(19) DANMARK

(10) DK/EP 2790721 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *A 61 K 38/08 (2006.01)* *A 61 K 38/12 (2006.01)* *A 61 K 38/25 (2006.01)*
A 61 P 3/04 (2006.01) *A 61 P 3/10 (2006.01)* *G 01 N 33/74 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2019-02-04**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2018-10-17**

(86) Europæisk ansøgning nr.: **12829142.4**

(86) Europæisk indleveringsdag: **2012-12-14**

(87) Den europæiske ansøgnings publiceringsdag: **2014-10-22**

(86) International ansøgning nr.: **IB2012002867**

(87) Internationalt publikationsnr.: **WO2013088241**

(30) Prioritet: **2011-12-15 US 201161576217 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

(73) Patenthaver: **Millendo Therapeutics SAS, 15, Chemin du Saquin , Espace Européen, 69130 Ecully, Frankrig**

(72) Opfinder: **VAN DER LELY, Aart Jan, Papavertuin 14, NL-2662 CW Bergschenhoek, Holland**
ABRIBAT, Thierry, 124 Chemin de Montray, F-69110 Sainte Foy Les Lyon, Frankrig

(74) Fuldmægtig i Danmark: **Plougmann Vingtoft A/S, Strandvejen 70, 2900 Hellerup, Danmark**

(54) Benævnelse: **FRAGMENTER AF IKKE-ACYLERET GHRELIN TIL ANVENDELSE TIL BEHANDLING AF PRADER-WILLI SYNDROM**

(56) Fremdragne publikationer:
EP-A1- 2 067 481
WO-A1-2005/039624
WO-A1-2008/145749
WO-A2-03/051389
US-A1- 2008 159 991
US-A1- 2011 245 160
DELPARIGI ANGELO ET AL: "High circulating ghrelin: A potential cause for hyperphagia and obesity in Prader-Willi syndrome.", JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, vol. 87, no. 12, December 2002 (2002-12), pages 5461-5464, XP055061123, ISSN: 0021-972X
RODRIGUEZ A ET AL: "Acylated and desacyl ghrelin stimulate lipid accumulation in human visceral adipocytes", INTERNATIONAL JOURNAL OF OBESITY, vol. 33, no. 5, May 2009 (2009-05), pages 541-552, XP002696090, ISSN: 0307-0565
Anonymous: "UnAcylated Ghrelin (UAG)", Alizé Pharma , 2013, XP002696091, Retrieved from the Internet: URL:http://www.alz-pharma.com/r-and-d-proj ets/unacylated-ghrelin-uag [retrieved on 2013-04-24]

DESCRIPTION

FIELD OF THE INVENTION

[0001] This invention relates to the use of unacylated ghrelin, fragments and/or analogs thereof for modulating ghrelin levels and/or ghrelin/unacylated ghrelin ratio in a subject wherein such modulation is beneficial to the subject. The invention also relates to the composition comprising unacylated ghrelin, fragments and/or analogs thereof modulating ghrelin levels and/or ghrelin/unacylated ghrelin ratio in a subject wherein such modulation is beneficial to the subject. The invention further relates to the use of ghrelin level and/or ghrelin/unacylated ghrelin ratio as biomarkers for determining a subject's likelihood of responding to and/or benefiting from administration of unacylated ghrelin.

BACKGROUND

[0002] Ghrelin (also referred as "acylated ghrelin" or abbreviated as "AG") is a 28 amino acid peptide, purified and identified from rat stomach and characterized by the presence of an *n*-octanoyl modification on the Ser3 residue (Ref. 1). Acylation of ghrelin is catalyzed by the enzyme ghrelin O-acyl transferase (GOAT). The expression of GOAT is mostly in the stomach and intestine. Ghrelin is the endogenous ligand of the growth hormone (GH) secretagogue receptor (GHSR-1a) (Refs. 2, 3). Ghrelin is now mostly recognized as a potent orexigenic factor stimulating food intake and modulating energy expenditure (Refs. 4, 5 and 6). At the peripheral level, Ghrelin exerts probably its major physiological action regulating glucose and lipid metabolism (Ref. 7). In fact, ghrelin has a diabetogenic action (Ref. 8) and suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance (Ref. 9). As such, elevated plasma ghrelin is of relevance in certain disorders of the metabolism and growth such as in diabetes and obesity. Elevated plasma ghrelin levels have also been demonstrated amongst adults and children with Prader-Willi Syndrome (PWS) (Ref. 10 and 11). PWS is a genetic obesity syndrome associated in most patients with GH deficiency. Children with PWS present a rapid weight gain along with a voracious appetite. Studies on the involvement of ghrelin in PWS have provided a significant rationale that the hyperphagia observed in PWS is positively correlated with elevated ghrelin levels, consistent with the known orexigenic effect of ghrelin (Ref. 12).

[0003] Unacylated ghrelin (also referred as "des-acyl ghrelin" or abbreviated as "UAG"), is the non-acylated form of ghrelin. Its concentration in plasma and tissue is higher compared to ghrelin. UAG has long been considered as a product with no physiological role as it fails to bind the only known ghrelin receptor GHSR-1a at physiological concentrations and has no physiological effect on GH secretion (Ref. 15). However, UAG is a biologically active peptide, particularly at the metabolic level and its administration has been shown to induce a negative energy balance by decreasing food intake and delaying gastric emptying (Ref. 16). Over-

expression of UAG in mice results in a decrease in fat accumulation with an increase in insulin sensitivity and glucose tolerance (Refs. 16 and 17).

[0004] UAG has been shown to prevent the hyperglycemic effects of ghrelin, when administered concomitantly, in healthy volunteers. see in particular U.S. Patent 7,825,090, herein incorporated in its entirety by reference. This initial observation was followed by several reports on the anti-diabetogenic potential of UAG (Refs. 18, 19, 30, 31 and 32).

[0005] *In vitro, in vivo* and clinical evidence indicate that UAG prevents the diabetogenic effects of ghrelin in healthy volunteers and in GH-deficient patients (Refs. 18 and 19). It inhibits both basal and ghrelin-induced glucose secretion by human hepatocytes (Ref. 31). In rats, UAG enhances portal insulin response to glucose (Ref. 32) and reduces fat deposition and triglycerides levels, as observed in transgenic mice overexpressing UAG (Ref. 16). *In vitro*, UAG stimulates insulin secretion from insulinoma cells (Ref. 32) and promotes proliferation and inhibits apoptosis of beta cells (Ref. 33).

[0006] The anti-diabetogenic effects and ghrelin-antagonizing effects of UAG, fragments and analogs thereof have been reported In U.S. Patent Number 7,485,620; U.S. Patent Number 8,222,217; U.S. Patent Number 8,318,664 and in WO 2008/145749.

[0007] Recent experiments on circulating angiogenic cells (CAC) indicates that UAG beneficially impacts the vascular remodeling process which is known to be impaired in type 2 diabetes patients. The effects of UAG on CAC have been reported in U.S. Patent Application Serial Number 2010/0016226 and in WO 2009/150214.

[0008] Obese mice and humans have been reported to present lower UAG levels than normal weight subjects, indicating that obesity might be correlated with a relative UAG deficiency (Refs. 34, 35 and 21). It has been observed that insulin-resistant obese subjects have an elevated AG/UAG ratio when compared to insulin-sensitive obese subjects (Refs. 20 and 22).

[0009] Treatments that target ghrelin and the GHS-R (*i.e.,* ghrelin antagonists) have been suggested as attractive pharmacologic avenues to fight against obesity and other conditions, disorders and diseases associated with ghrelin. Several GHS-R ligands and anti-obesity vaccines have been proposed (Ref. 24). Other pharmacological approaches inducing antibodies against ghrelin, ghrelin enantiomers and inhibition of ghrelin acyl-transferase (GOAT) (Ref. 25) have been investigated: however, due to lack of efficacy, non-selectivity and lack of sustained weight loss, these pharmacological approaches have not yet reached the market (Ref. 26).

[0010] Therefore, there exists a need in the art for an efficient and more direct way of modulating circulating ghrelin levels and/or circulating ghrelin/unacylated ghrelin ratio in subjects wherein such modulation is beneficial to the subject and for more efficient ways of identifying those subjects that can benefit from modulation of ghrelin levels and ghrelin/unacylated ghrelin ratio.

SUMMARY OF THE INVENTION

[0011] The invention provides fragments of unacylated ghrelin consisting of the peptide as set forth in SEQ ID NO: 6 or as set forth in SEQ ID NO: 25, and a pharmaceutically acceptable salt thereof, for their use in the treatment of Prader-Willi Syndrome in a subject having elevated ghrelin levels, particularly elevated circulating ghrelin levels. The invention particularly provides unacylated ghrelin fragments and salt thereof for their use in the treatment of Prader-Willi Syndrome in a subject having elevated ghrelin levels, wherein it comprises administering an effective amount of said unacylated ghrelin fragment and salt from about 0.001 µg/kg to about 10 mg/kg, more preferably from about 1 µg/kg to about 1 mg/kg.

[0012] According to the invention, said unacylated ghrelin fragment and pharmaceutically acceptable salts thereof may be administered through a route selected from the group consisting of intravenous, subcutaneous, transdermal, oral, buccal, sublingual, nasal and inhalation.

[0013] The invention also provides fragments of unacylated ghrelin consisting of the peptide as set forth in SEQ ID NO: 6 or as set forth in SEQ ID NO: 25, and a pharmaceutically acceptable salt thereof, for their use in the treatment of Prader-Willi Syndrome in a subject having elevated ghrelin levels,, wherein the patient was identified by

- determining a level of circulating ghrelin from the subject, and
- processing the level of circulating ghrelin at least in part based on a reference level of circulating ghrelin to derive information conveying whether the level of circulating ghrelin is elevated.

BRIEF DESCRIPTION OF THE FIGURES

[0014]

Figure 1 is a schematic representation of a study protocol involving Type 2 Diabetes Mellitus (T2DM) subjects according to one embodiment of the present invention. SBM refers to Standard Breakfast Meal.

Figures 2A and 2B are graphs illustrating the effect of UAG infusion on AG serum levels in T2DM subjects. In Figure 2A, AG serum levels in pg/ml were measured following placebo or UAG administration before and one hour after SBM. In Figure 2B, UAG serum levels in pg/ml were measured following placebo or UAG administration before and one hour after SBM.

Figures 3A and 3B are graphs illustrating the mean post-prandial glucose levels in T2DM

subjects as measured with a continuous glucose monitoring device (CGMS® iPro™ Continuous Glucose Recorder, Medtronic trading. The Netherlands). Figure 3A shows the mean absolute glucose levels after SBM and following placebo, UAG 3 mcg/kg/h and UAG 10 mcg/kg/h infusions. Repeated measures ANOVA $p<0.0001$; Bonferroni's Multiple Comparisons placebo vs. 3 mcg NS; placebo vs. 10 mcg $p<0.001$; 3 mcg vs. 10 mcg $p<0.001$. Figure 3B shows the mean t0-t180 glucose levels for the three treated groups.

Figures 4A and 4B are graphs illustrating the mean post-prandial glucose levels in T2DM subjects. Figure 4A shows the change in glucose levels from pre-meal baseline following placebo, UAG 3 mcg/kg/h and UAG 10 mcg/kg/h infusions. Repeated measures ANOVA $p<0.0001$; Sonferroni's Multiple Comparisons placebo vs. 3 mcg $p<0.001$; placebo vs. 10 mcg $p<0.001$; 3 mcg vs. 10 mcg $p<0.05$. Figure 4B shows the mean t0-t180 glucose levels for the three treated groups.

Figures 5A and 5B are graphs illustrating the peak plasma glucose levels after SBM in the eight subjects of the study following placebo, UAG 3 mcg/kg/h and UAG 10 mcg/kg/h infusions. Wilcoxon matched-pairs signed rank test; *: 10 mcg vs. Placebo, $p<0.05$.

Figures 6A, 6B and 6C are graphs indicating the existence of a correlation between fasting basal AG and/or UAG concentrations and glycemic response to UAG administration. The graph in Figure 6A illustrates the correlation between fasting basal AG levels and change (following UAG vs. placebo administration) in peak glucose levels after SBM. The graph in Figure 6B illustrates the correlation between fasting basal AG levels and change (following UAG vs. placebo administration) in AUC glucose levels after SBM using iPro continuous glucose measurements. The graph in Figure 6C illustrates the correlation between the ratio of fasting basal AG over UAG levels and change (following UAG vs. placebo administration) in AUC glucose levels after SBM using iPro continuous glucose measurements.

Figure 7A is a schematic representation of a Hyperinsulinemic-Euglycemic Clamp study protocol in T2DM subjects according to a further embodiment of the present invention.

Figure 7B is a graph illustrating the effects of UAG on the M-index, reflective of insulin sensitivity, during the protocol as schematized in Figure 7A. The change in M-index from baseline was measured following placebo and UAG infusions. *: $p<0.05$, One-tailed Mann Whitney test.

Figures 7C and 7D are graphs illustrating the effects of a short UAG infusion period on basal AG levels in T2DM subjects. Figure 7C shows circulating AG levels before UAG infusion and following a 2.5 hour UAG infusion. Figure 7D shows the AG change from baseline after a 2.5 hour UAG infusion ([AG] level at 12:00 - [AG] levels at 9:30). Two-tailed Wilcoxon matched-pairs signed rank test; *: $p<0.05$; **: $p<0.01$.

Figure 8 is a graph illustrating the effects of cyclic UAG fragment (6-13) on AG-induced food intake over the indicated study period. *$p<0.001$: Kruskal-Wallis One Way ANOVA. Differences between groups were evaluated by the Dunn's test.

DETAILED DESCRIPTION

[0015] The present invention stems from, but is not limited to, the findings by the Inventors that administration of UAG decreases levels of circulating AG in subjects with T2DM. The present invention further stems from the findings that the higher the level of basal AG, the more important are the effects of UAG on reducing AG levels and on reducing the deleterious effects associated with AG levels such as, for example, obesity, hyperglycemia, insulin resistance, fat deposition, hyperphagia and obesity associated with insulin resistance. The Inventors have also found that the higher the ratio of circulating AG/UAG, the more efficient is UAG in decreasing such ratio and in suppressing the deleterious effects associated with AG/UAG ratio.

[0016] In view of this, circulating AG level and circulating AG/UAG ratio may each be used as biomarkers for identifying a subject's likelihood of responding to and/or benefiting from administration of UAG. These biomarkers may thus be used for identifying within a population of subjects suffering from a condition such as, but not limited to, obesity, diabetes, insulin resistance, Prader-Willi, hyperphagia and hyperghrelinemia, which of the subjects are likely to respond to and/or benefit from administration of UAG. The higher the circulating AG levels and/or the higher the circulating AG/UAG ratio in a subject, the more this subject is likely to respond to and/or benefit from the administration of UAG.

[0017] To this date, studies have reported that UAG counteracts the peripheral actions of ghrelin on, for example, glucose and insulin metabolisms. The present study provides the first evidence that administration of UAG also suppresses circulating ghrelin levels and provides the first evidence of the existence of a correlation between the level of circulating AG and the efficacy of UAG in improving metabolic parameters affected by AG levels and/or by AG/UAG ratio.

[0018] The surprising demonstrations presented therein allow to expand the applications and the indications for which unacylated ghrelin can be used so as to include the facilitation, amelioration and/or treatment of conditions that result from AG levels and/or AG/UAG ratio. These demonstrations also allow to expand the applications and the indications for which unacylated ghrelin can be used so as to include the facilitation, amelioration and/or treatment of conditions that result from elevated AG levels and/or elevated AG/UAG ratio. As used herein, the expression "elevated AG level(s)" refers to a level of circulating AG that is above the AG level observed in normal and/or healthy subjects. In some implementations, the expression "elevated AG level(s)" refers to a level of circulating AG at which one or more deleterious physiological symptoms associated with AG appear, persist or are worsen in a subject.

[0019] As used herein, the expression "elevated AG/UAG ratio" refers to a ratio of circulating AG/UAG that is above the AG/UAG ratio observed in normal and/or healthy subjects. In some implementations, the expression "elevated AG/UAG ratio" refers to a ratio of circulating

AG/UAG at which one or more deleterious physiological symptoms associated with the AG/UAG ratio appear or persist or are worsen in a subject.

[0020] It is to be understood that several factors may affect the levels of circulating ghrelin and unacylated ghrelin in normal subjects. Examples of such factors include, but are not limited to, gender, age, fitness, body mass index (BMI), eating habits, etc.

[0021] As used herein, the expression "inhibition of ghrelin" refers to an impairment of the biological activity of ghrelin which occurs due to a decrease in ghrelin levels and/or due to an impairment of its biological activity.

[0022] A person skilled in the art will be familiar with the techniques and assays for measuring AG and UAG levels in a subject. Such techniques may include techniques that involve the use of protease inhibitors such as 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) or other cocktail of protease inhibitors.

[0023] In one implementation of this embodiment, an obese subject is characterized as having a body weight approximately 20%, approximately 25%, approximately 30% or greater than the normal body weight for said subject. Normal body weight may be determined by a comparison of the weight of the subject at a prior point in time, such as when AG levels were normal and/or when AG/UAG ratio was normal, or by a comparison of the AG levels and/or AG/UAG ratio of the subject as compared to averages of other subjects of a similar age and/or condition.

[0024] In another implementation of this embodiment, an overweight subject is characterized as having a body weight approximately 5% greater to approximately 20% greater than the normal body weight for said subject. Normal body weight may be determined by a comparison of the weight of the subject at a prior point in time, such as as when AG levels were normal and/or when AG/UAG ratio was normal, or by a comparison of the AG levels and/or AG/UAG ratio as compared to averages of other subjects of a similar age and/or condition.

[0025] In another implementation of this embodiment, a normal subject is characterized as having a body weight approximately 5% greater than to approximately 5% less than the normal body weight for said subject. Normal body weight may be determined by a comparison of the weight of the subject at a prior point in time, such as when AG levels were normal and/or when AG/UAG ratio was normal, or by a comparison of the AG levels and/or AG/UAG ratio as compared to averages of other subjects of a similar age and/or condition. A normal weight subject may have a BMI in the approximate range of 19-22.

[0026] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of skill in the art to which the invention pertains.

i) Unacylated ghrelin, and fragments

[0027] The terms "unacylated ghrelin", "des-acyl ghrelin" and the abbreviation "UAG" are intended to mean peptides that have the amino acid sequence specified in SEQ ID NO: 1 which amino acid sequence is:

Gly-Ser-Ser-Phe-Leu-Ser-Pro-Glu-His-Gln-Arg-Val-Gln-Gln-Arg-Lys-Glu-Ser-Lys-Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg (SEQ ID NO: 1)

[0028] Unacylated ghrelin may also be referred to as UAG (1-28).

[0029] By "peptide", "polypeptide" or "protein" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylate or phosphorylation), or chemical modification, or those containing unnatural or unusual amino acids such as D-Tyr. ornithine, amino-adipic acid. The terms are used interchangeably in the present application.

[0030] The expressions "fragments" and "fragments thereof" refer to amino acid fragments of a peptide such as UAG.

[0031] Fragments of UAG are shorter than the amino acid sequence depicted in SEQ ID NO: 1, therefore are shorter than 28 amino acid residues. Fragments of UAG may therefore be 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5 or 4 amino acid residues in length. For example, fragments of UAG may have the amino acid sequences depicted in Table 1 below:

Table 1:

Fragment	SEQ ID NO:	Amino Acid Sequence
UAG (1-14)	2	Gly-Ser-Ser-Phe-Leu-Ser-Pro-Glu-His-Gln-Arg-Val-Gln-Gln
UAG (1-18)	3	Gly-Ser-Ser-Phe-Leu-Ser-Pro-Glu-His-Gln-Arg-Val-Gln-Gln-Arg-Lys-Glu-Ser
UAG (1-5)	4	Gly-Ser-Ser-Phe-Leu
UAG (17-28)	5	Glu-Ser-Lys-Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg
UAG (6-13)	6	Ser-Pro-Glu-His-Gln-Arg-Val-Gln
UAG (8-13)	7	Glu-His-Gln-Arg-Val-Gln
UAG (8-12)	8	Glu-His-Gln-Arg-Val
UAG (6-18)	9	Ser-Pro-Glu-His-Gln-Arg-Val-Gln-Gln-Arg-Lys-Glu-Ser

Fragment	SEQ ID NO:	Amino Acid Sequence
UAG (8-11)	10	Glu-His-Gln-Arg
UAG (9-12)	11	His-Gln-Arg-Val
UAG (9-11)	29	His-Gln-Arg
UAG (14-1)	30	Gln Gln Val Arg Gln His Glu Pro Ser Leu Phe Ser Ser Gly

Some UAG fragments have been reported in U.S. Patent Number 8,222,217; U.S. Patent Number 8,318,664 and in WO/2008/145749, wherein it has been demonstrated that the smallest UAG fragment to retain the biological activity of UAG is UAG (9-12) depicted herein as SEQ ID NO: 11.

[0032] In a first embodiment of the invention, the fragment of UAG is UAG (6-13) as set forth in SEQ ID NO: 6.

[0033] In one embodiment, the polypeptides such as UAG, fragments or analogs thereof, are used in a form that is "purified", "isolated" or "substantially pure". The polypeptides are "purified", "isolated" or "substantially pure" when they are separated from the components that naturally accompany them. Typically, a compound is substantially pure when it is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, by weight, of the total material in a sample.

[0034] Certain peptides according to the present invention may also be in cyclic form, such that the Nor C-termini are linked head-to-tail. Such peptide derivatives may have improved stability and bioavailability relative to the non-cyclized peptides.

[0035] Examples of cyclic peptides include: cyclic UAG (1-14), cyclic UAG (1-18), cyclic UAG (17-28), cyclic UAG (6-13), cyclic UAG (8-13), cyclic UAG (8-12), cyclic UAG (8-11), cyclic UAG (9-12) and cyclic UAG (9-11).

[0036] In another embodiment of the invention, the fragment of UAG is cyclic UAG (6-13) as set forth in SEQ ID NO: 25.

[0037] Methods for cyclizing peptides are well known in the art and for example may be accomplished by disulfide bond formation between two side chain functional groups, amide or ester bond formation between one side chain functional group and the backbone α-amino or carboxyl function, amide or ester bond formation between two side chain functional groups, or amide bond formation between the backbone α-amino and carboxyl functions. These cyclization reactions have been traditionally carried out at high dilution in solution. Cyclization is commonly accomplished while the peptide is attached to the resin. One of the most common ways of synthesizing cyclic peptides on a solid support is by attaching the side chain of an

amino acid to the resin. Using appropriate protection strategies, the C-and N-termini can be selectively deprotected and cyclized on the resin after chain assembly. This strategy is widely used, and is compatible with either tert-butyloxycarbonyl (Boc) or 9-fluorenylmethoxycarbonyl (Fmoc) protocols. However, it is restricted to peptides that contain appropriate side chain functionality to attach to the solid support. A number of approaches may be used to achieve efficient synthesis of cyclic peptides. One procedure for synthesizing cyclic peptides is based on cyclization with simultaneous cleavage from the resin. After an appropriate peptide sequence is assembled by solid phase synthesis on the resin or a linear sequence is appended to resin, the deprotected amino group can react with its anchoring active linkage to produce protected cyclic peptides. In general, a final deprotection step is required to yield the target cyclic peptide.

[0038] Lactamazation, a form of cyclization, may be performed to form a lactam bridge using Fmoc synthesis, amino acids with different protecting groups at the lateral chains may be introduced, such as, but not limited to, aspartic acid (or glutamic) protected with allyl ester at the beta ester (or gamma ester for glutamic acid) and lysine protected with allyloxy carbamate at the N-ε. At the end of the synthesis, with the N-terminus of the peptide protected with Fmoc, Boc or other protecting group different from Alloc, the allyl and alloc protecting groups of aspartic acid and lysine may be deprotected with, for example, palladium (0) followed by cyclization using PyAOP (7-Azabenzotriazol-1-yloxytris(pyrrolidino) phosphonium-hexafluorophosphate) to produce the lactam bridge.

[0039] Unless otherwise indicated, an amino acid named herein refers to the L-form. Well recognized abbreviations in the art will be used to describe amino acids, including levorotary amino acids (L-amino acids or L or L-form) and dextrorotatory amino acids (D-amino acids or D or D-form), Alanine (Ala or A), Arginine (Arg or R), Asparagine (Asn or N), Aspartic acid (Asp or D), Cysteine (Cys or C), Glutamic acid (Glu or E), Glutamine (Gin or Q), Glycine (Gly or G), Histidine (His or H), Isoleucine (Ile or I), Leucine (Leu or L), Lysine (Lys or K), Methionine (Met or M), Phenylalanine (Phe or F), Proline (Pro or P), Serine (Ser or S), Threonine (Thr or T), Tryptophan (Trp or W), Tyrosine (Tyr or Y) and Valine (Val or V). An L-amino acid residue within the native peptide sequence may be altered to any one of the 20 L-amino acids commonly found in proteins or any one of the corresponding D-amino acids, rare amino acids, such as, but not limited to, 4-hydroxyproline or hydroxylysine, or a non-protein amino acid, such as P-alanine or homoserine.

[0040] Certain aspects of the invention use UAG polynucleotides. These include isolated polynucleotides which encode the UAG polypeptides, fragments and analogs defined in the application.

[0041] As used herein, the term "polynucleotide" refers to a molecule comprised of a plurality of deoxyribonucleotides or nucleoside subunits. The linkage between the nucleoside subunits can be provided by phosphates, phosphonates, phosphoramidates, phosphorothioates, or the like, or by nonphosphate groups as are known in the art, such as peptoid-type linkages utilized in peptide nucleic acids (PNAs). The linking groups can be chiral or achiral. The

oligonucleotides or polynucleotides can range in length from 2 nucleoside subunits to hundreds or thousands of nucleoside subunits. While oligonucleotides are preferably 5 to 100 subunits in length, and more preferably, 5 to 60 subunits in length, the length of polynucleotides can be much greater (e.g., up to 100). The polynucleotide may be any of DNA and RNA. The DNA may be in any form of genomic DNA, a genomic DNA library, cDNA derived from a cell or tissue, and synthetic DNA. Moreover, the present invention may, in certain aspects, use vectors which include bacteriophage, plasmid, cosmid, or phagemid.

[0042] The expressions "biological activity" or "biological property", or the term "activity" in reference to the polypeptides of the present invention, are used interchangeably herein and refer to the pharmacological, biological or cellular abilities of the polypeptides of the invention and include, but are not limited to, the capacity of replacing UAG in the biological functions of UAG as described in the present application, such as, but not limited to, modulating AG; inhibiting AG; decreasing circulating AG levels; decreasing circulating elevated AG levels; decreasing circulating AG/UAG ratio; decreasing circulating elevated AG/UAG ratio; ameliorating the symptoms induced by AG levels and/or AG/UAG levels; facilitating, preventing and/or treating conditions associated with circulating AG and/or circulating AG/UAG ratio and facilitating, preventing and/or treating conditions associated with elevated circulating AG and/or elevated circulating AG/UAG ratio.

[0043] The actions of UAG have previously been shown to be conserved by fragments UAG (6-13) (SEQ ID NO: 6), UAG (8-13) (SEQ ID NO: 7), UAG (8-12) (SEQ ID NO: 8), UAG (8-11) (SEQ ID NO: 12), UAG (9-12) (SEQ ID NO: 11) and UAG (9-11) (SEQ ID NO: 29). U.S. Patents 8,222,217 and 8,318,664, have shown that these fragments retain the activity of UAG full length on glucose, insulin and lipid metabolisms. A peptide with the inverse sequence of UAG (1-14) (SEQ ID NO: 3) and named UAG (14-1) (SEQ IDNO: 30) was used as a negative control in the experiments testing UAG fragments. UAG (8-11) (SEQ ID NO: 10) was shown to be the smallest UAG fragment to retain UAG activities. The results provided herein further indicate that UAG fragments, UAG (6-13) (SEQ ID NO: 6) and cyclic UAG (6-13) (SEQ ID NO: 25) retain UAG's ability to decrease AG levels and decrease AG/UAG ratio.

[0044] UAG, fragments and/or analogs thereof are used to reduce the elevated AG levels associated with Prader-Willi Syndrome (PWS). People who suffer from PWS suffer from slowed development, severe obesity and an insatiable appetite. Their hunger is so strong that it often requires custodial enforcement of food availability to avert early death as a result of hyperphagia. AG concentrations in these subjects are higher than normal. This correlation between hyperphagia and increase AG levels is consistent with the known orexigenic effect of AG. The data present herein demonstrate that administration of UAG can decrease the elevated AG levels in PWS subjects. The fragments of UAG of the invention can be used to help patients with Prader-Willi syndrome reduce their ghrelin levels to more normal/healthier levels, curb their appetite, and/or ameliorate other manifestations of this disorder. This decrease in AG is expected to translate into a decrease in appetite and in a subsequent reduction in fat mass associated with PWS.

[0045] As used herein, the term "hyperphagia" refers to excessive hunger and abnormally large intake of solids by mouth. Hyperphagic conditions may occur in association with for example, central nervous system (CNS) disorders including gangliocytoma of the third ventricle, hypothalmic astrocytoma, Kleine-Levin Syndrome, Froehlich's Syndrome, Parkinson's Disease, genetic disorders including Praeder-Willi Syndrome, major psychiatric disorders including anxiety, major depressive disorder, depressive phase of bipolar disorder, seasonal affective disorder, and schizophrenia, psychotropic medication, including delta-9 tetrahydrocannabinol, antidepressants and neuroleptics and sleep disorders including sleep apnea. Hyperphagia may occur in psychiatric disorders such as depression, anxiety and schizophrenia.

[0046] As used herein, the term "treatment" refers to both therapeutic treatments as well as to prophylactic measures. Those in need of treatment include those already with the disorder, disease or condition as well as those in which the disease, disorder or condition is to be prevented. Those in need of treatment are also those in which the disorder, disease or condition has occurred and left after-effects or scars. Treatment also refers to administering a therapeutic substance effective to improve or ameliorate, diminish symptoms associated with a disease, a disorder or a condition to lessen the severity of or cure the disease, disorder or condition, or to prevent the disease, disorder or condition from occurring or reoccurring.

[0047] Studies have demonstrated a persistent increase in plasma AG levels up to one year following a diet-induced weight loss in obese subjects (Refs. 28 and 29). The reduction of elevated AG levels in these subjects could thus prevent obesity relapse while the subject is under diet.

[0048] For therapeutic and/or pharmaceutical uses, the polypeptides as defined herein may be formulated for, but not limited to, intravenous, subcutaneous, transdermal, topical, oral, buccal, sublingual, nasal, inhalation, pulmonary, or parenteral administration according to conventional methods. Intravenous injection may be by bolus or infusion over a conventional period of time. The polypeptides as defined herein may also be administered directly to a target site within a subject e.g., by biolistic delivery to an internal or external target site or by catheter to a site in an artery.

[0049] In one embodiment, the polypeptides defined herein are administered as a bolus. Accordingly, in one implementation of this embodiment, the medicament is administered as a bolus prior to meal, wherein the bolus comprises an effective amount of UAG, a fragment and/or an analog thereof of a salt thereof. The bolus may be administered one, twice, three times or more daily or may be administered according to other dosage regimens.

[0050] Suitable dosage regiments are determined taking into account factors well known in the art such as, but not limited to, type of subject being dosed, the age, the weight, the sex and the medical condition of the subject, the route of administration, the desired affect, etc.

[0051] Active ingredients, such as the polypeptides defined herein, may be administered orally

as a suspension and can be prepared according to techniques well known in the art of pharmaceutical formulation and may contain, but not be limited to, microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners/flavoring agents. As immediate release tablets, these compositions may contain, but are not limited to microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants. The active ingredients may be administered by way of a controlled-release delivery system.

[0052] Administered by nasal aerosol or inhalation formulations may be prepared, for example, as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, employing fluorocarbons, and/or employing other solubilizing or dispersing agents.

[0053] The polypeptides of the invention may be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form. When administered by injection, the injectable solution or suspension may be formulated using suitable non-toxic, parenteral-acceptable diluents or solvents, well known in the art.

[0054] The polypeptides of the invention may also be formulated for topical administration. The term "topical" as used herein includes any route of administration that enables the compounds to line the skin or mucosal tissues.

[0055] The formulation suitable for topical application may be in the form of, for example, cream, lotion, solution, gel, ointment, paste, plaster, paint, bioadhesive, or the like, and/or may be prepared so as to contain liposomes, micelles, microparticles and/or microspheres. The formulation may be aqueous, i.e., contain water, or may be non-aqueous and optionally used in combination with an occlusive overlayer so that moisture evaporating from the body surface is maintained within the formulation upon application to the body surface and thereafter.

[0056] Ointments, as is well known in the art of pharmaceutical formulation, are semisolid preparations that are typically based on petrolatum or other petroleum derivatives.

[0057] Formulations may also be prepared with liposomes, micelles, microparticles and/or microspheres. Liposomes are microscopic vesicles having a lipid wall comprising a lipid bilayer, and can be used as drug delivery systems. Micelles are known in the art to be comprised of surfactant molecules arranged so that their polar head groups form an outer spherical shell, while the hydrophobic, hydrocarbon chains are oriented towards the center of the sphere, forming a core. Microparticles are particulate carrier systems in the micron size range, normally prepared with polymers, which can be used as delivery systems for drugs or vaccines that are usually trapped within the particles. Microspheres, similarly, may be incorporated into the present formulations and drug delivery systems. Like liposomes and micelles, microspheres essentially encapsulate a drug or drug-containing formulation. Microspheres are generally, although not necessarily, formed from synthetic or naturally occurring biocompatible

polymers, but may also be comprised of charged lipids such as phospholipids.

[0058] Preparations of formulations suitable for topical administration are well known in the art and described in the pertinent texts and literature.

[0059] In general, pharmaceutical compositions will comprise at least one of the polypeptides of the invention together with a pharmaceutically acceptable carrier which will be well known to those skilled in the art. The compositions may further comprise for example, one or more suitable excipients, diluents, fillers, solubilizers, preservatives, carriers, salts, buffering agents and other materials well known in the art depending upon the dosage form utilized. Methods of composition are well known in the art.

[0060] In the present context, the term "pharmaceutically acceptable carrier" is intended to denote any material, which is inert in the sense that it substantially does not have any therapeutic and/or prophylactic effect *per se* and that are non-toxic. A pharmaceutically acceptable carrier may be added to the polypeptides of the invention with the purpose of making it possible to obtain a pharmaceutical composition, which has acceptable technical properties. Examples of such carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts, or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, and PEG.

[0061] Carriers for topical or gel-based forms of polypeptides include polysaccharides such as sodium carboxymethylcellulose or methylcellulose, polyvinylpyrrolidone, polyacrylates, polyoxyethylene-polyoxypropylene-block polymers, PEG, and wood wax alcohols.

[0062] The polypeptides used for *in vivo* administration must be sterile. This may be accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The polypeptides ordinarily will be stored in lyophilized form or in solution. Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0063] The fragments of UAG of the invention may be used in an article of manufacture or a commercial package or kit, comprising: a container, a label on the container, and a composition comprising the polypeptides of the invention as an active agent within the container when used at the indicated level, wherein the composition is effective for, *inter alia*, modulating AG; inhibiting AG; decreasing circulating AG levels; decreasing circulating elevated AG levels; decreasing circulating AG/UAG ratio; decreasing circulating elevated AG/UAG ratio; ameliorating the symptoms induced by AG levels and/or AG/UAG levels; facilitating, preventing and/or treating conditions associated with circulating AG and/or circulating AG/UAG ratio and/or facilitating, preventing and/or treating conditions associated with elevated circulating AG

and/or elevated circulating AG/UAG ratio.

[0064] An "effective amount" or a "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the peptides noted herein may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the compound are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as to modulate AG; inhibit AG; decrease circulating AG levels; decrease circulating elevated AG levels; decrease circulating AG/UAG ratio; decrease circulating elevated AG/UAG ratio; ameliorate the symptoms induced by AG levels and/or AG/UAG levels; facilitate, prevent and/or treat conditions associated with circulating AG and/or circulating AG/UAG ratio and/or facilitate, prevent and/or treat conditions associated with elevated circulating AG and/or elevated circulating AG/UAG ratio. A prophylactically effective amount can be determined as described above for the therapeutically effective amount. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

[0065] For example, a therapeutically effective amount or effective dose of the peptides of the invention (also referred to herein as "active compound") is an amount sufficient to modulate AG; inhibit AG; decrease circulating AG levels; decrease circulating elevated AG levels; decrease circulating AG/UAG ratio; decrease circulating elevated AG/UAG ratio; ameliorate the symptoms induced by AG levels and/or AG/UAG levels; facilitate, prevent and/or treat conditions associated with circulating AG and/or circulating AG/UAG ratio and/or facilitate, prevent and/or treat conditions associated with elevated circulating AG and/or elevated circulating AG/UAG ratio. The methods and/or assays for measuring such parameters are known to those of ordinary skill in the art.

[0066] The therapeutically effective amount of the invention will generally vary from about 0.001 µg/kg to about 10 mg/kg, more particularly from about 0.01 µg/kg to about 10 mg/kg, and even more particularly from about 1 µg/kg to about 1 mg/kg. Therapeutically effective amounts or effective doses that are outside this range but that have the desired therapeutic effect are also encompassed by the present invention.

[0067] In a one embodiment, the subject noted above is a mammal, in a further aspect, a human.

[0068] In a further embodiment, the present polypeptides may be administered in combination with additional pharmacologically active substances or may be administered in combination with another therapeutic method. The combination may be in the form of a kit-in-part system, wherein the combined active substances may be used for simultaneous, sequential or

separate administration.

iii) AG levels and AG/UAG ratio as biomarkers

[0069] According to another embodiment, the present invention relates to the use of circulating AG levels and the use of circulating AG/UAG ratio as biomarkers for the identification of subjects suffering from Prader Willi Syndrome that are likely to respond to and/or benefit from a treatment comprising the administration of a therapeutically effective amount of UAG, a fragment thereof and/or an analog thereof.

[0070] In one implementation of this embodiment, circulating AG levels and/or circulating AG/UAG ratio are measured in a blood sample obtained from a subject according to known methods in the art. The level of circulating AG and/or the ratio of circulating AG/UAG are then processed in part based on reference circulating levels of AG and reference circulating AG/UAG ratios (derived, for example, from the subject himself or from a population of control subjects) so as to derive information which conveys whether the subject has an abnormal level of circulating AG (i.e., below or above a normal or healthy level) and/or an abnormal ratio of circulating AG/UAG (i.e., below or above a normal or healthy ratio). An information which conveys that the subject has an elevated circulating AG level and/or an elevated circulating AG/UAG ratio indicates that the subject is likely to respond to administration of UAG, a fragment thereof or an analog thereof. Administration of UAG, a fragment thereof or an analog thereof in such subject is thus likely to decrease circulating AG levels and/or circulating AG/UAG ratio as well as to lessen the effects associated with elevated circulating AG levels and elevated circulating AG/UAG ratio.

[0071] In another implementation of this embodiment, the biomarkers may be used for identifying subjects within a population of subjects that are likely to respond to and/or benefit from administration of a therapeutically effective amount of UAG fragment. In this implementation, the subjects of the population suffer from Prader-Willi Syndrome (PWS)

EXPERIMENTS AND DATA ANALYSIS

[0072] The data present herein reports a strong suppressing effect of UAG on serum AG levels and on serum AG/UAG ratio in T2DM subjects.

[0073] A continuous overnight (15 hours) infusion of two doses of UAG (3 µg UAG/kg/hr and 10 µg UAG/kg/hr) versus placebo in a cross-over model on the AG concentrations was performed in eight overweight subjects with type 2 diabetes. Figure 1 depicts a schematic representation of the study protocol. Glucose and insulin responses to a standard breakfast meal (SBM) in the subjects and reasonable metabolic control were assessed. During the infusions with UAG, subjects did not report more side effects than placebo. Laboratory

evaluations showed no significant changes in chemistry or parameters and the side effects were not dose-dependent.

Infusion of UAG decreases plasma AG levels

[0074] The data present in Figures 2A and 2B show the changes in serum AG levels (Figure 2A) and UAG levels (Figure 2B) before and after SBM. Before initiation of SBM, AG levels are significantly decreased from 21.01 ± 8.9 pg/ml (mean ± SD) during placebo infusion to 3.0 ± 6.7 pg/ml in the presence 3 mcg/kg.hr UAG infusion and to 1.4 ± 3.2 pg/ml in the presence of 10 mcg/kg.hr UAG infusion. AG levels are also decreased following SMB from 14.03 ± 9.4 pg/ml in placebo to 0.8 ± 1.8 pg/ml in the presence 3 mcg/kg.hr UAG infusion and to 0.8 ± 1.8 pg/ml in the presence of 10 mcg/kg.hr UAG infusion (Figure 2A). In parallel, an overnight infusion of UAG resulted in an increase in UAG levels (Figure 2B). UAG levels increased from 105.9 ± 31.4 (mean ± SD) pg/ml in placebo infusion and before start of the SBM to 10998 ± 2623 pg/ml in the presence 3 mcg/kg.hr UAG infusion and to 12085 ± 1616 pg/ml in the presence of 10 mcg/kg.hr UAG infusion. These results indicate that administration of UAG reduces serum AG levels in T2DM subjects.

Infusion of UAG fragment counteracts AG-induced food intake

[0075] A cyclized fragment of UAG, namely cyclic UAG (6-13) as depicted in SEQ ID NO: 25, was able to counteract the orexigenic effects induced by AG in rat (Figure 8). These results demonstrate that UAG fragments as defined herein which retain the core sequence responsible for UAG-related actions/activities also retain their effects on AG levels. As discussed above, there is a benefit of inhibiting the effect of AG or its levels (e.g. devcreasing food consumption) in certain subjects. These results demonstrate a beneficial role for UAG in the treatment of patients with Prader Willi syndrome, in whom elevated AG levels are associated with hyperphagia. Inhibition of AG levels and/or biological effects should thus result in decreased appetite and/or food consumption.

Infusion of UAG decreases post-prandial plasma glucose levels

[0076] An overnight infusion of UAG significantly depressed post-prandial glucose levels as assessed by iPro continuous glucose monitoring (Figures 3A and 3B). The area under the curves decreased from 1618 mmol/3hrs for placebo infusion to 1601 mmol/3 hrs and 1540 mmol/3 hrs for the 3 and 10 mcg UAG infusions respectively. Figures 4A and 4B depict the results obtained as changes from the glucose pre-SBM baseline. The area under the curves decreased for the 3 and 10 mcg UAG infusions doses when compared to placebo. Figures 5A and 5B indicate a decrease in plasma glucose peak after SBM when UAG is administered. An overall decrease in post-prandial plasma glucose peaks can be observed in the subjects

(Figure 5B). Overall, these data indicate that administration of UAG decreases post-prandial plasma glucose levels in subjects with T2DM.

UAG-induced reduction in plasma AG levels and AG/UAG ratio correlates with reduction in glucose levels

[0077] The inventors were able to show the existence of a correlation between fasting AG levels and/or UAG levels and glycemic response following UAG administration using a standard glucokinase assay. Further to demonstrating such correlation, Figure 6A shows that the more elevated the fasting AG levels are, the best are the hypoglycemic effect of UAG administration. Figure 6B also shows the same correlation and effect using iPro continuous glucose measurements. Fasting AG/UAG ratio also correlates with the glycemic response following UAG administration (Figure 6C). The higher AG/AUG ratios correlate with the best hypoglycemic effect of UAG infusion using iPro continuous glucose measurements.

Infusion of UAG improves insulin sensitivity

[0078] Insulin sensitivity in T2DM subjects was assessed using the hyperinsulinemic-euglycemic clamp protocol as depicted in Figure 7A. In patients receiving effective euglycemic insulin clamp at the start of a 2.5 hr placebo/UAG infusion, the M-index change from baseline was increased by 36% in the UAG vs. placebo group (p=0.02) (Figure 7B). These results demonstrate that UAG infusion improves insulin resistance in T2DM subjects.

Shorter UAG infusion is sufficient to decrease plasma AG levels

[0079] AG levels were measured in T2DM subjects prior to UAG infusion and following a 2.5 hour-long UAG infusion. The data presented in Figure 7C shows that the shortened UAG infusion period was sufficient to decrease plasma AG levels. Figure 7D indicates the changes in AG levels from baseline demonstrating that a short UAG infusion period suffices to decrease plasma AG levels.

[0080] These data demonstrate, *inter alia,* that administration of UAG improves glucose levels during a SBM through a reduction in AG. A significant decrease in peak glucose levels after meal was also observed. No significant change in serum insulin levels during the infusion of low and high dose of UAG infusions was observed (data not shown), which indicates that the improved glycemic control correlates with an improved insulin sensitivity. The data also show that administration of UAG improves hyperglycemia in a ghrelin concentration dependent manner thus making the UAG hypoglycemic effects stronger at higher AG levels or at higher AG/UAG ratio.

[0081] These results are the first indication that UAG is a potent inhibitor of ghrelin levels making UAG a strong candidate for the development of a ghrelin inhibitor in the treatment of metabolic disorders.

MATERIALS AND TECHNICAL PROTOCOLS

[0082] *Study design* - Single-center, investigator initiated, double blind and placebo controlled randomized study. During the first visit medical history, medication use and vital signs of subjects were checked. Blood samples were also taken for chemistry and hematology analysis. The study consisted of three rounds of hospitalization (visit 2, 3 and 4) of two days each.

[0083] The first day started at approximately 15:00 and continued until 14:00 the next day. The eight subjects were divided into 3 groups for the study. The dosages used during these rounds were either 3 mcg/kg.hr UAG, 10 mcg/kg.hr UAG or placebo/saline solution prepared by the hospital pharmacy and delivered in 3 bags for each subject. Neither subjects nor researchers were aware of the drug given in these rounds. A washout period of one week was performed between the treatment periods.

[0084] Before, during and after the study, blood samples were taken for, AG, UAG, chemistry, hematology and CAC cells. Blood samples for glucose were also taken via i.v catheter. For continuous glucose monitoring a Continuous Glucose monitor (iPro2, Medtronic trading, The Netherlands) was placed in the abdomen of the subjects. Weight and blood pressure were measured during each visit. A washout period of at least 1 week was performed between the treatment periods.

[0085] *Subjects* - Eight subjects were enrolled (2 females and 6 males; mean age of 53 yrs (ranging from 31-65 years old) with mean body mass index (BMI) of 31.5 kg/m^2, range 26-36 kg/m^2. Seven of the eight subjects used metformin daily. All subjects were diagnosed with type 2 diabetes for at least 3 months prior to enrollment. Metformin monotherapy for at least 3 months prior to screening was allowed, but metformin treatment was stopped 1 day prior to start of each treatment period. In the population mean glycosylated hemoglobin level (HbA1c) was 52 mmol/mol range from 48 mmol/mol to 57 mmol/mol (6.9%; range 6.5-7.4%).) and Body Mass Index was above 25 kg/m^2. Exclusion criteria consisted in history or presence of long-term type 2 diabetes complications; clinically significant abnormalities in laboratory evaluation at screening, and use of systemic corticosteroids within 60 days prior to screening. Prior to infusion, subjects received two indwelling catheters: the first catheter was inserted prior to treatment; and the second catheter was inserted prior to the SBM for blood sampling. The second catheter was kept patent by slow infusion of isotonic saline. *Study drug* - UAG used in this study was produced by Bachem AG, Hauptstrasse 144, Bubendorf CH-4416, Switzerland. UAG was delivered as lyophilized powder (vials containing 5 mg of drug) and stored at the local pharmacy according to the manufacturer's specifications.

[0086] *Study procedures* - Infusions were performed at the local clinical research unit for 15 hours from 9 pm to 12 am. Each volume of UAG solution was filtered and diluted in 0.9% of saline solution to obtain the appropriate dose for administration. Placebo consisted in 0.9% of saline solution. The dose was calculated based on the subject's weight. Each vial was reconstituted with 5 ml water for injection, filtered through a 0.22 µm filter. The dose was then injected in a 500 ml bag of 0.9% NaCl. Three bags were prepared to ensure a continuous 15-hour infusion at 100 ml/hour.

[0087] Standard breakfast meal (SBM) consisted of:

- 3 slices wheat bread;
- 3 portions of margarine;
- 2 slices of cheese (48 % fat);
- 1 portion of jam;
- 1 cup of whole milk; and
- 1 boiled egg;

for a total of 714 kcal (17% proteins; 46 % fat; and 37 % carbohydrates). The SBM had to be consumed within 15 minutes, from 8:00 am to 8:15 am. At each of the 3 visits, the following safety parameters were assessed: hemoglobin, hematocrit, platelet count, WBC count, RBC count, and differential and were determined using Sysmex XE 2100, Firma Sysmex, Ecustraat 11, 4879 NP Etten-Leur. AST, ALT, alkaline phosphatase, total bilirubin, creatine phophokinase (CPK), lactate dehydrogenase (LDH), creatinine, urea, amylase, lipase, uric acid, glucose, cholesterol, LDL, HDL, triglycerides, sodium, potassium, calcium, chloride, protein and albumin are determined using the Hitachi Modular P800, Roche Transistorstraat 41, 1332 CK Almere. Blood glucose levels were measured using a continuous glucose monitoring device (Medtronic CGMS iPro™ Continuous Glucose Recorder, Medtronic; The Netherlands) that was subcutaneously inserted for the whole treatment period. Serum glucose levels (using the in-house glucokinase assay) were also assessed every 30 minutes and starting before, and continuing for 4 hours after, the SBM. During the CGMS, all subjects had to perform at least four capillary glycemic tests per day. The data collected were entered into the CGMS monitor to obtain correlation coefficients between the SMBG and the CGMS values. All SMBG tests were performed using a digital glucometer (Contour, Bayer). AG and UAG levels were assessed before the start of the overnight infusion, 10 minutes before the start and 30 minutes after the SBM. To preserve acylation of ghrelin, blood samples were collected directly into EDTA tubes, then within 2 minutes 1 ml of EDTA-blood was added to 1 ml of preservative solution on ice (0.0295 N HCl containing 72 mM NaCl, 58 mM NaF, 4 mM 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (a water soluble, irreversible serine protease inhibitor; AEBSF), pH 3.0, 295 mOsm/KgPlasma was prepared by centrifugation at 4°C, then 1 ml was acidified with 100 µl 1N HCl and stored at - 80°C until assays were performed.

[0088] The ghrelin assay utilized MSD ELISA 96-well plates (Meso Scale Discovery (MSD), Gaithersburg, MD, USA) coated by incubation with 30 µl/well of capture antibody (D4 diluted to 1 µg/ml in PBS; anti-C-terminal ghrelin (Gutierrez et al. 2008 PNAS 105:6320-6325)), overnight at room temperature. The capture antibody was removed and wells were blocked with 150 µl

casein buffer (Pierce) for 1 h at room temperature with shaking. Standards for AG and UAG were prepared using eight 4x serial dilutions in casein buffer starting at 8 ng/ml and 30 ng/ml, respectively. Preserved plasma was diluted 1:1 in casein buffer. Separate plates were used for detection of AG and UAG. Standards and samples (25 µl/well) were loaded onto coated ELISA plates, and incubated at room temperature with shaking for 2 hours, washed 3x with PBS-T (150 µl/well). The C2-4a1 and E8 detection antibodies (N-terminal AG and N-terminal UAG, respectively) were sulfotagged using the standard protocol from MSD. They were then diluted 1:10000 in 0.2X casein/0.05% Tween 20 and added to AG or UAG plates, respectively, at 25 µl/well. Plates were incubated at room temperature for 1 hour with shaking. Plates were washed three times with PBS-T (150 µl/well). Finally, 150 µl of 1X Read Buffer (MSD) was added to each well, and the plates were immediately read on an MSD Sector Imager 6000. AG and UAG values for samples were calculated by interpolation from their respective standard curves using Sector Imager software.

[0089] *Clamp study* - Randomized 2-period, 2-treatment, double-blind study of UAG vs. vehicle infusion, evaluating one dose (10 µg/kg/h) of UAG administrated by continuous iv infusion for 2.5 hrs. In patients receiving effective euglycemic insulin clamp at the start of a 2.5 hr placebo/UAG infusion.

[0090] *Statistical analyses* - Data analyses were performed with the Graph Pad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA 92037 USA). The results are given as means (±SE). Comparisons were calculated using Bonferroni's Multiple Comparisons, Wilcoxon matched-pairs signed-rank tests and ANOVA analyses.

[0091] With respect to the experimental data presented in Figure 8, Sprague-Dawley rats of 7 weeks of age, weighing between 275g and 300g, were fed a pellet diet *ad libitum* and were singly housed in plastic cage. The experiment was performed at 2.5 h after the onset of the light cycle in freely fed rats. The rates were i.p. injected simultaneously with vehicle plus vehicle, vehicle + AG (13 µg/kg) or cyclised UAG (6-13) (SEQ ID NO: 25) (42 µg/kg) + AG (13 µg/kg). Immediately after the completion of the i.p. injection in rats, the *night-ad-libitum* food was removed and replaced by 2 pellets for each animal, previously weighed, placed into the top of the cage. Food intake was calculated as the difference between the food weight before and after the feeding period at each time interval (30 min, 1h, and 2h). Cumulative food intake was calculated by summating the values of the different time periods.

[0092] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

REFERENCE LIST

[0093]

1. 1. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H and. Kangawa K; Ghrelin is a growth-hormone-releasing acylated peptide from stomach, Nature 402:656-660 (1999).
2. 2. Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, Bhattacharya S, Carpenter R, Grossman AB and Korbonits M; The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans, J. Clin. Endocrinol. Metab. 87:2988-2991 (2002).
3. 3. Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PL, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee K-K, Pong S-S, Chaung L-Y, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji D, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Schaeffer JA, Smith RG, Van der Ploeg LHT; A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273:974-977 (1996).
4. 4. Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K, Nakao K.; Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab. Oct;86(10):4753-8 (2001)
5. 5. Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K, Nakazato M. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology 123(4):1120-1128, 2002.
6. 6. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402(6762):656-660, 1999.
7. 7. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 407(6806):908-913, 2000.
8. 8. Broglio F, Gianotti L, Destefanis S, Fassino S, Abbate Daga G, Mondelli V, Lanfranco F, Gottero C, Gauna C, Hofland L, Van Der Lely AJ, Ghigo E. The endocrine response to acute ghrelin administration is blunted in patients with anorexia nervosa, a ghrelin hypersecretory state. Clin Endocrinol (Oxf) 60(5):592-599, 2004.
9. 9. Tong J, Prigeon RL, Davis HW, Bidlingmaier M, Kahn SE, Cummings DE, Tschöp MH, D'Alessio D. Ghrelin suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance in healthy humans. Diabetes Sep;59(9):2145-51 (2010).
10. 10. Cummings DE, Clement K, Purnell JQ, Vaisse C, Foster KE, Frayo RS, Schwartz MW, Basdevant A, Weigle DS. Elevated plasma ghrelin levels in Prader Willi syndrome. Nat Med 8(7):643-644 (2002).
11. 11. Haqq AMF, Farooqi IS, O'Rahilly S, Stadler DD, Rosenfeld RG, Pratt KL, Lafranchi SH, Purnell JQ. Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. J Clin Endocrinol Metab 88(1):174-178 (2003).
12. 12. Mifune H, Nishi Y, Tajiri Y, Masuyama T, Hosoda H, Kangawa K, Kojima M. Increased production of active ghrelin is relevant to hyperphagia in nonobese spontaneously

diabetic Torii rats. Metabolism. 2011 Oct 14. [Epub ahead of print]

13. 13. Misra M, Miller KK, Kuo K, Griffin K, Stewart V, Hunter E, Herzog DB, Klibanski A. Secretory dynamics of ghrelin in adolescent girls with anorexia nervosa and healthy adolescents. Am J Physiol Endocrinol Metab 289(2):E347-E356 (2005).
14. 14. Nikolopoulos D, Theocharis S, Kouraklis G. Ghrelin: a potential therapeutic target for cancer. Regul Pept 163(1-3):7-17 (2010).
15. 15. van der Lely AJ, Tschop M, Heiman ML, Ghigo E; Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. Endocr Rev 25:426-457 (2004).
16. 16. Asakawa A, Inui A, Fujimiya M, Sakamaki R, Shinfuku N, Ueta Y, Meguid MM, Kasuga M. Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. Gut. Jan;54(1):18-24 (2005).
17. 17. Zhang W, Chai B, Li JY, Wang H, Mulholland MW. Effect of des-acyl ghrelin on adiposity and glucose metabolism. Endocrinology. Sep;149(9):4710-6. (2008)
18. 18. Broglio F, Gottero C, Prodam F, Gauna C, Muccioli G, Papotti M, Abribat T, Van Der Lely AJ, Ghigo E. Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humans. J Clin Endocrinol Metab. Jun;89(6):3062-5 (2004).
19. 19. Gauna C, Meyler FM, Janssen JA, Delhanty PJ, Abribat T, van Koetsveld P, Hofland LJ, Broglio F, Ghigo E, van der Lely AJ. Administration of acylated ghrelin reduces insulin sensitivity, whereas the combination of acylated plus unacylated ghrelin strongly improves insulin sensitivity. J Clin Endocrinol Metab. Oct;89(10):5035-42 (2004).
20. 20. St-Pierre DH, Karelis AD, Coderre L, Malita F, Fontaine J, Mignault D, Brochu M, Bastard JP, Cianflone K, Doucet E, Imbeault P, Rabasa-Lhoret R. Association of acylated and nonacylated ghrelin with insulin sensitivity in overweight and obese postmenopausal women. J Clin Endocrinol Metab. Jan;92(1):264-9 (2007).
21. 21. Pacifico L, Poggiogalle E, Costantino F, Anania C, Ferraro F, Chiarelli F, Chiesa. Acylated and nonacylated ghrelin levels and their associations with insulin resistance in obese and normal weight children with metabolic syndrome. Eur J Endocrinol. Dec;161(6):861-70 (2009).
22. 22. Barazzoni R, Zanetti M, Ferreira C, Vinci P, Pirulli A, Mucci M, Dore F, Fonda M, Ciocchi B, Cattin L, Guarnieri G. Relationships between desacylated and acylated ghrelin and insulin sensitivity in the metabolic syndrome. J Clin Endocrinol Metab. Oct;92(10):3935-40 (2007).
23. 23. Rodríguez A, Gómez-Ambrosi J, Catalán V, Gil MJ, Becerril S, Sáinz N, Silva C, Salvador J, Colina I, Frühbeck G. Acylated and desacyl ghrelin stimulate lipid accumulation in human visceral adipocytes. Int J Obes (Lond). May;33(5):541-52 (2009).
24. 24. Schellekens H, Dinan TG, Cryan JF. Lean mean fat reducing "ghrelin" machine: Hypothalamic ghrelin and ghrelin receptors as therapeutic targets in obesity. Neuropharmacology 58(1):2-19 (2010).
25. 25. Gualillo O, Lago F, Dieguez C. Introducing GOAT: a target for obesity and anti-diabetic drugs? Trends Pharmacol Sci 29(8):398-401 (2008).
26. 26. Hillman JB, Tong J, Tschop M. Ghrelin biology and its role in weight-related

disorders. Discov Med. Jun;11(61):521-8 (2011).
27. 27. Kumar R, Salehi A, Rehfeld JF, Höglund P, Lindström E, Håkanson R. Proghrelin peptides: Desacyl ghrelin is a powerful inhibitor of acylated ghrelin, likely to impair physiological effects of acyl ghrelin but not of obestatin A study of pancreatic polypeptide secretion from mouse islets. Regul Pept. Sep 24;164(2-3):65-70 (2010)
28. 28. Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med. May 23;346(21):1623-30 (2002).
29. 29. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, Proietto J. Long-term persistence of hormonal adaptations to weight loss. N Engl J Med. Oct 27;365(17):1597-604 (2011).
30. 30. Kiewiet RM, van Aken MO, van der Weerd K, Uitterlinden P, Themmen AP, Hofland LJ, de Rijke YB, Delhanty PJ, Ghigo E, Abribat T, van der Lely AJ. Effects of acute administratio of acylated and unacylated ghrelin on glucose and insulin concentratios in morbidly obese subjects without overt diabetes. Eur J Endocrinol 161:567-573 (2009).
31. 31. Gauna C, Delhanty PJD, Hofland LJ, Janssen J, Broglio F, Ross RJM, Ghigo E, van der Lely AJ. Ghrelin stimulates, whereas des-octanoyl ghrelin inhibits, glucose output by primary hepatocytes. Journal of Clinical Endocrinology and Metabolism 90:1055-1060 (2005).
32. 32. Gauna C, Kiewiet RM, Janssen JA, van de Zande B, Delhanty PJ, Ghigo E, Hofland LJ, Themmen AP, van der Lely AJ. Unacylated ghrelin acts as a potent insulin secretagogue in glucose-stimulated conditions. Am J Physiol Endocrinol Metab 293:E697-704 (2007).
33. 33. Granata R, Settanni F, Gallo D, Trovato L, Biancone L, Cantaluppi V, Nano R, Annunziata M, Campiglia P, Arnoletti E, Ghe C, Volante M, Papotti M, Muccioli G, Ghigo E. Obestatin promotes survival of pancreatic beta-cells and human islets and induces expression of genes involved in the regulation of beta-cell mass and function. Diabetes 57:967-979 (2008).
34. 34. Longo KA, Charoenthongtrakul S, Giuliana DJ, Govek EK, McDonagh T, Qi Y, DiStefano PS, Geddes BJ. Improved insulin sensitivity and metabolic flexibility in ghrelin receptor knockout mice. Regul Pept 150:55-61 (2008).
35. 35. Nonogaki K, Nozue K, Oka Y. Hyperphagia alters expression of hypothalamic 5-HT2C and 5-HT1B receptor genes and plasma des-acyl ghrelin levels in Ay mice. Endocrinology 147:5893-5900 (2006).
36. 36. Kitamura S, Yokota I, Hosoda H, Kotani Y, Matsuda J, Naito E, Ito M, Kangawa K, Kuroda Y. Ghrelin concentration in cord and neonatal blood: relation to fetal growth and energy balance. J Clin Endocrinol Metab. 2003 Nov;88(11):5473-7.

SEQUENCE LISTING

[0094]

<110> ALIZE PHARMA SAS Aart Jan van der Lely Thierry Abribat

<120> MODULATION OF GHRELIN LEVELS AND GHRELIN/UNACYLATED GHRELIN RATIO USING UNACYLATED GHRELIN

<130> D31874

<150> 61/576,217
<151> 2011-12-15

<160> 30

<170> PatentIn version 3.5

<210> 1
<211> 28
<212> PRT
<213> Homo sapiens

<400> 1
Gly Ser Ser Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys
1 5 10 15

Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg
20 25

<210> 2
<211> 14
<212> PRT
<213> Homo sapiens

<400> 2
Gly Ser Ser Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln
1 5 10

<210> 3
<211> 18
<212> PRT
<213> Homo sapiens

<400> 3
Gly Ser Ser Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys
1 5 10 15

Glu Ser

<210> 4
<211> 5
<212> PRT
<213> Homo sapiens

<400> 4
Gly Ser Ser Phe Leu
1 5

<210> 5
<211> 12
<212> PRT
<213> Homo sapiens

<400> 5
Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg
1 5 10

<210> 6
<211> 8
<212> PRT
<213> Homo sapiens

<400> 6
Ser Pro Glu His Gln Arg Val Gln
1 5

<210> 7
<211> 6
<212> PRT
<213> Homo sapiens

<400> 7
Glu His Gln Arg Val Gln
1 5

<210> 8
<211> 5
<212> PRT
<213> Homo sapiens

<400> 8
Glu His Gln Arg Val
1 5

<210> 9
<211> 13
<212> PRT
<213> Homo sapiens

<400> 9
Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser
1 5 10

<210> 10
<211> 4
<212> PRT

<213> Homo sapiens

<400> 10
Glu His Gln Arg
1

<210> 11
<211> 4
<212> PRT
<213> Homo sapiens

<400> 11
His Gln Arg Val
1

<210> 12
<211> 8
<212> PRT
<213> Homo sapiens

<400> 12
Ser Pro Asp His Gln Arg Val Gln
1 5

<210> 13
<211> 8
<212> PRT
<213> Homo sapiens

<400> 13
Ser Pro Glu His Gln Lys Val Gln
1 5

<210> 14
<211> 8
<212> PRT
<213> Homo sapiens

<400> 14
Gly Pro Glu His Gln Arg Val Gln
1 5

<210> 15
<211> 8
<212> PRT
<213> Homo sapiens

<400> 15
Ala Pro Glu His Gln Arg Val Gln

1 5

<210> 16

<211> 8

<212> PRT

<213> Homo sapiens

<400> 16

Ser Ala Glu His Gln Arg Val Gln
1 5

<210> 17

<211> 8

<212> PRT

<213> Homo sapiens

<400> 17

Ser Pro Ala His Gln Arg Val Gln
1 5

<210> 18

<211> 8

<212> PRT

<213> Homo sapiens

<400> 18

Ser Pro Glu Ala Gln Arg Val Gln
1 5

<210> 19

<211> 8

<212> PRT

<213> Homo sapiens

<400> 19

Ser Pro Glu His Ala Arg Val Gln
1 5

<210> 20

<211> 8

<212> PRT

<213> Homo sapiens

<400> 20

Ser Pro Glu His Gln Ala Val Gln
1 5

<210> 21

<211> 8

<212> PRT

<213> Homo sapiens

<400> 21

Ser Pro Glu His Gln Arg Ala Gln
1 5

1 5

<210> 22
<211> 8
<212> PRT
<213> Homo sapiens

<400> 22
Ser Pro Glu His Gln Arg Val Ala
1 5

<210> 23
<211> 8
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (1)..(1)
<223> ACETYLATION

<400> 23
Ser Pro Glu His Gln Arg Val Gln
1 5

<210> 24
<211> 8
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (1)..(1)
<223> ACETYLATION

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> D-amino acid

<400> 24
Ser Pro Glu His Gln Arg Val Gln
1 5

<210> 25
<211> 8
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE

<222> (1)..(8)
<223> Cyclic

<400> 25
Ser Pro Glu His Gln Arg Val Gln
1 5

<210> 26
<211> 8
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (3)..(6)
<223> Cyclic

<400> 26
Ser Pro Glu His Gln Lys Val Gln
1 5

<210> 27
<211> 8
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (1)..(1)
<223> ACETYLATION

<220>
<221> MISC_FEATURE
<222> (3)..(6)
<223> Cyclic

<400> 27
Ser Pro Glu His Gln Lys Val Gln
1 5

<210> 28
<211> 8
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (1)..(1)
<223> ACETYLATION

<400> 28
Ser Pro Glu His Gln Lys Val Gln
1 5

<210> 29
<211> 3
<212> PRT
<213> Homo sapiens

<400> 29
His Gln Arg
1

<210> 30
<211> 14
<212> PRT
<213> Homo sapiens

<400> 30
Gln Gln Val Arg Gln His Glu Pro Ser Leu Phe Ser Ser Gly
1 5 10

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US7825090B [0004]
- US7485620B [0006]
- US8222217B [0006] [0031] [0043]
- US8318664B [0006] [0031] [0043]
- WO2008145749A [0006] [0031]
- US20100016226A [0007]
- WO2009150214A [0007]
- WO61576217A [0094]

Non-patent literature cited in the description

- **GUTIERREZ et al.**PNAS, 2008, vol. 105, 6320-6325 [0088]
- **KOJIMA MHOSODA HDATE YNAKAZATO MMATSUO HKANGAWA K**Ghrelin is a growth-hormone-releasing acylated peptide from stomachNature, 1999, vol. 402, 656-660 [0093]
- **GNANAPAVAN SKOLA BBUSTIN SAMORRIS DGMCGEE PFAIRCLOUGH PBHATTACHARYA SCARPENTER RGROSSMAN ABKORBONITS MT**he tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humansJ. Clin. Endocrinol. Metab., 2002, vol. 87, 2988-2991 [0093]
- **HOWARD ADFEIGHNER SDCULLY DFARENA JPLIBERATOR PLROSENBLUM CIHAMELIN MHRENIUK DLPALYHA OCANDERSON J**A receptor in pituitary and hypothalamus that functions in growth hormone releaseScience, 1996, vol. 273, 974-977 [0093]
- **ARIYASU HTAKAYA KTAGAMI TOGAWA YHOSODA KAKAMIZU TSUDA MKOH TNATSUI KTOYOOKA S**Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humansJ Clin Endocrinol Metab., 2001, vol. 86, 104753-8 [0093]
- **DATE YMURAKAMI NTOSHINAI KMATSUKURA SNIIJIMA AMATSUO HKANGAWA KNAKAZATO MT**he role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in ratsGastroenterology, 2002, vol. 123, 41120-1128 [0093]
- **KOJIMA MHOSODA HDATE YNAKAZATO MMATSUO HKANGAWA K**Ghrelin is a growth-hormone-releasing acylated peptide from stomachNature, 1999, vol. 402, 6762656-660 [0093]
- **TSCHOP MSMILEY DLHEIMAN ML**Ghrelin induces adiposity in rodentsNature, 2000, vol. 407, 6806908-913 [0093]
- **BROGLIO FGIANOTTI LDESTEFANIS SFASSINO SABBATE DAGA GMONDELLI VLANFRANCO FGOTTERO CGAUNA CHOFLAND LT**he endocrine response to acute ghrelin administration is blunted in patients with anorexia nervosa, a ghrelin hypersecretory stateClin Endocrinol (Oxf), 2004, vol. 60, 5592-599 [0093]
- **TONG JPRIGEON RLDAVIS HWBIDLINGMAIER MKAHN SECUMMINGS DETSCHÖP MHD'ALESSIO D**Ghrelin suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance in healthy humansDiabetes, 2010, vol. 59, 92145-51 [0093]
- **CUMMINGS DECLEMENT KPURNELL JQVAISSE CFOSTER KEFRAYO RSSCHWARTZ MWBASDEVANT AWEIGLE DS**Elevated plasma ghrelin levels in Prader Willi syndromeNat Med, 2002, vol. 8, 7643-644 [0093]
- **HAQQ AMFFAROOQI ISO'RAHILLY SSTADLER DDROSENFELD RGPRATT KLLAFRANCHI SHPURNELL JQ**Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndromeJ Clin Endocrinol Metab, 2003, vol. 88, 1174-178 [0093]

- **MIFUNE HNISHI YTAJIRI YMASUYAMA THOSODA HKANGAWA KKOJIMA M**Increased production of active ghrelin is relevant to hyperphagia in nonobese spontaneously diabetic Torii ratsMetabolism, 2011, [0093]
- **MISRA MMILLER KKKUO KGRIFFIN KSTEWART VHUNTER EHERZOG DBKLIBANSKI A**Secretory dynamics of ghrelin in adolescent girls with anorexia nervosa and healthy adolescentsAm J Physiol Endocrinol Metab, 2005, vol. 289, 2E347-E356 [0093]
- **NIKOLOPOULOS DTHEOCHARIS SKOURAKLIS G**Ghrelin: a potential therapeutic target for cancerRegul Pept, 2010, vol. 163, 1-37-17 [0093]
- **VAN DER LELY AJTSCHOP MHEIMAN MLGHIGO E**Biological, physiological, pathophysiological, and pharmacological aspects of ghrelinEndocr Rev, 2004, vol. 25, 426-457 [0093]
- **ASAKAWA AINUI AFUJIMIYA MSAKAMAKI RSHINFUKU NUETA YMEGUID MMKASUGA M**Stomach regulates energy balance via acylated ghrelin and desacyl ghrelinGut., 2005, vol. 54, 118-24 [0093]
- **ZHANG WCHAI BLI JYWANG HMULHOLLAND MW**Effect of des-acyl ghrelin on adiposity and glucose metabolismEndocrinology, 2008, vol. 149, 94710-6 [0093]
- **BROGLIO FGOTTERO CPRODAM FGAUNA CMUCCIOLI GPAPOTTI MABRIBAT TVAN DER LELY AJGHIGO E**Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humansJ Clin Endocrinol Metab., 2004, vol. 89, 63062-5 [0093]
- **GAUNA CMEYLER FMJANSSEN JADELHANTY PJABRIBAT TVAN KOETSVELD PHOFLAND LJBROGLIO FGHIGO EVAN DER LELY AJ**Administration of acylated ghrelin reduces insulin sensitivity, whereas the combination of acylated plus unacylated ghrelin strongly improves insulin sensitivityJ Clin Endocrinol Metab., 2004, vol. 89, 105035-42 [0093]
- **ST-PIERRE DHKARELIS ADCODERRE LMALITA FFONTAINE JMIGNAULT DBROCHU MBASTARD JPCIANFLONE KDOUCET E**Association of acylated and nonacylated ghrelin with insulin sensitivity in overweight and obese postmenopausal womenJ Clin Endocrinol Metab., 2007, vol. 92, 1264-9 [0093]
- **PACIFICO LPOGGIOGALLE ECOSTANTINO FANANIA CFERRARO FCHIARELLI FCHIESA**Acylated and nonacylated ghrelin levels and their associations with insulin resistance in obese and normal weight children with metabolic syndromeEur J Endocrinol., 2009, vol. 161, 6861-70 [0093]
- **BARAZZONI RZANETTI MFERREIRA CVINCI PPIRULLI AMUCCI MDORE FFONDA MCIOCCHI BCATTIN L**Relationships between desacylated and acylated ghrelin and insulin sensitivity in the metabolic syndromeJ Clin Endocrinol Metab., 2007, vol. 92, 103935-40 [0093]
- **RODRÍGUEZ AGÓMEZ-AMBROSI JCATALÁN VGIL MJBECERRIL SSÁINZ NSILVA CSALVADOR JCOLINA IFRÜHBECK G**Acylated and desacyl ghrelin stimulate lipid accumulation in human visceral adipocytesInt J Obes (Lond), 2009, vol. 33, 5541-52 [0093]
- **SCHELLEKENS HDINAN TGCRYAN JF**Lean mean fat reducing "ghrelin" machine: Hypothalamic ghrelin and ghrelin receptors as therapeutic targets in

obesityNeuropharmacology, 2010, vol. 58, 12-19 [0093]

- **GUALILLO OLAGO FDIEGUEZ C**Introducing GOAT: a target for obesity and anti-diabetic drugs?Trends Pharmacol Sci, 2008, vol. 29, 8398-401 [0093]
- **HILLMAN JBTONG JTSCHOP M**Ghrelin biology and its role in weight-related disordersDiscov Med., 2011, vol. 11, 61521-8 [0093]
- **KUMAR RSALEHI AREHFELD JFHÖGLUND PLINDSTRÖM EHÅKANSON R**Proghrelin peptides: Desacyl ghrelin is a powerful inhibitor of acylated ghrelin, likely to impair physiological effects of acyl ghrelin but not of obestatin A study of pancreatic polypeptide secretion from mouse isletsRegul Pept., 2010, vol. 164, 2-365-70 [0093]
- **CUMMINGS DEWEIGLE DSFRAYO RSBREEN PAMA MKDELLINGER EPPURNELL JQ**Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgeryN Engl J Med., 2002, vol. 346, [0093]
- **SUMITHRAN PPRENDERGAST LADELBRIDGE EPURCELL KSHULKES AKRIKETOS APROIETTO J**Long-term persistence of hormonal adaptations to weight lossN Engl J Med., 2011, vol. 365, 171597-604 [0093]
- **KIEWIET RMVAN AKEN MOVAN DER WEERD KUITTERLINDEN PTHEMMEN APHOFLAND LJDE RIJKE YBDELHANTY PJGHIGO EABRIBAT T**Effects of acute administratio of acylated and unacylated ghrelin on glucose and insulin concentratios in morbidly obese subjects without overt diabetesEur J Endocrinol, 2009, vol. 161, 567-573 [0093]
- **GAUNA CDELHANTY PJDHOFLAND LJJANSSEN JBROGLIO FROSS RJMGHIGO EVAN DER LELY AJ**Ghrelin stimulates, whereas des-octanoyl ghrelin inhibits, glucose output by primary hepatocytesJournal of Clinical Endocrinology and Metabolism, 2005, vol. 90, 1055-1060 [0093]
- **GAUNA CKIEWIET RMJANSSEN JAVAN DE ZANDE BDELHANTY PJGHIGO EHOFLAND LJTHEMMEN APVAN DER LELY AJ**Unacylated ghrelin acts as a potent insulin secretagogue in glucose-stimulated conditionsAm J Physiol Endocrinol Metab, 2007, vol. 293, E697-704 [0093]
- **GRANATA RSETTANNI FGALLO DTROVATO LBIANCONE LCANTALUPPI VNANO RANNUNZIATA MCAMPIGLIA PARNOLETTI E**Obestatin promotes survival of pancreatic beta-cells and human islets and induces expression of genes involved in the regulation of beta-cell mass and functionDiabetes, 2008, vol. 57, 967-979 [0093]
- **LONGO KACHAROENTHONGTRAKUL SGIULIANA DJGOVEK EKMCDONAGH TQI YDISTEFANO PSGEDDES BJ**Improved insulin sensitivity and metabolic flexibility in ghrelin receptor knockout miceRegul Pept, 2008, vol. 150, 55-61 [0093]
- **NONOGAKI KNOZUE KOKA Y**Hyperphagia alters expression of hypothalamic 5-HT2C and 5-HT1B receptor genes and plasma des-acyl ghrelin levels in Ay miceEndocrinology, 2006, vol. 147, 5893-5900 [0093]
- **KITAMURA SYOKOTA IHOSODA HKOTANI YMATSUDA JNAITO EITO MKANGAWA KKURODA Y**Ghrelin concentration in cord and neonatal blood: relation to fetal growth and energy balanceJ Clin Endocrinol Metab., 2003, vol. 88, 115473-7 [0093]

Patentkrav

1. Fragmenter af ikke-acyleret ghrelin bestående af peptidet som angivet i SEQ ID NO: 6 eller som angivet i SEQ ID NO: 25, og et farmaceutisk acceptabelt salt deraf, til deres anvendelse til behandling af Prader-Willi syndrom hos en patient med forhøjede ghrelin-niveauer.

2. Ikke-acylerede ghrelin-fragmenter og salt deraf til deres anvendelse ifølge krav 1, hvor den omfatter indgivelse af en effektiv mængde af nævnte ikke-acylerede ghrelin-fragment og salt fra ca. 0,001 µg/kg til ca. 10 mg/kg.

3. Ikke-acylerede ghrelin-fragmenter og salt deraf til deres anvendelse ifølge et af kravene 1 eller 2, hvor den omfatter indgivelse af en effektiv mængde af nævnte ikke-acylerede ghrelin-fragment og salt fra ca. 1 µg kg til ca. 1 mg/kg.

4. Ikke-acylerede ghrelin-fragmenter og salt deraf til deres anvendelse ifølge et af kravene 1 til 3, hvor nævnte ikke-acylerede ghrelin-fragment og farmaceutisk acceptable salte deraf indgives via en rute valgt fra gruppen bestående af intravenøs, subkutan, transdermal, oral, bukkal, sublingual, nasal og inhalering.

5. Ikke-acylerede ghrelin-fragmenter og salt deraf til deres anvendelse ifølge et af kravene 1 til 4, hvor de forhøjede ghrelin-niveauer er forhøjede cirkulerende ghrelin-niveauer.

6. Ikke-acylerede ghrelin-fragmenter og salt deraf til deres anvendelse ifølge et af kravene 1 til 5, hvor patienten blev identificeret ved

- at bestemme et niveau af cirkulerende ghrelin fra patienten, og
- at bearbejde niveauet af cirkulerende ghrelin mindst delvist baseret på et referenceniveau af cirkulerende ghrelin for at opnå information der tilkendegiver, hvorvidt niveauet af cirkulerende ghrelin er forhøjet.

DRAWINGS

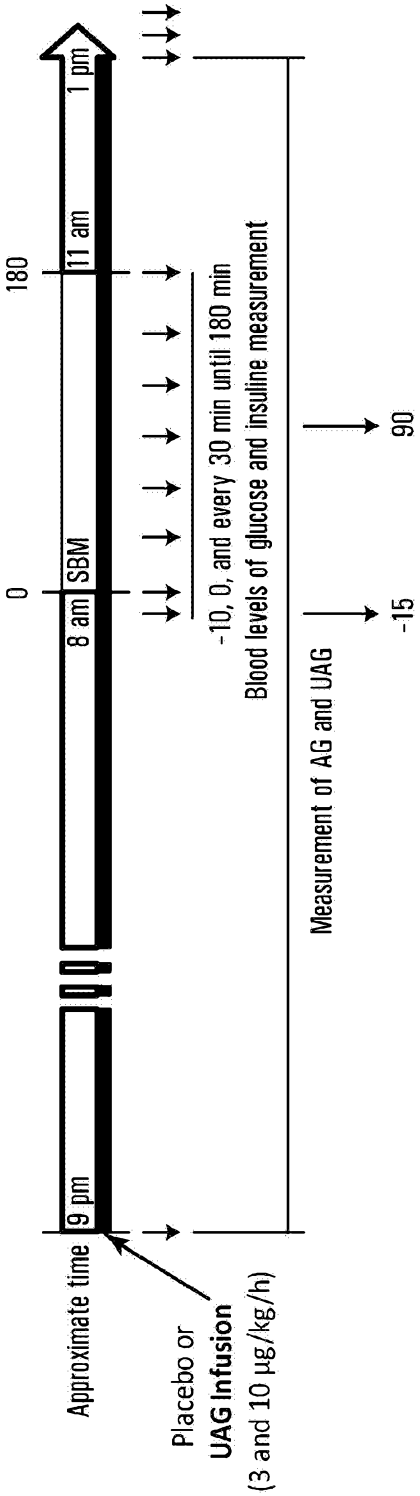

FIG. 1

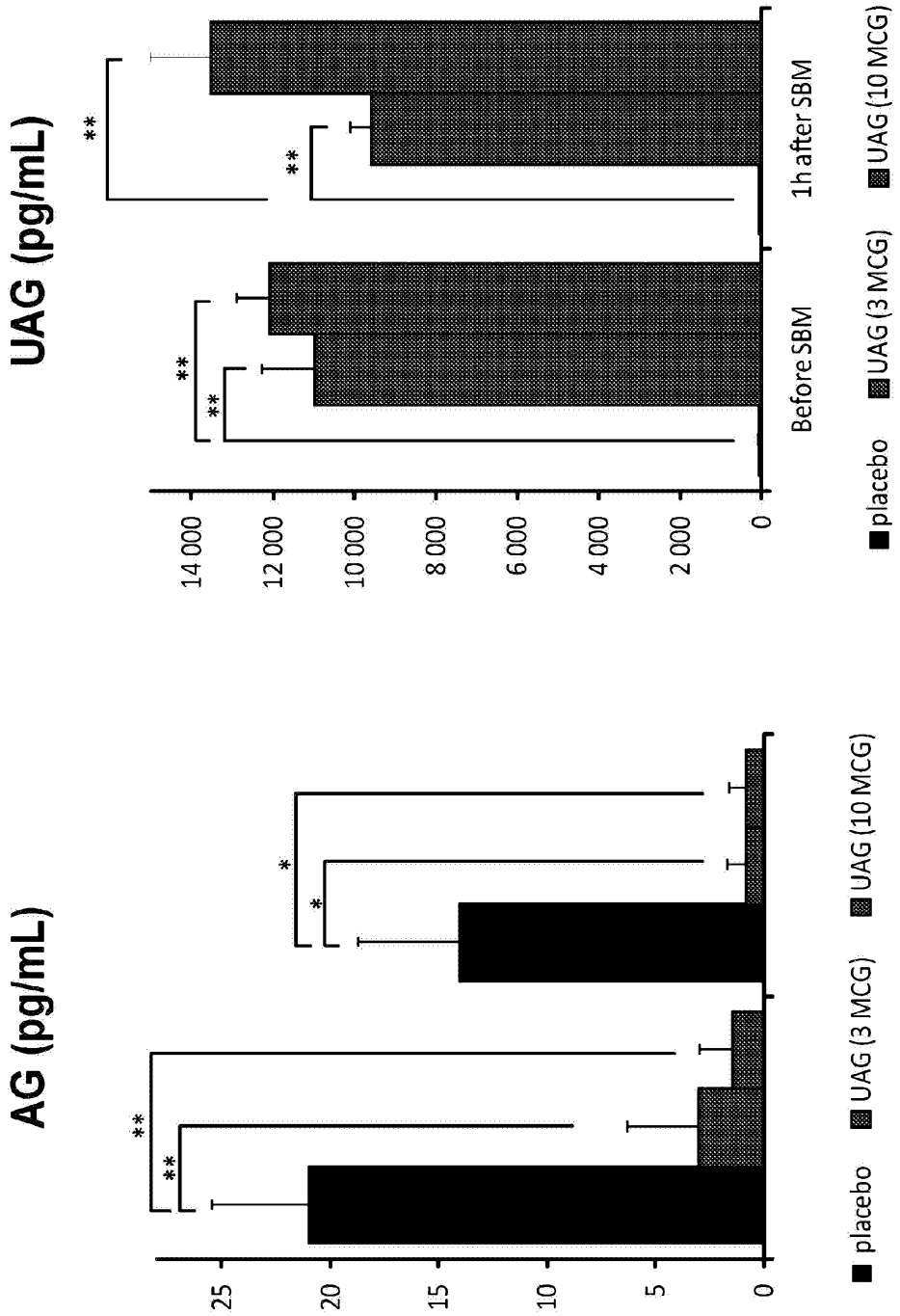
UAG (pg/mL)
14 000
12 000
10 000
8 000
6 000
4 000
2 000
0
**
**
**
**
Before SBM
1h after SBM
placebo
UAG (3 MCG)
UAG (10 MCG)

FIG. 2B

AG (pg/mL)
25
20
15
10
5
0
**
**
*
*
placebo
UAG (3 MCG)
UAG (10 MCG)

FIG. 2A

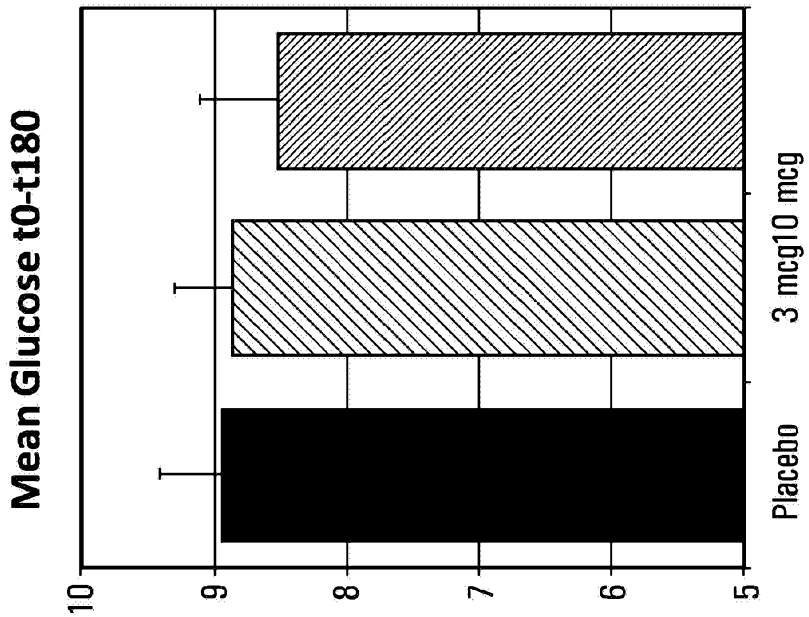
Mean Glucose t0-t180
10
9
8
7
6
5
Placebo
3 mcg
10 mcg

FIG. 3B

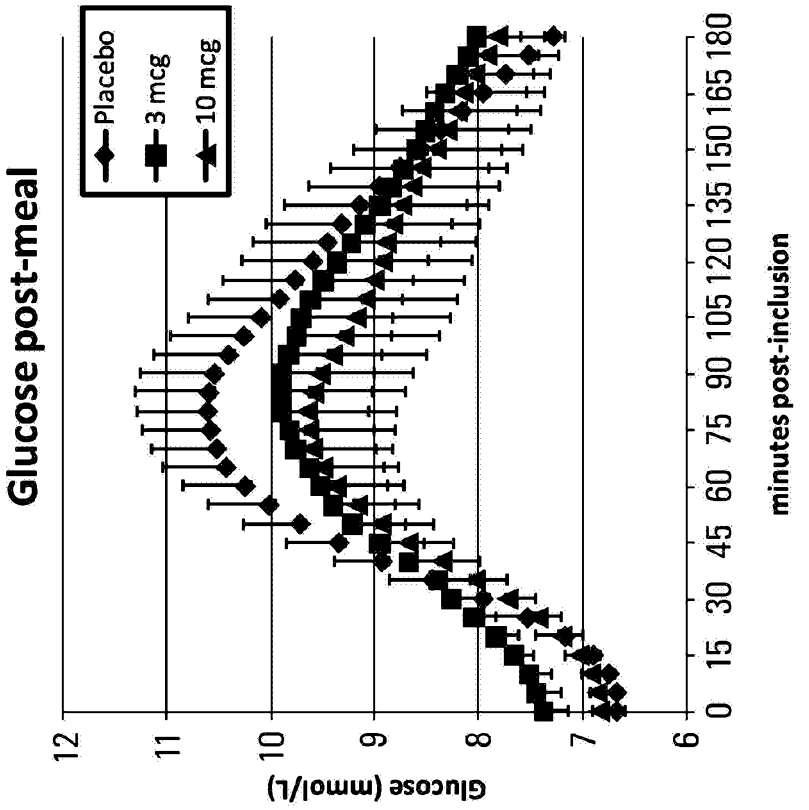
Glucose post-meal
Placebo
3 mcg
10 mcg
Glucose (mmol/L)
12
11
10
9
8
7
6
0 15 30 45 60 75 90 105 120 135 150 165 180
minutes post-inclusion

FIG. 3A

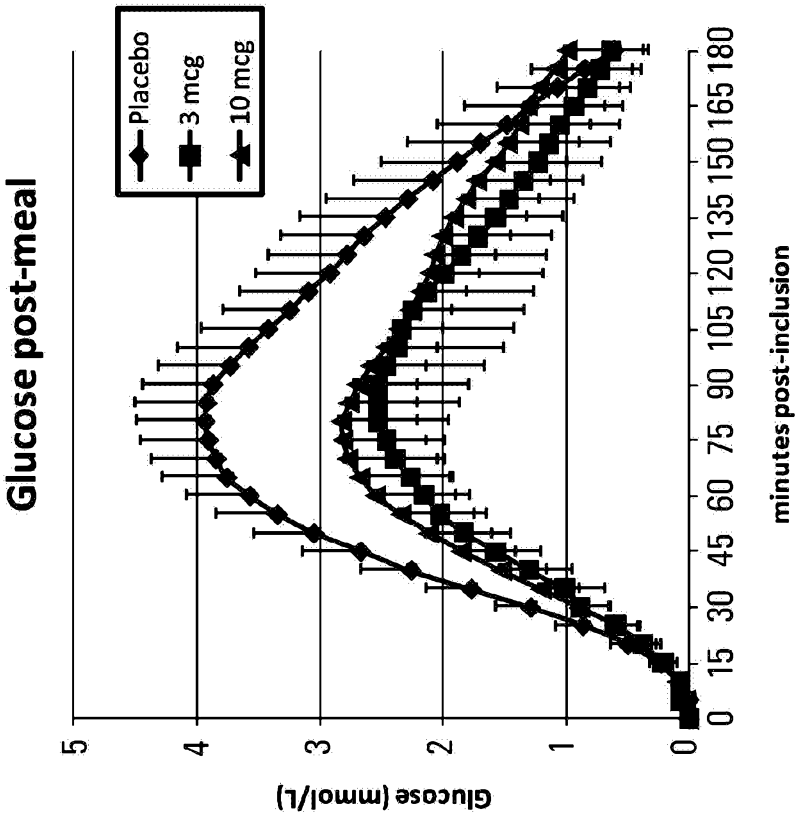
Glucose post-meal
Placebo
3 mcg
10 mcg
Glucose (mmol/L)
0
1
2
3
4
5
0 15 30 45 60 75 90 105 120 135 150 165 180
minutes post-inclusion

FIG. 4A

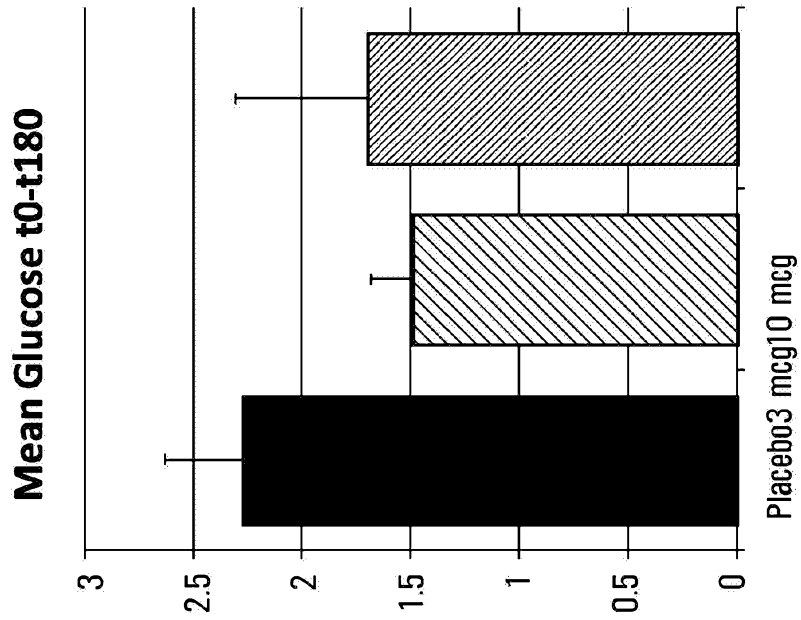
Mean Glucose t0-t180
0
0.5
1
1.5
2
2.5
3
Placebo3 mcg10 mcg

FIG. 4B

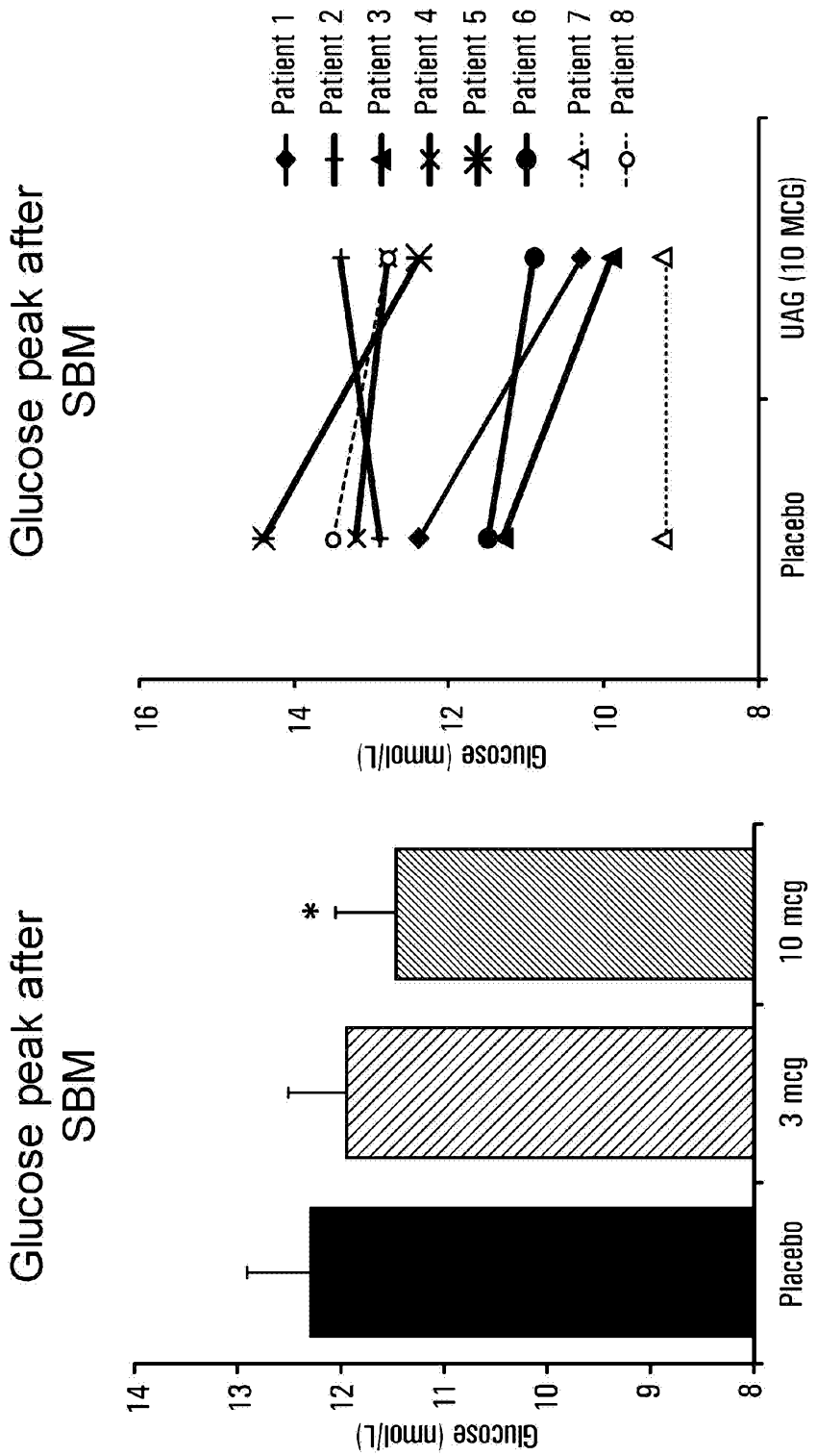
Glucose peak after SBM
Glucose (mmol/L)
16
14
12
10
8
Placebo
UAG (10 MCG)
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8

FIG. 5B

Glucose peak after SBM
Glucose (mmol/L)
14
13
12
11
10
9
8
*
Placebo
3 mcg
10 mcg

FIG. 5A

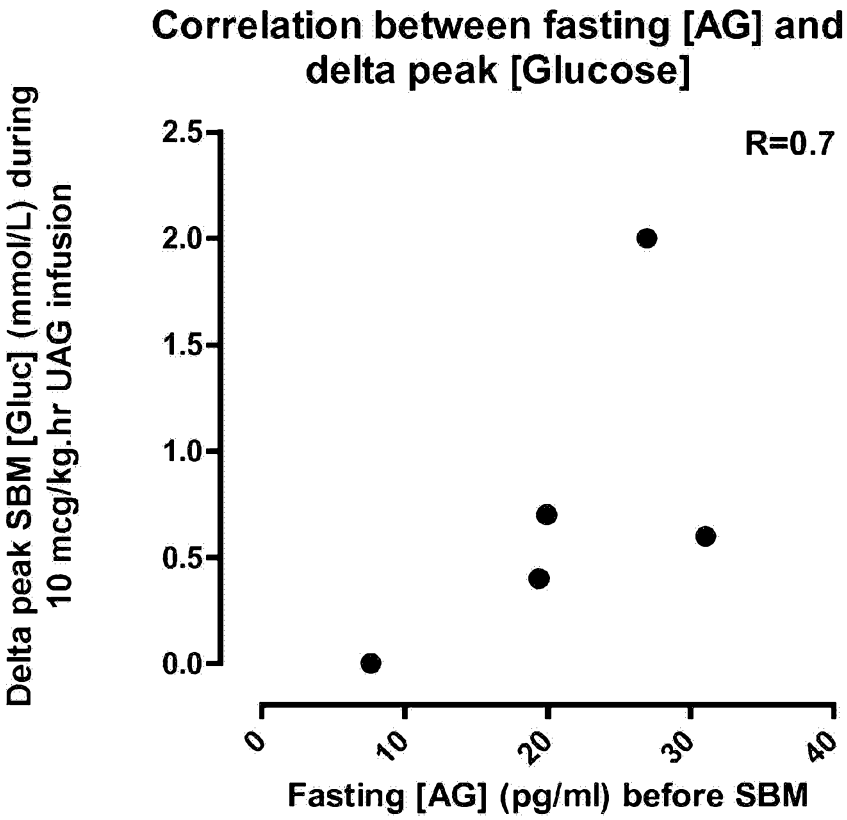
Correlation between fasting [AG] and delta peak [Glucose]
R=0.7
Delta peak SBM [Gluc] (mmol/L) during 10 mcg/kg.hr UAG infusion
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
Fasting [AG] (pg/ml) before SBM

FIG. 6A

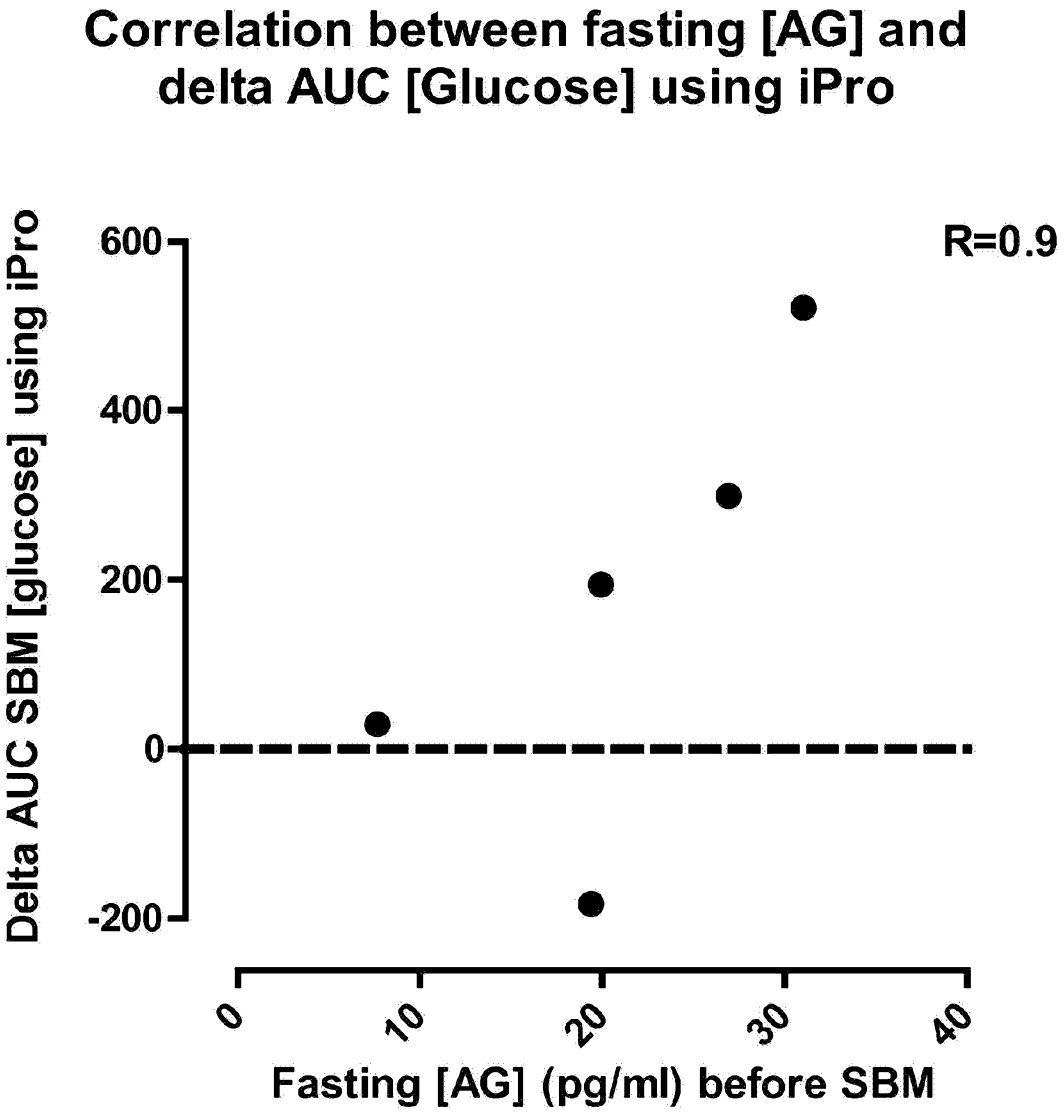
Correlation between fasting [AG] and delta AUC [Glucose] using iPro
Delta AUC SBM [glucose] using iPro
600
400
200
0
-200
R=0.9
0
10
20
30
40
Fasting [AG] (pg/ml) before SBM

FIG. 6B

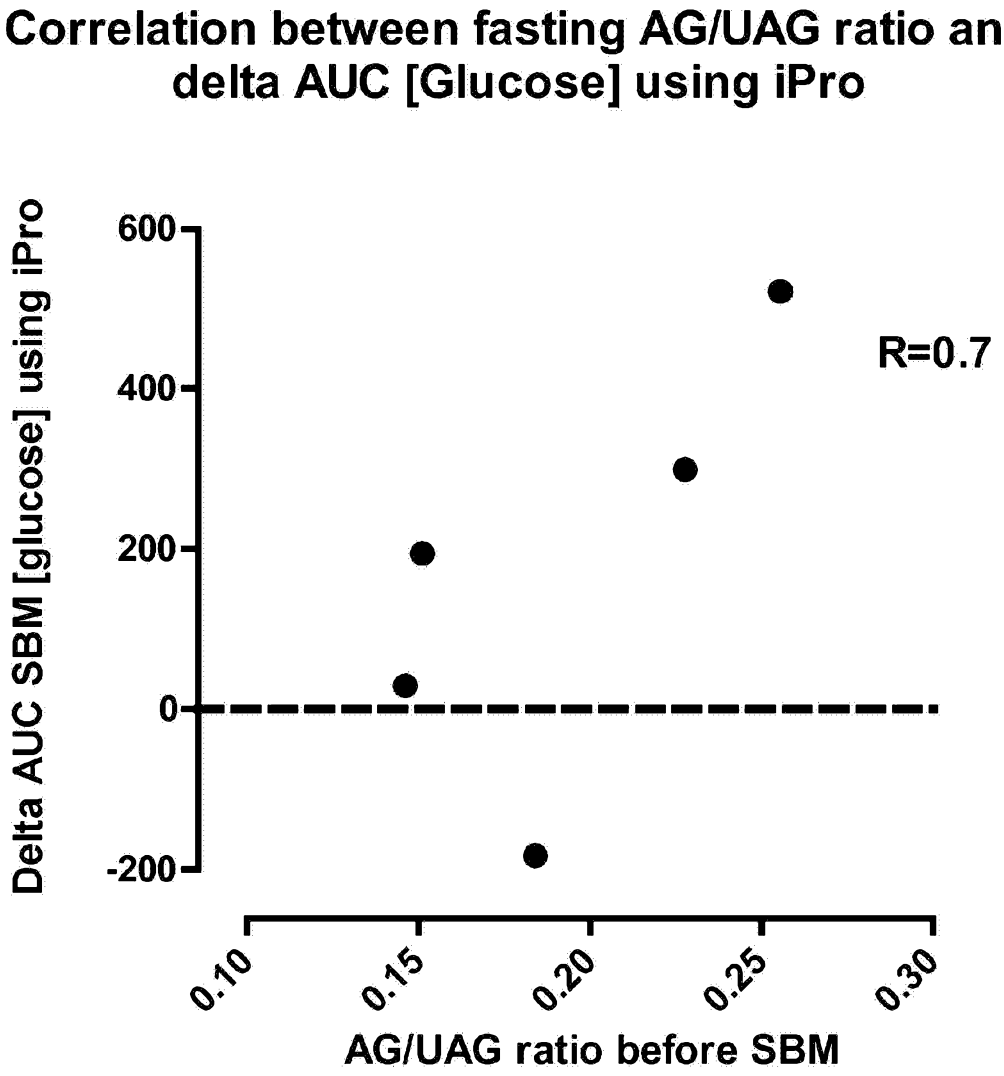
Correlation between fasting AG/UAG ratio and delta AUC [Glucose] using iPro
Delta AUC SBM [glucose] using iPro
600
400
200
0
-200
R=0.7
0.10
0.15
0.20
0.25
0.30
AG/UAG ratio before SBM

FIG. 6C

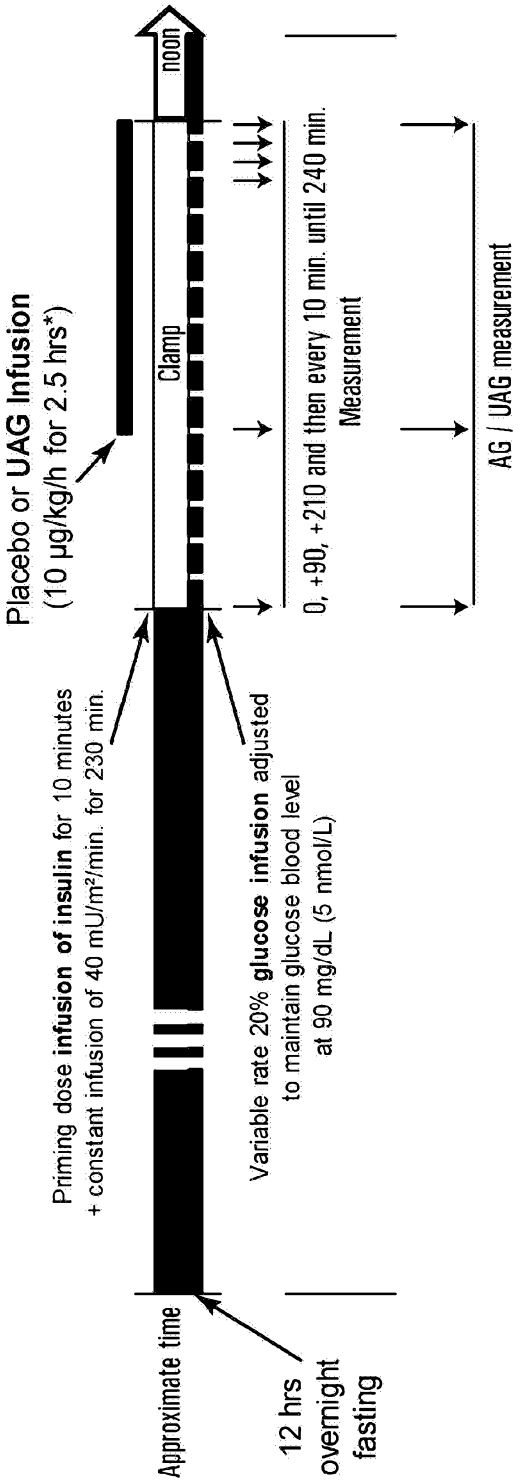
Placebo or UAG Infusion
(10 µg/kg/h for 2.5 hrs*)
Priming dose infusion of insulin for 10 minutes
+ constant infusion of 40 mU/m²/min. for 230 min.
Variable rate 20% glucose infusion adjusted
to maintain glucose blood level
at 90 mg/dL (5 nmol/L)
Clamp
noon
0, +90, +210 and then every 10 min. until 240 min.
Measurement
AG / UAG measurement
Approximate time
12 hrs
overnight
fasting

FIG. 7A

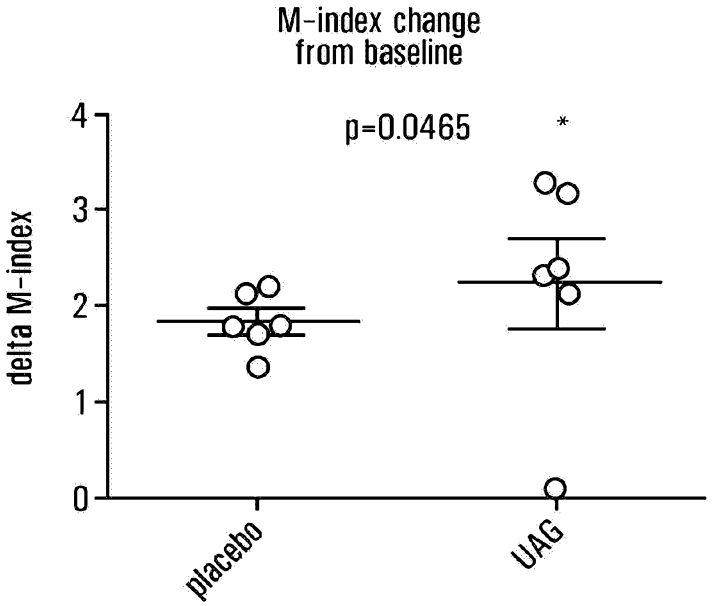
M-index change
from baseline
p=0.0465
*
delta M-index
4
3
2
1
0
placebo
UAG

FIG. 7B

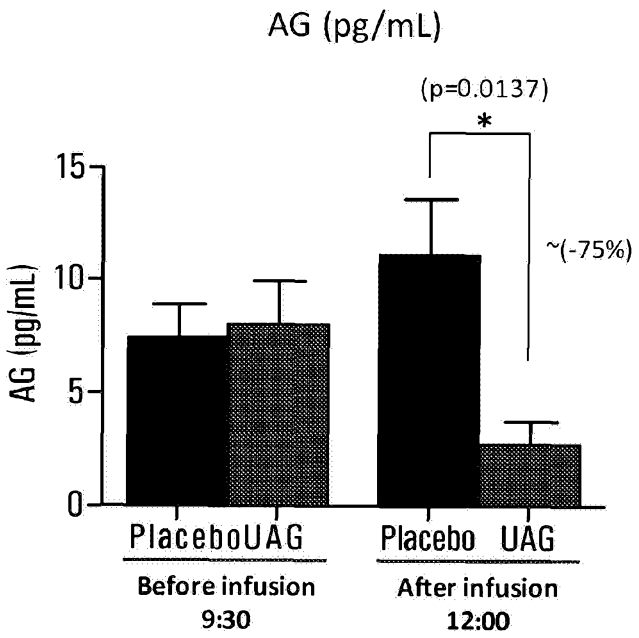
AG (pg/mL)
(p=0.0137)
*
~(-75%)
15
10
5
0
AG (pg/mL)
PlaceboUAG
Placebo UAG
Before infusion
9:30
After infusion
12:00

FIG. 7C

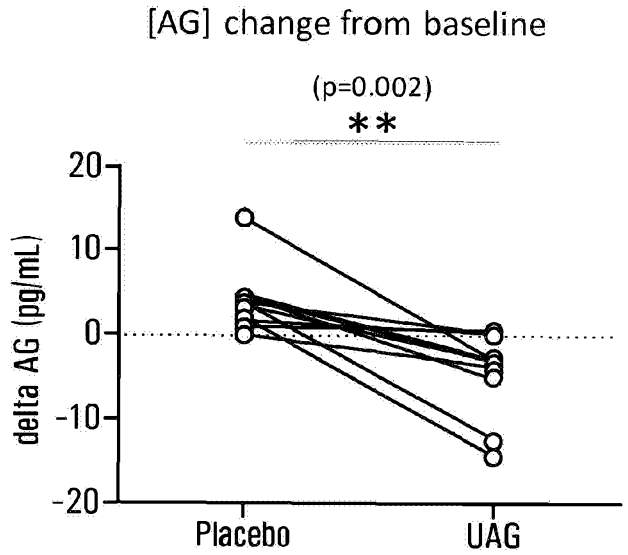
[AG] change from baseline
(p=0.002)
**
20
10
0
-10
-20
delta AG (pg/mL)
Placebo
UAG

FIG. 7D

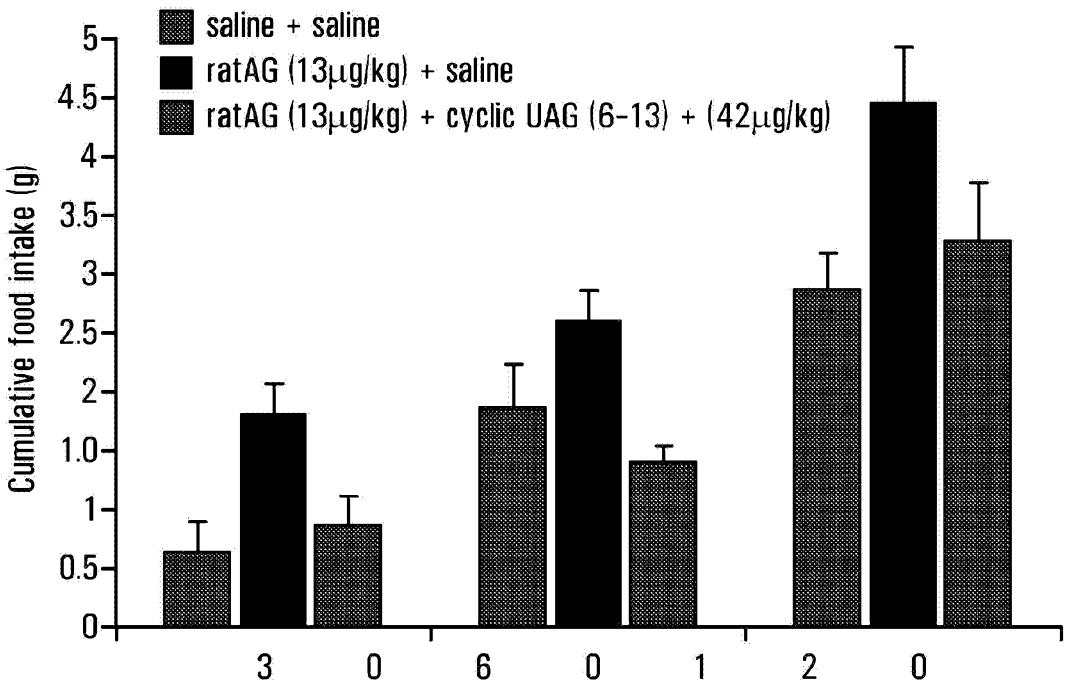
saline + saline
ratAG (13μg/kg) + saline
ratAG (13μg/kg) + cyclic UAG (6-13) + (42μg/kg)
Cumulative food intake (g)
5
4.5
4
3.5
3
2.5
2
1.0
1
0.5
0
3
0
6
0
1
2
0

FIG. 8