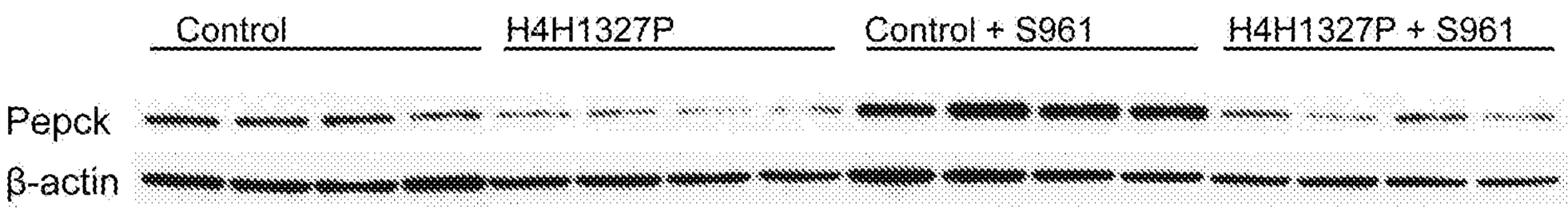
Control
H4H1327P
Control + S961
H4H1327P + S961
Pepck
β-actin

FIG. 3A

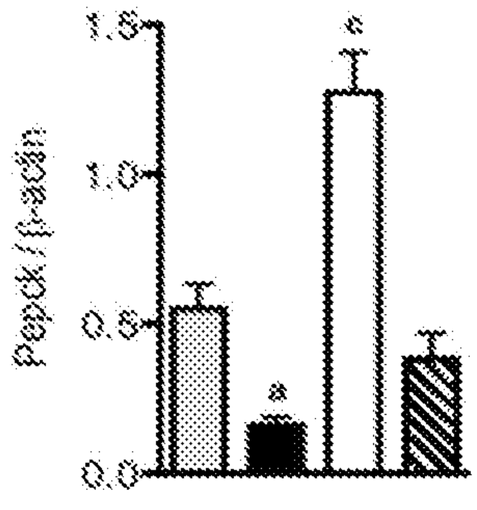
Pepck / β-actin
1.5
1.0
0.5
0.0
*
*

FIG. 3B

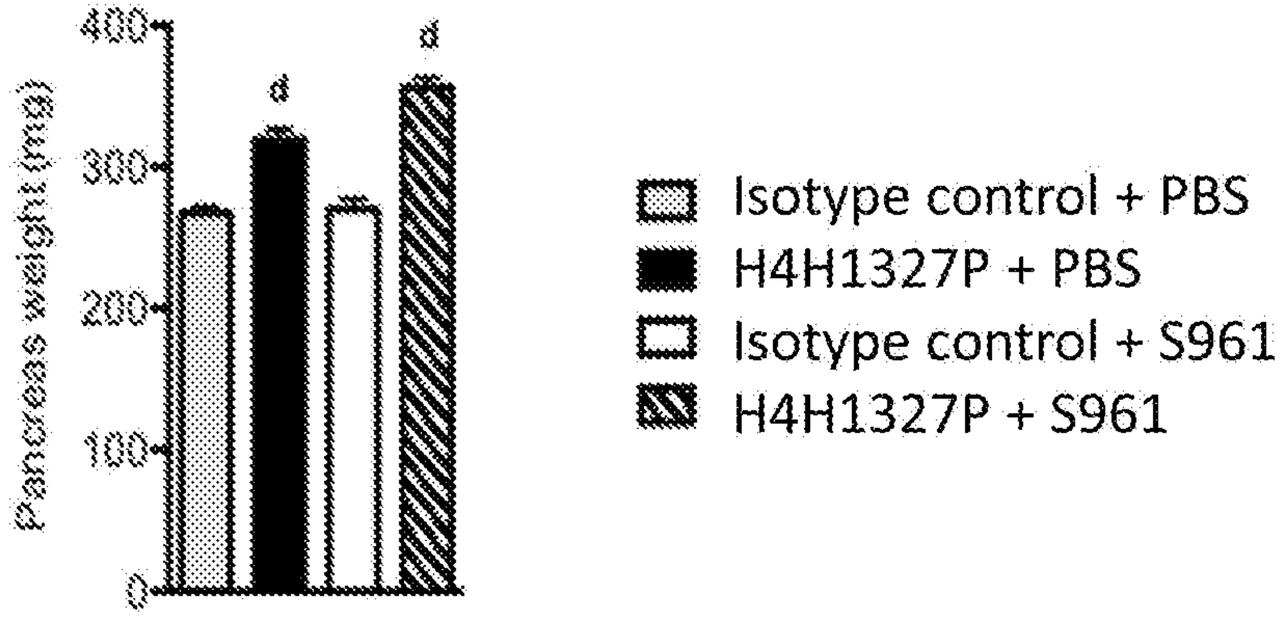
Pancreas weight (mg)
400
300
200
100
0
a
a
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 4A


α-cell mass (mg)
a
a
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 4B

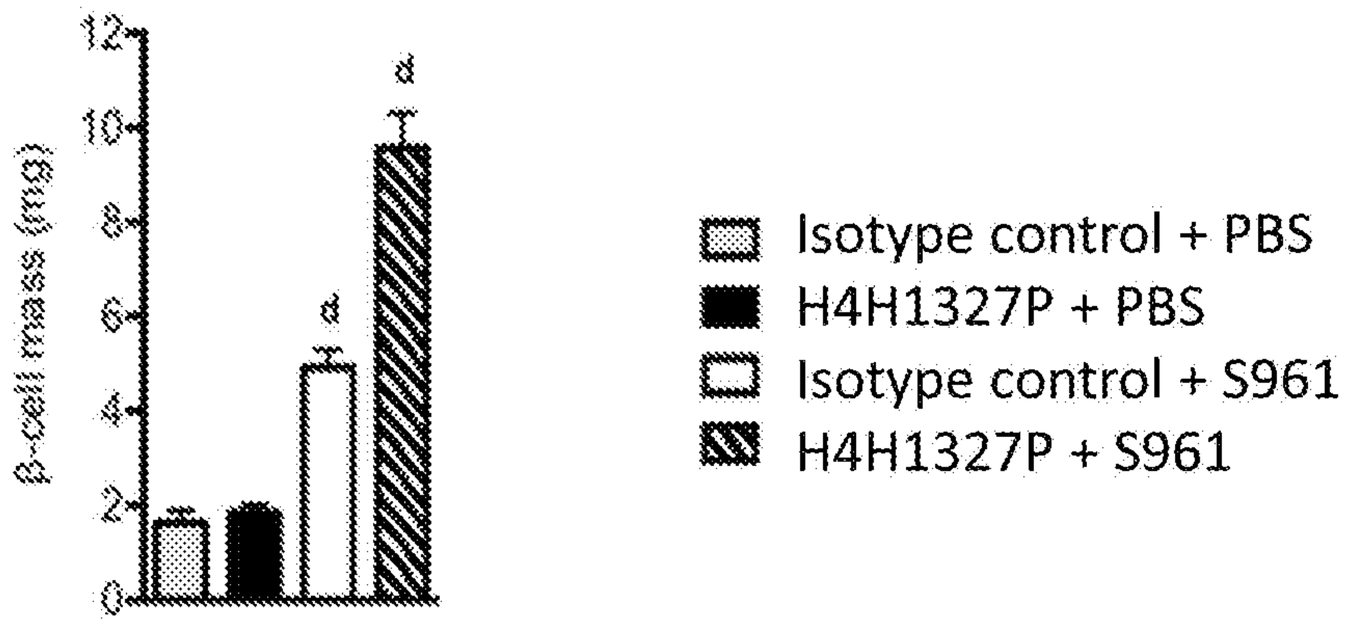
β-cell mass (mg)
0
2
4
6
8
10
12
a
a
a
a
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 4C

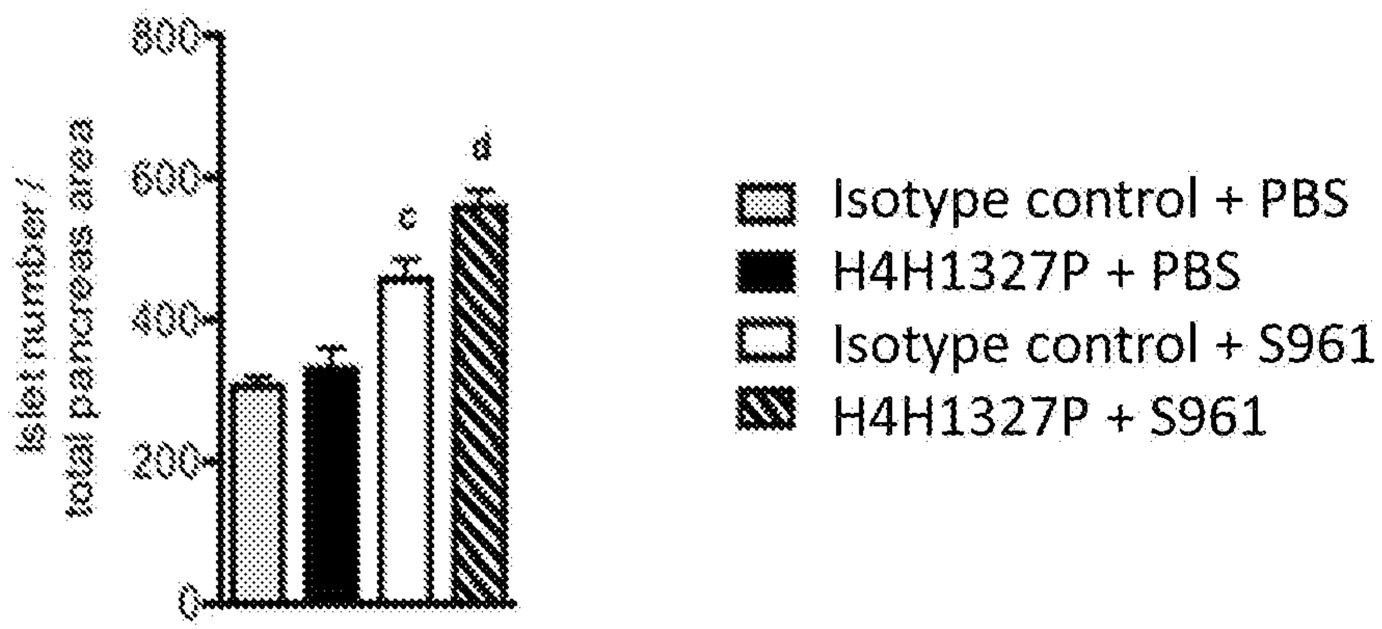
Islet number / total pancreas area
0
200
400
600
800
b
c
a
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961

FIG. 4D

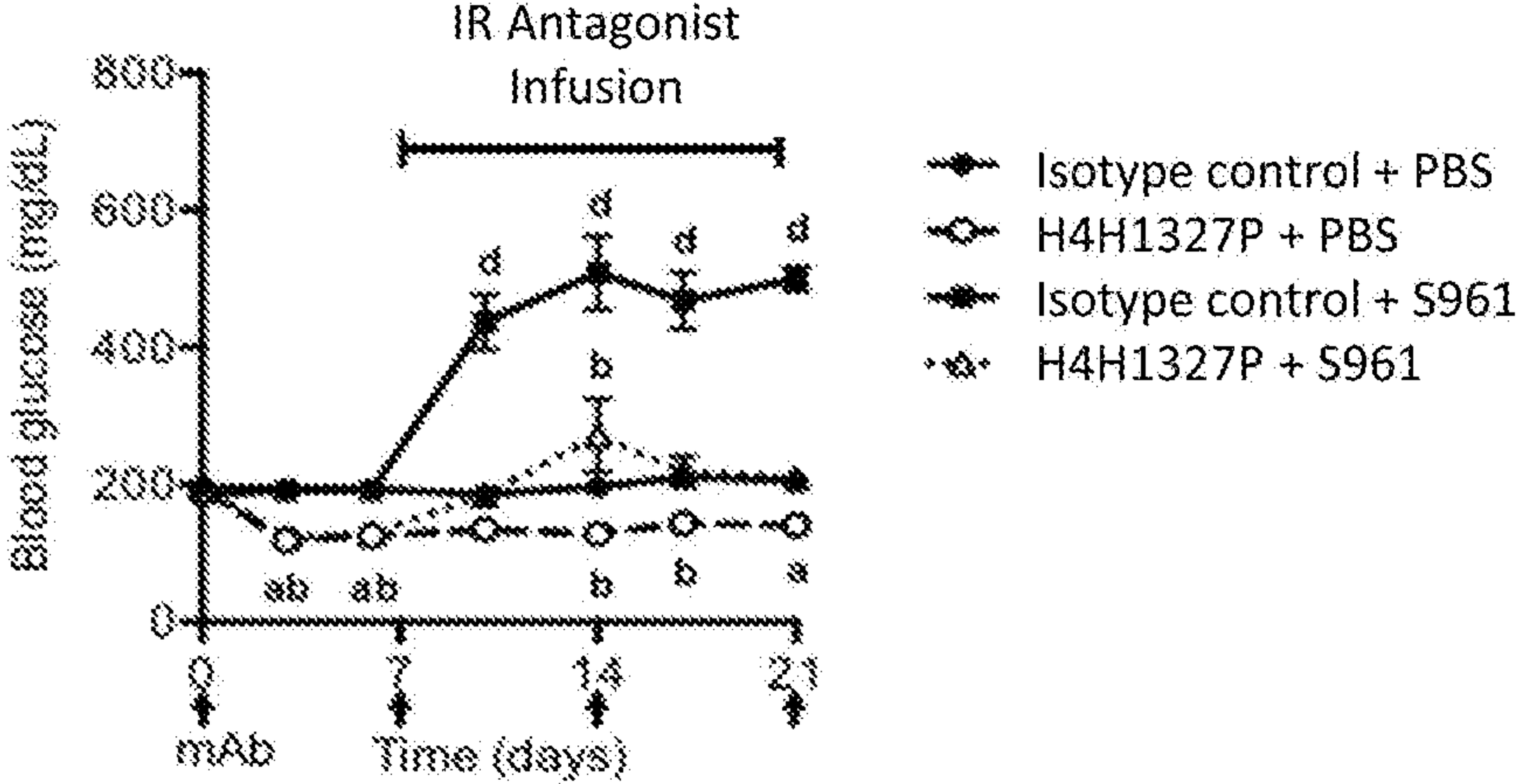
IR Antagonist
Infusion
Isotype control + PBS
H4H1327P + PBS
Isotype control + S961
H4H1327P + S961
800
600
400
200
0
mAb
Time (days)

FIG. 1A