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(54) Title: USES OF SECRETAGOGUES FOR TREATMENT OF ORGAN TRANSPLANT PATIENTS

(57) Abstract: The invention relates to the use of a GH secretagogue compound for the preparation of a medicament for the stimulation of appetite, food intake and/or weight gain in an organ transplantation patient in need of such treatment. Furthermore, the invention relates to a method for stimulating appetite, food intake and/or weight gain in an organ transplantation patient, said method comprising administration of a secretagogue to said patient.

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Uses of secretagogues for treatment of organ transplant patients

5 This application claims priority from Danish patent application number PA 2004 01658, filed 27th October 2004, which is hereby incorporated by reference in its entirety. All patent and non-patent references cited in the present application are also hereby incorporated by reference in their entirety.

Field of the invention

10 The present invention relates to the medicaments for the stimulation of appetite and/or food intake and/or weight gain in organ transplantation patients in need thereof, as well as to methods of stimulating appetite and/or food intake and/or weight gain in an organ transplantation patient in need thereof.

15 Background of the inventionGhrelin

Ghrelin is a bioactive peptide which originally was described to be involved in the control of GH secretion but later found to be a major regulator of appetite, food intake and energy homeostasis (Kojima M et al., Trends Endocrinol Metab 12:118-122; Nakazato M et al., 2001, Nature 409:194-198). Similar to many other bioactive peptides, ghrelin probably acts both as a hormone, a paracrine substance and as a neurotransmitter. The story of ghrelin, its receptor and synthetic compounds acting through this receptor unraveled in a unique "reverse" order. In the eighties a synthetic hexa-peptide from a series of opioid-like peptides was found to be able to
20 release growth hormone (GH) from isolated pituitary cells (Bowers CY et al., 1980, Endocrinology 106:663-667). Since this action was independent of the growth hormone releasing hormone (GHRH) receptor, several pharmaceutical companies embarked upon drug discovery projects based on this hexa-peptide GH secretagogue (GHS) and its putative receptor. Several series of potent and efficient
25 peptide as well as non-peptide GH secretagogues were consequently described in the mid nineties (Bowers CY et al., Endocrinology 114:1537-1545; Patchett AA et al., 1995; Proc Natl Acad Sci U S A 92:7001-7005; Smith RG et al., Science 260:1640-1643). However, it was only several years later that the receptor through which these artificial GH secretagogues acted was eventually cloned and shown to
30 be a member of the 7TM G protein coupled receptor family (Howard AD et al.,
35

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Science 273:974-977; Smith RG et al., 1997 Endocr Rev 18:621-645). In 1999, the endogenous ligand for this receptor, the hormone ghrelin, was finally discovered (Kojima M et al., 1999, Nature 402:656-660). The main site for ghrelin production is the stomach, where the peptide is found in classical endocrine cells in the gastric mucosa.

From here, ghrelin is secreted in the pre-meal situation which results in a sharp, short-lived surge in plasma levels of ghrelin before the meal and starting 1-2 hours before and lasting a short while after initiation of the meal. Since ghrelin is the only peripherally produced orexigenic (appetite promoting) substance it is believed that the increase in plasma levels of ghrelin is crucial for the initiation of the meal.

In its role as a key initiator of appetite, ghrelin released from the endocrine cells in the mucosa of the GI tract may act both locally as a paracrine substance and centrally as a hormone.

Previously, ghrelin has been administered by continuous infusions for 270 minutes, which has shown that an increase in food intake can be obtained through intravenous administration of ghrelin (Wren et al JCEM 2001; 86(12)5992-5995).

Organ transplant patients

Weight loss is also common among patients with end stage kidney, liver, heart and pulmonary disease, and low body weight has been found to be a predictor of increased morbidity and mortality in these conditions. In patients referred to lung transplantation, low body mass index (BMI) or low lean body mass is associated with an increased waiting list mortality (Schwebel et al. Eur Respir J 2000;16:1050-1055; Schell et al. J Heart Lung Transplant 1998;17:1097-1103). Similarly, in patients undergoing kidney, liver, heart, or lung transplantation the postoperative mortality is higher among patients with a poor preoperative nutritional status (Madill et al. J Heart Lung Transplant 2001;20:288-96; Lietz et al. Transplantation 2001;72:277-283; Bilbao et al. Clin Transplant 2003;17:401-411). Weight gain in the first year after lung transplantation has been shown to reduce the risk of subsequent mortality (Singer et al. J Heart Lung Transplant 2003;22:894-902).

Summary of the Invention

In a first aspect, the invention relates to the use of a secretagogue compound for the preparation of a medicament for the stimulation of appetite, food intake and/or weight gain in an organ transplantation patient in need of such treatment.

5

Furthermore, the invention relates to a method for stimulating appetite, food intake and/or weight gain in an organ transplantation patient, said method comprising administration of a secretagogue to said patient.

10 Accordingly, the invention relates to the use of a secretagogue compound for the preparation of a medicament for

a) stimulation of appetite, and/or

b) stimulation of food intake, and/or

15 c) stimulation of weight gain, and/or

d) increasing body fat mass, and/or

e) increasing lean body mass,

including any combination of the above,

20

by administering a dosage of said medicament in an individual, wherein the individual is a patient that will undergo, is undergoing or has undergone an organ transplantation, such as a transplantation of the lung, liver, kidney or heart.

25 Preferred combinations are: a); b); c); d); and e); in isolation; as well as a)+b); a) + c); a) + d); a) + e); b) + c); b) + d); b) + e); d) +e); c)+ d)+ e) ; a) + c) + d); a) + c) + e); ; a) + d) + e); a) + c) + d) + e); b) + c) + d); b) + c) + e); b) + d) + e); and b) + c) + d) + e).

30 Preferably, said secretagogue is a ghrelin-like compound which comprises a structure defined herein below.

The orexigenic and metabolic effects of secretagogues, such as ghrelin, reduce the morbidity and mortality in patients undergoing organ transplantation. Furthermore,
35 these effects improve their quality of life.

Patients particularly in need of treatment are ones having a lean body mass of less than 80% of normal, such as less than 60% of normal and/or a body mass index below 17 kg/m².

5

The medicament may be given during the pre-operative period, the peri-operative period and/or post-operative period. In order to optimise the recipient's nutritional status prior to, during and after organ transplantation, secretagogue therapy is preferably initiated at the time of referral to transplantation and continued throughout the peri- and postoperative period until body weight has been normalised. Thus, in a preferred embodiment, the medicament is given during the pre-operative, the peri-operative and the post-operative period.

The medicament is preferably given until the lean body mass is more than 60% of normal, preferably more than 80% of normal, more preferably more than 90% of normal.

In a preferred aspect of the invention the ghrelin-like compound is administered with a substance capable of increasing the half-life of the ghrelin-like compound, for example by incorporating the ghrelin-like compound into liposomes, micelles, iscoms, and/or microspheres or other transport molecules, in particular to protect the modified amino acid from being desacylated.

In all embodiments of the present invention, the medicament can be administered as a bolus injection or by fast running infusion, i.e. an infusion preferably lasting less than 120 minutes, such as less than 90 minutes, for example less than 60 minutes, such as less than 45 minutes, such as less than 30 minutes, for example less than 25 minutes, such as less than 20 minutes, such as less than 15 minutes, for example less than 12 minutes, such as less than 10 minutes, such as less than 8 minutes, for example less than 6 minutes, such as less than 5 minutes, such as less than 4 minutes, for example less than 3 minutes, such as less than 2 minutes, such as less than 1 minute.

The bolus injection or the fast running infusion can be administered prior to a meal or during a meal as described in more detail herein below. In one preferred

embodiment the medicament is administered as a bolus. The bolus is preferably administered subcutaneously.

5 Detailed Description of the Invention

Definitions

Affinity: the strength of binding between receptors and their ligands, for example between the GHR1a receptor and a ghrelin-like compound, The affinity may be e.g.
10 described using a dissociation constant, K_d.

Amino Acid Residue: An amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are preferably in the "L" isomeric form. However, the amino acid encompasses every
15 amino acid such as L-amino acid, D-amino acid, alpha -amino acid, beta -amino acid, gamma -amino acid, natural amino acid and synthetic amino acid or the like as long as the desired functional property is retained by the polypeptide. NH₂ refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In
20 keeping with standard polypeptide, abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

25	SYMBOL		
	1-Letter	3-Letter	AMINO ACID
	Y	Tyr	tyrosine
	G	Gly	glycine
	F	Phe	phenylalanine
	M	Met	methionine
30	A	Ala	alanine
	S	Ser	serine
	I	Ile	isoleucine
	L	Leu	leucine
	T	Thr	threonine
35	V	Val	valine

	P	Pro	proline
	K	Lys	lysine
	H	His	histidine
	Q	Gln	glutamine
5	E	Glu	glutamic acid
	Z	Glx	Glu and/or Gln
	W	Trp	tryptophan
	R	Arg	arginine
	D	Asp	aspartic acid
10	N	Asn	asparagine
	B	Asx	Asn and/or Asp
	C	Cys	cysteine
	X	Xaa	Unknown or other

15 It should be noted that all amino acid residue sequences represented herein by formulae have a left-to-right orientation in the conventional direction of amino terminus to carboxy terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence and modified and non-naturally occurring amino acids. Furthermore, it should be noted

20 that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues or a covalent bond to an amino-terminal group such as NH₂ or acetyl or to a carboxy-terminal group such as COOH.

25 Appetite: Appetite in an individual is assessed by measuring the amount of food ingested and by assessing the individual's desire to eat. Appetite (for example, hunger) is typically assessed with a short questionnaire given to individuals on a random basis several times a week. Typically, subjects rate their hunger, preoccupation with food, and desire to eat greater quantities and different types of

30 food by answering the questions using analogue scales ranging from 1, not at all, to 5, extremely.

BMI measures your height/weight ratio. It is determined by calculating weight in kilograms divided by the square of height in meters. The BMI "normal" range is 19-

35 25.

- Body fat mass: Body fat mass can be measured e.g. by the fat fold technique: In this technique, a pincer-type caliper is used to measure subcutaneous fat by determining skin fold thickness at representative sites on the body. These skin fold measurements are then used to compute body fat by either adding the scores from the various measurements and using this value as an indication of the relative degree of fatness among individuals or by using the measurements in mathematical equations that have been developed to predict percent body fat. Another method for measuring body fat mass is using a DEXA scan or MRI.
- Concentration equivalent: A concentration equivalent is an Equivalents dosage being defined as the dosage of a ghrelin-like compound having in vitro and/or in vivo the same response as evaluated from a dosage-response curve of wild-type ghrelin.
- Dissociation constant, K_d : a measure to describe the strength of binding (or affinity or avidity) between receptors and their ligands, for example the GHR1a receptor and a ghrelin-like compound. The smaller K_d the stronger binding.
- Fusion Polypeptide: A polypeptide comprised of at least two polypeptides and a linking sequence to operatively link the two polypeptides into one continuous polypeptide. The two polypeptides linked in a fusion polypeptide are typically derived from two independent sources, and therefore a fusion polypeptide comprises two linked polypeptides not normally found linked in nature.
- Ghrelin: a polypeptide as described in Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656-660. Human 28 aa ghrelin has the amino acid of SEQ ID NO: 1.
- Ghrelin-like compound: the term "ghrelin-like compound" as used herein refers to any compound which mimics the function of wild-type ghrelin, such as further described in the section below entitled "Functionality", for example wild-type human ghrelin. The ghrelin-like compound may be defined by the formula I:
- $$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2, \text{ wherein}$$

Z¹ is an optionally present protecting group

5 each X¹ is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X² is any amino acid selected from naturally occurring and synthetic occurring amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

10

each X³ is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

15 wherein one or more of X¹ and X³ optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

Z² is an optionally present protecting group,

20 m is an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

Furthermore, a ghrelin-like compound has a functionality leading to the desired therapeutic effects described herein.

25

GHS: growth hormone secretagogue

GHS-R 1a: the receptor for GHS. GHS-R 1a is also denoted GHS 1a, or the ghrelin receptor 1a.

30

Individual: A living animal or human. In preferred embodiments, the subject is a mammal, including humans and non-human mammals such as dogs, cats, pigs, cows, sheep, goats, horses, rats, and mice. In the most preferred embodiment, the subject is a human.

35

Isolated: is used to describe the various ghrelin-like compounds, polypeptides and nucleotides disclosed herein, that have been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified.

Lean body mass and "increased or maintained lean body mass":

Lean body mass is defined herein as body weight minus body fat; primarily muscle, bone and other non-fat tissue. A number of methods may be used for calculating an individual's percentage lean body mass:

1) Body mass index (BMI) calculated as body weight (kg) divided by the square of height (m) is used to describe the severity of obesity. BMI correlates reasonably well with fat mass and using age- and sex- specific prediction equations, the relative fat mass can be predicted with an error of ~5% in most subjects (Gallagher,D., et al., 1996 Am J Epidemiol 143:228-239).

2) Waist-to-hip ratio (WHR): in the mid-eighties WHR was shown to be particularly well correlated to increased visceral adipose tissue as measured by computed tomography (CT) or magnetic resonance (MRI) waist scans ($r=0.88$, $P<0.001$) (Seidell,J.C., et al., 1989, INT.J.OBES. 13:289-303; Ashwell,M., et al., 1985,. Br Med J (Clin Res Ed) 290:1692-1694). Furthermore, WHR has been tightly correlated to total body fat as assessed by CT ($r=0.97$, $p<0.0001$) (Despres,J.P., et al., 1991 American journal of clinical nutrition 54:471-477). It has since been used as a valuable surrogate measure of visceral adipose tissue. WHR is a useful measure to quantify either inter-individual differences or individual changes over time (Gallagher,D., et al., 1996 Am J Epidemiol 143:228-239).

3) Computed Tomography (CT) provides unique direct information on body composition and permits quantification of all major tissue system level components (adipose tissue, skeletal muscle, visceral organs and bone) (Hemmingsson,A et al., 1982, Acta Radiol 23:149-151). CT moreover has the advantage of permitting the partition of the total adipose tissue (AT) mass into its subcutaneous and visceral components (Tokunaga,K., et al., INT.J.OBES. 7:437-445). This leaves CT with a significant advantage for body composition studies because of its potential for monitoring changes in the visceral AT and subcutaneous AT compartments

separately, information, which is at present not available with any alternative in vivo technique except for MRI. CT scan for the assessment of body composition can be performed as either multi-slice covering the whole body and wherefrom calculations of whole body and regional AT can be derived. The CT method has been extensively validated. Animal and human cadaver studies have been used to verify the accuracy of the AT-estimate and its anatomical distribution showing a high correlation and therefore by some suggested as a "gold standard" against which to compare other non-invasive methods (Kvist, H., et al., 1988, INT.J.OBES. 12:249-266). At present, the most accurate in vivo methods of measuring body composition are multi-slice MRI and CT (Rossner,S., 1990, INT.J.OBES. 14:893-902). The highest precision is obtained using multi-slice CT scans (CV<1%) (Jensen,M.D., et al., 1995, American journal of clinical nutrition 61:274-278). However, due to radiation exposure, multi-slice CT is unsuitable for studies requiring repeated measurements on the same subject. A regional scan assessing the AT at the abdominal level is highly correlated with total AT mass in both men and women (r=0.92 and 0.97, respectively) (Despres,J.P., et al., American journal of clinical nutrition 54:471-477; Koester,R.S., et al., 1992, Int J Obes Relat Metab Disord 16:543-554; Lemieux,S., et al., 1993, American journal of clinical nutrition 58:463-467).

4) Magnetic Resonance Imaging (MRI) has the same imaging properties as CT, but does not require the use of radiation and therefore may be preferred to CT.

5) Bioelectric Impedance Analysis (BIA): In BIA an alternating current at one or more frequencies is introduced via electrodes and the body impedance (=resistance) to the electrical flow is measured. Body water poses the least impedance to the electric current, whereas body fat and bone provides the most (Heymsfield,S.B., et al., 1997, *Annu Rev Nutr* 17:527-558). BIA is inexpensive, easy to use and portable, and is therefore frequently used in combination with anthropometric measurements to predict body composition. BIA was reviewed by an expert panel in 1996, evaluating the method to provide a reliable estimate under most conditions and to be useful in healthy individuals and diseases where no major disturbances of water distribution are prominent.

6) Dual energy X-ray Absorptiometry ("DXA" or "DEXA"): DXA is a direct, operator independent, non-invasive method to estimate body composition. Measurements are based on the differential attenuation of two X-rays as they pass through the body. It distinguishes bone mineral from soft tissue and subsequently divides the

latter into FM and FFM (Pietrobelli, A., et al., 1996, *Am J Physiol* 271:E941-E951). The analyzed data yield information about composition of the whole body but also permits regional body composition determination. DXA has been widely validated and appear to be a precise (CV~1%(bone), CV~3%(LBM and FM) and simple way of measuring total and regional FM and LBM. Exposure to radiation is minimal (2-5 μ Sv) in most DXA machines.

In one embodiment of the invention described herein, it is preferred that the increase or maintenance of lean body mass is measured using DXA. In another preferred embodiment, said increase or maintenance of lean body mass is measured using MRI.

"Increasing lean body mass" and variations on this phrase can mean e.g. either increasing total lean body mass in an individual and/or increasing an individual's overall percentage lean body mass (e.g. as compared to total body mass), such as increasing an individual's percentage lean body mass by more than 0.5 %, such as more than 0.75%, such as more than 1%, for example more than 1.25%, such as more than 1.5%, for example more than 1.75%, such as more than 2%, for example more than 2.25%, such as more than 2.5%, for example more than 2.75%, such as more than 3%, for example more than 3.25%, such as more than 3.5%, for example more than 3.75%, such as more than 4%, for example more than 4.25%, such as more than 4.5%, for example more than 4.75%, such as more than 5%, for example more than 5.25%. In one embodiment it is preferred that the increase lean body mass is caused by an increase in muscle mass, as measured using for instance MRI or CT. In another preferred embodiment, said increase of lean body mass may be with respect to a control group of individuals not treated with the GH secretagogue. By "maintaining" lean body mass and grammatical variants thereof, is meant that said GH secretagogue acts to counteract loss of an individual's lean body mass, by preventing or reducing a decrease in total amount of lean body mass (as measured using for instance DEXA scans). In all cases, by "increase or maintenance of lean body mass" herein is meant that the increase or maintenance of lean body mass is caused by the beneficial effects of the secretagogue itself on the individual thus treated, instead of being caused by e.g. increased exercise or other factors not directly related to a GH secretagogue's metabolic effects.

Modified amino acid: an amino acid wherein an arbitrary group thereof is chemically modified. In particular, a modified amino acid chemically modified at the alpha - carbon atom in an alpha -amino acid is preferable.

- 5 Non-acylated ghrelin-like compound: a ghrelin like-compound as defined herein, which does not contain an acyl group attached to any of its constituent amino acids.

Organ transplantation patient: An individual which will undergo, is undergoing or has undergone an organ transplantation, such as a transplantation of the lung, liver,
10 kidney or heart. Accordingly, the term includes patients that will undergo organ transplantation, but are e.g. preparing for the transplantation or on a waiting list.

One group of patients in need of treatment are those having a lean body mass of less than 80% of normal, such as less than 60% of normal and/or a body mass
15 index below 17 kg/m².

The medicament is preferably given until the lean body mass is more than 60 % of normal, preferably more than 80% of normal, more preferably more than 90%
20 of normal.

Polypeptide: The phrase polypeptide refers to a molecule comprising amino acid residues which do not contain linkages other than amide linkages between adjacent amino acid residues.

25 Receptor: A receptor is a molecule, such as a protein, glycoprotein and the like, that can specifically (non-randomly) bind to another molecule.

Secretagogue: a GHS-R1a secretagogue, ie. a substance stimulating growth hormone release, such as ghrelin or a ghrelin-like compound. A secretagogue
30 according to the invention may for example be selected from the group of:

L-692-429, L-692-585 (Benzoelactam compounds)

MK677 (Spiroindaner)

G-7203, G-7039, G-7502 (Isonipectic acid peptidomimetic)

NN703, ipamorelin.

35 GHRP-1 (Bower)

	GHRP-2	(Bower)
	GHRP-6	(Bower)
	Hexarelin	(Europeptide)
	Ipamorelin	(Novo Nordisk)
5	NNC 26-0235	(Novo Nordisk)
	G-7039	(Genentech)
	G-7502	(Genentech)
	NNC26-0323	(Genentech)
	NN703	(Novo Nordisk)
10	NNC26-0722(NN)	(Novo Nordisk)
	L-692,429	(Merck)
	L-692,585	(Merck)
	NNC26-0610(NN)	(Novo Nordisk)
	MK-677	(Merck)
15	L-163,540	(Merck)
	CP-424,391	(Pfizer)
	EP51319	(Theratechnology)
	RC-1291	(Rejuvenon)
	BIM28125	(IPSEN)
20	BIM28131	(IPSEN)

In particular the secretagogue is a ghrelin-like compound, including 28 aa human ghrelin. The secretagogue may in one embodiment be non-acylated, for instance a non-acylated form of ghrelin or a non-acylated ghrelin-like compound. For example, another bulky group (such as e.g. a cholesterol or tryptophan moiety) replaces the usual acyl group on said non-acylated ghrelin-like compound.

Surfactant molecule: Molecule comprising a hydrophobic part and a hydrophilic part, i.e. molecule capable of being present in the interphase between a lipophilic phase and a hydrophilic phase.

Patients in need of treatment

The present invention is directed to the treatment of organ transplantation patients.

In one embodiment, the organ transplantation patient is a lung transplantation patient. In another embodiment, the organ transplantation patient is a kidney transplantation patient. In a further embodiment, the organ transplantation patient is a liver transplantation patient. In a further embodiment, the organ transplantation patient is a heart transplantation patient.

One preferred patient group is thus an organ transplantation patient undergoing, having undergone, or undergoing in the future, a solid organ transplant. Said solid organ may preferably be heart, lung, liver, kidney, pancreas, intestine or an extremity.

Another preferred patient group is an organ transplantation patient undergoing, having undergone, or undergoing in the future, a Hematopoietic Stem Cell Transplantation (autologous or allogeneic). Said stem cell transplantation may preferably be e.g. Bone marrow transplantation and/or Peripheral blood stem cell transplantation.

In another aspect of the present invention, the patient treated is a patient undergoing, having undergone, or undergoing in the future, a Major Elective or Acute Surgical Procedure, such as e.g. selected from the group consisting of the following:

- Cardiac surgery
- Lung surgery
- Gastrointestinal surgery
- Gynecological surgery
- Orthopedic surgery, including back surgery
- Reconstructive plastic surgery
- Neurosurgery

Thus, said surgery can be cardiac surgery or lung surgery. In another embodiment, said surgery can be Gynecological surgery. In another embodiment, said surgery can be Orthopedic surgery, such as back surgery. In another embodiment, said surgery can be reconstructive plastic surgery, such as reconstructive facial surgery

or reconstructive surgery after burns. In another embodiment, said surgery can be neurosurgery.

Stimulation of appetite, food intake, weight gain, increase of body fat mass

5 Facilitating a weight gain or facilitating maintenance of weight, in particular in individuals suffering from a pathological weight loss, is not only a matter of stimulating appetite and/or food intake but rather correcting the imbalance between energy intake and energy consumption, i.e. total body metabolism.

10 However, some individuals will still benefit from stimulation of appetite, particularly those individuals for whom a pathological process has led to a lowered appetite, which will naturally lead to an unhealthy weight loss. Thus, in one aspect the present invention relates to the stimulation of appetite by administering a GH-secretagogue, such as a ghrelin-like compound. The stimulation of appetite may be measured
15 using for instance a visual analog scale for measuring appetite, feeling of hunger or satiety level as described in e.g. Example 8 of published patent application WO 2005/014032 (Gastrotech Pharma: "Use of secretagogues like ghrelin in cancer cachexia and for stimulating appetite"). In a preferred embodiment of the invention, the stimulation is at least 5% compared to prior to the treatment (i.e. total appetite at
20 least 105% of appetite prior to treatment), such as 10% higher, more preferably 20% higher or even more preferably 30%, 40% or 50% higher

Stimulation of appetite does not necessarily lead to an increase in food intake, and accordingly, the present invention further relates to another aspect: the stimulation
25 of food intake by administering a GH-secretagogue, such as a ghrelin-like compound. The food intake can be measured using a multitude of techniques including self-reporting using e.g. diaries or questionnaires, measurements of calorie-intake from a buffet meal, using weighing of food prior to ingestion, or weighing and analysis of paired quantities of food. The food intake may be
30 measured on a meal basis, a daily basis, a weekly basis or a monthly basis. In a preferred embodiment of the invention, the treatment results in at least a 1% increase in food intake, such as an increase of 2%, more preferably at least 3%, or at least 5% or at least 7%, and even more preferred 10% above average food intake prior to initiation of treatment. In another embodiment, the treatment leads to
35 increase in calorie intake irrespective of changes in food intake, since amount of

food ingested may not be directly related to the ingested calorie intake, as the various food items such as fat, carbohydrates and proteins, contain different amounts of calories per amount food. In a preferred embodiment of the invention, the treatment results in at least a 1% increase in calorie intake, such as an increase
5 of at least 2%, more preferably at least 3%, or at least 5% or at least 7%, and even more preferred at least 10% in calorie intake.

In a third aspect the present invention relates to stimulation of weight gain, reducing a weight loss or maintaining a stable body-weight by administering a ghrelin-like
10 compound to an organ transplant patient. Thus, preferably, the GH-secretagogue, such as a ghrelin-like compound, is useful for stimulating food intake and weight gain, more preferably the secretagogue, such as a ghrelin-like compound, is useful for stimulating weight gain, for reducing a weight loss or for maintaining stable body weight.

15 As discussed below it is preferred that the GH-secretagogue, such as a ghrelin-like compound, is administered prior to a meal, such as within 180 minutes prior to a meal, such as within 150 minutes prior to a meal, for example within 120 minutes prior to a meal, such as within 100 minutes prior to a meal, for example within 80
20 minutes prior to a meal, such as within 60 minutes prior to a meal, for example within 45 minutes prior to a meal, such as within 30 minutes prior to a meal, for example within 15 minutes prior to a meal.

In particular the present invention is useful for treatment of under weight subjects, or
25 for preventing loss of weight to a stage of under weight. Under weight subjects include those having a body weight about 3% or less, 5% or less, 10% or less, 20% or less, or 30% or less, than the lower end of "normal" weight range or Body Mass Index ("BMI"). "Normal" weight ranges are well known in the art and take into account factors such as a patient age, height, and body type. Furthermore, the
30 invention is suitable for treating patients who have experienced an involuntary weight-loss prior to commencement of treatment, such as a weight-loss of 1% or less per month, 2% or less per month, or 5% or less per 6 months.

An increase in the body fat mass of an individual can be readily assessed by the
35 skilled person using a number of state of the art techniques. In one embodiment the

invention relates to an increase in body fat mass without the individual gaining weight overall. A preferred embodiment of the invention leads to an increase in body fat of at least 1%, such as at least 2% compared to prior to the initiation of treatment, more preferably at least 4%, such as at least 5%, and at least 8% and at
5 least 10 %, even more preferably at least 20% or at least 40% above pre-treatment values.

In another preferred embodiment the invention leads to an increase in lean body mass of at least 1%, such as at least 2% compared to prior to the initiation of
10 treatment, more preferably at least 3%, such as at least 4%, such as at least 5%, such as at least 6%, such as at least 7%, such as at least 8% or at least 10 %, or least 15% above pre-treatment values.

It is furthermore envisaged that administration of a GH secretagogue may be used
15 as part of the "supportive care regimen" for treatment of a patient in need thereof, e.g. to encourage intake of food and/or counteract any metabolic changes caused by the patient's state and/or therapy, and/or increase the patients functional status and/or increase the patient's life quality. Such patients in need thereof include, but are not restricted to, transplant patients either before and/or after transplant. In one
20 preferred embodiment, said GH secretagogue is administered as supportive care to optimise nutritional status in a patient, more preferably acting to increase or maintain lean body mass. In one embodiment, the administration of a GH secretagogue as part of a supportive care regimen may be started up to a month before, such as two weeks before, for example a week before, the primary treatment (such as the
25 transplant operation) is due to start, with the GH secretagogue being administered e.g. 1-2 times daily.

As part of the above-mentioned supportive care regime to increase an individual's nutritional status, it is envisaged that GH secretagogue administration may
30 preferably act to counteract loss of lean body mass, such as acting to increase the individual's lean body mass, and/or increasing the individual's percentage lean body mass.

Quality of Life

In all embodiments of the present invention, it is preferred that the treatment method and/or pharmaceutical compositions and/or compounds of the present invention are capable of affording the individual thus treated an improved quality of life (QOL), for example as is caused by improved appetite and/or body weight and/or nutritional status. Thus, in one aspect the invention relates to improvements of Quality of Life using a secretagogue, such as ghrelin or a ghrelin-like compound as described herein. In another embodiment, said improvement in an individual's life quality is assessed using a "Quality of life" questionnaire, as is known to one skilled in the art.

Two validated quality of life surveys preferred for use in assessing improved quality of life as caused by the administration of the compounds of the present invention are as follows:

(i) *Medical Outcomes Study Short-Form Health Survey (SF-36)*. The SF-36 contains 36 questions that assess eight aspects of the patients' QOL; physical functioning (PF), role-physical functioning (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional functioning (RE), and mental health (MH). According to the manual and interpretation guide responses to questions within scales are summed and linearly transformed to scale scores that range from 0, representing poor health status, to 100, representing optimal health status. The Swedish version has been validated and normative data have been presented for the general Swedish population (Sullivan MKJ, Ware J. Hälsoenkät: svensk manual och tolkningsguide (SF-36 Health Survey. Swedish manual and interpretation guide). Göteborg: Sahlgrenska University Hospital; 1994.)

(ii) *EORTC QLQ-C30 (+3) questionnaire*. The EORTC QLQ-C30 (version 1.0) is a 30 item core questionnaire intended for assessment of QOL among patients, the instrument is developed by the EORTC Quality of Life Study group. The first version has been validated in cancer patients and reference data from general populations have been published. The questionnaire comprises five functional scales; physical functioning (five questions), role functioning (two questions), emotional functioning (four questions), cognitive functioning (two questions) and social functioning (two questions). There are three symptom scales; fatigue (three questions), nausea and vomiting (two questions) and pain (two questions), and there are six single items on dyspnoea, insomnia, loss of appetite, constipation, diarrhea and financial difficulties.

Two global questions are asking about the patient's health status and overall QOL. All scales and single-items measures range in score from 0 to 100. A high score for the functioning scales and the global health status and QOL represents a high level of functioning / health status and QOL. A high score for the symptom / item scales represents a high level of symptoms / problems. The QOL scores can be calculated according to the EORTC QLQ-C 30 scoring manual.

Preferred questionnaires for assessing a patient's improved quality of life after treatment with one or more secretagogue compounds are given in Example 8 of published patent application WO 2005/014032 (Gastrotech Pharma: "Use of secretagogues like ghrelin in cancer cachexia and for stimulating appetite"): Examples of questionnaires assessing patient quality of life:

- A) EORTC QLQ-C30
- B) Taste questionnaire
- C) Hunger-Appetite-Feeling of Supersaturation-Nausea-Anxiety-Tiredness – Visual analogue scale

In preferred embodiments of the present invention, treatment of patients with the described conditions results in a significant improvement in the patients quality of life. Preferably, the treatment results in a significant increase in quality of life as measured using any method for testing the quality of life including, but not limited to, the above mentioned questionnaires, e.g. an increase in the quality of life score(s), or a composite quality of life score, as appropriate for the individual measuring tool, or a decrease in score(s) related to the symptoms and/or problems, respectively. This increase or decrease, respectively, is preferably 1% above the score obtained prior to initiation of the treatment, more preferably 2% above, even more preferred 5%, such as 10%, even more preferred 20%, 50% or 75% above the pre-treatment score. In another embodiment, the treatment results in measurable increases in quality of life score such that the score after treatment is equal to the average score found in a comparable healthy subject pool, or close to such a "normal" score, i.e. more than 50% of the score, even more preferably 60% of the score, or more preferably 75% of the score. Further, in another embodiment, the treatment results in a decrease in the score(s) related to the symptoms and/or problems of at least 1%, more preferably 3%, even more preferably 5% or more preferred 10%, 20%, 30% or 50% of the score(s) prior to initiation of treatment. These increases or

reductions, respectively, may refer to one, several, or all of the aspects of the individual quality of life measuring tool, or a composite score when appropriate.

Ghrelin-like compound

5 Any secretagogue, such as ghrelin or a ghrelin-like compound, may be used in the present invention. One preferred type of ghrelin-like compound according to the invention described herein is a compound comprising a structure defined by formula I:

10 Formula I: $Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$, wherein

Z^1 is an optionally present protecting group

15 each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic occurring amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

20

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

25 wherein one or more of X^1 and X^3 optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

Z^2 is an optionally present protecting group,

m is an integer in the range of from 1-10

30

n is 0 or an integer in the range of from 1-35.

35 Accordingly, the term "secretagogue" includes the naturally occurring 28 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 1, as well as the naturally occurring 27 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 2.

Thus, the present invention relates to the use of ghrelin or a peptide homologous thereto. Ghrelin is described by Kojima in Nature (1999), vol. 402,656-660.

5 The present invention includes diastereomers as well as their racemic and resolved enantiomerically pure forms. Secretagogues can contain D-amino acids, L-amino acids, alpha-amino acid, beta-amino acid, gamma-amino acid, natural amino acid and synthetic amino acid or the like or a combination thereof. Preferably, amino acids present in a ghrelin-like compound are the L-enantiomer.

10 The ghrelin-like compound preferably comprises an amino acid modified with a bulky hydrophobic group. The number of amino acids N-terminally to the modified amino acid is preferably within the range of from 1-9. Accordingly, m is preferably an integer in the range of from 1-9, such as of from 1-8, such as of from 1-7, such as of from 1-6, such as of from 1-5, such as of from 1-4, such as of from 1-3, such as of
15 from 1-2, such as 2.

It is more preferred that the number of amino acids N-terminally to the modified amino acid is low, such as of from 1-3, such as of from 1-2. Most preferably 2 amino acids are positioned N-terminal to the modified amino acid.

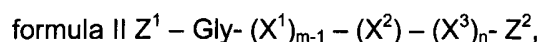
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In a preferred embodiment $(X^1)_m$ has a Gly residue in the N-terminal part of the sequence. Accordingly, in preferred embodiment $(X^1)_m$ is selected from the sequences:

25 Gly, Gly-Ser, Gly-Cys, Gly-Lys, Gly-Asp, Gly-Glu, Gly-Arg, Gly-His, Gly-Asn, Gly-Gln, Gly-Thr, and Gly-Tyr.

More preferably $(X^1)_m$ is Gly-Ser or Gly-Cys, most preferably Gly-Ser.

30 In other words, in a preferred embodiment the ghrelin-like compound is selected from a compound of



35 formula III $Z^1 - \text{Gly} - \text{Ser} - (X^2) - (X^3)_n - Z^2$, and

formula IV $Z^1 - \text{Gly} - (X^2) - (X^3)_n - Z^2$.

And more preferably the ghrelin-like compound has formula III.

5

As described above, X^2 may be any amino acid modified with a bulky hydrophobic group. In particular X^2 is selected from the group of modified Ser, Cys, Asp, Lys, Trp, Phe, Ile, and Leu. More preferably X^2 is selected from the group of modified Ser, modified Cys and modified Lys, and most preferably X^2 is modified Ser.

10

Furthermore, $(X^1)_m - (X^2)$ is preferably Gly-Xaa-Ser*, or Gly-Xaa-Cys*, wherein Xaa is any amino acid, more preferably $(X^1)_m - (X^2)$ is Gly-Ser-Ser*, or Gly-Ser-Cys*, wherein * indicates that the amino acid residue is modified with a bulky hydrophobic group.

15

$(X^3)_n$ preferably comprises a sequences which is a fragment of ghrelin, such as human ghrelin. Accordingly, $(X^3)_n$ preferably comprises a sequence selected from one or more of the sequences shown below:

20

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

Phe Leu Ser Pro Glu

25

Phe Leu Ser Pro

Phe Leu Ser

30

Phe Leu

Phe

35

In a preferred embodiment the length of the ghrelin-like compound is substantially similar to the length of human ghrelin, i.e. 27 or 28 amino acids. Accordingly, n is

preferably an integer in the range of from 1-25, such as of from 1-24, such as from 1-15, such as of from 1-10, or such as of from 10-25, such as of from 10-24, such as of from 15-25, such as of from 15-24.

- 5 Most preferably a ghrelin-like compound includes the naturally occurring 28 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 1, as well as the naturally occurring 27 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 2.
- 10 $(X^3)_n$ may be selected from any fragment of ghrelin, such as human ghrelin, and accordingly, $(X^3)_n$ may be selected from one or more of the sequences shown below or a homologue thereof:

15 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

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln Pro

20 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu

25 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys

30 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro

35 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro

24

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys

5 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu

10 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln

15 Phe Leu Ser Pro Glu His Gln Arg Val Gln

Phe Leu Ser Pro Glu His Gln Arg Val

20 Phe Leu Ser Pro Glu His Gln Arg

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

25 Phe Leu Ser Pro Glu

Phe Leu Ser Pro

30 Phe Leu Ser

Phe Leu

Phe

35 Or selected from

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln Pro Arg

5 Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln Pro

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln

10

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu

15

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala

20

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro

25

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser

30

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg

35

26

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln

Phe Leu Ser Pro Glu His Gln Lys Val Gln

5 Phe Leu Ser Pro Glu His Gln Lys Val

Phe Leu Ser Pro Glu His Gln Lys

Or selected from

10

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln Pro Arg

15

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln ProPhe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln

20

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys LeuPhe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys

25

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro

30

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys

35

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu

5 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg

10 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln

Phe Leu Ser Pro Glu His Gln Arg Ala Gln

Phe Leu Ser Pro Glu His Gln Arg Ala

15 Or selected from

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln Pro Arg

20 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln Pro

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro

25 Ala Lys Leu Gln

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu

30 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala

35

28

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro

5 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser

10

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys

15 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln

Phe Leu Ser Pro Glu His Gln Lys Ala Gln

20

Phe Leu Ser Pro Glu His Gln Lys Ala

In another embodiment $(X^3)_n$ comprises or consists of a sequence selected from the sequences

25

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

30 Phe Leu Ser Pro Glu

Phe Leu Ser Pro

Phe Leu Ser

35

Phe Leu

Phe

5 Further suitable GH secretagogues for use in the present invention are disclosed in PCT patent application no. PCT/DK2004/000529, Danish patent application no. PA 200401875, and PCT applications with publication numbers WO0192292 (Merck and Co. Inc), WO0134593 (Novo Nordisk AS) and WO0107475 ("Novel peptides", Kangawa et al.); said documents all being incorporated herein by reference.

10

Methods for production of GH secretagogues are well known to those skilled in the art, for example in Example 2 of PCT patent application PCT/DK2004/000519 (Gastrotech Pharma), incorporated herein by reference.

15 **Functionality**

The GH secretagogues used herein, in particular ghrelin-like compounds, are active at the receptor for GHS as described above, i.e. the receptor GHS-R 1a. The compounds can bind to the receptor, and preferably, stimulate receptor activity.

20 The receptor activity can be measured using different techniques such as detecting a change in the intracellular conformation of the receptor, in the G-protein coupled activities, and/or in the intracellular messengers.

25 One simple measure of the ability of a ghrelin like compound to activate the ghrelin receptor is to measure its EC₅₀, i.e. the dose at which the compound is able to activate the signalling of the receptor to half of the maximal effect of the compound.

30 The receptor can either be expressed endogenously on primary cells cultures, for example pituitary cells, or heterologously expressed on cells transfected with the ghrelin receptor. Whole cell assays or assays using membranes prepared from either of these cell types can be used depending on the type of assay.

35 As the receptor is generally believed to be primarily coupled to the Gq signalling pathway, any suitable assay which monitor activity in the Gq/G11 signalling pathway can be used, for example:

- 1) an assay measuring the activation of Gq / G11 performed for example by measurement of GTPγS binding combined with, e.g., anti-G-α-q or -11 antibody precipitation in order to increase the signal to noise ratio. This assay may also detect coupling to other G-proteins than Gq/11.
 - 2) An assay which measure the activity of phospholipase C (PLC) one of the first down-stream effector molecules in the pathway, for example by measuring the accumulation of inositol phosphate which is one of the products of PLC.
 - 3) More down stream in the signalling cascade is the mobilization of calcium from the intracellular stores
 - 4) Further more down stream signalling molecules such as the activity of different kinds of MAP kinases (p38, jun, ect.), NF-κ-B translocation and CRE driven gene transcription may also be measured.
 - 5) Binding of fluorescently tagged arrestin to the activated ghrelin receptor
- Other examples of suitable protocols for use in determining secretagogue functionality are given in Example 5 of published patent application WO 2005/014032 (Gastrotech Pharma: "Use of secretagogues like ghrelin in cancer cachexia and for stimulating appetite"): *Functional tests on the ghrelin receptor*
- A ghrelin-like compound used in the invention preferably has at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, functional activity relative to 28 aa human ghrelin as determined using the assay described herein above, and/or an EC₅₀ greater than about 1,000 nM, greater than about 100 nM, or greater than about 50 nM, or greater than about 10 nM or greater than 1 nM. Greater refers to potency and thus indicates a lesser amount is needed to achieve binding inhibition.
- In one embodiment of the use of the invention, the compound has a potency (EC₅₀) on the GHS-R 1A of less than 500 nM. In another embodiment the compound has a potency (EC₅₀) on the GHS-R 1A of less than 100 nM, such as less than 80 nM, for

example less than 60 nM, such as less than 40 nM, for example less than 20 nM, such as less than 10 nM, for example less than 5 nM, such as less than 1 nM, for example less than 0.5 nM, such as less than 0.1 nM, for example less than 0.05 nM, such as less than 0.01 nM.

5

In a further embodiment the dissociation constant (K_d) of the compound is less than 500 nM. In a still further embodiment the dissociation constant (K_d) of the ligand is less than 100 nM, such as less than 80 nM, for example less than 60 nM, such as less than 40 nM, for example less than 20 nM, such as less than 10 nM, for example less than 5 nM, such as less than 1 nM, for example less than 0.5 nM, such as less than 0.1 nM, for example less than 0.05 nM, such as less than 0.01 nM.

10

Binding assays can be performed using recombinantly-produced receptor polypeptides present in different environments. Such environments include, for example, cell extracts and purified cell extracts containing the receptor polypeptide expressed from recombinant nucleic acid or naturally occurring nucleic acid; and also include, for example, the use of a purified GHS receptor polypeptide produced by recombinant means or from naturally occurring nucleic acid which is introduced into a different environment.

15

20

Using a recombinantly expressed GHS receptor offers several advantages such as the ability to express the receptor in a defined cell system, so that a response to a compound at the receptor can more readily be differentiated from responses at other receptors. For example, the receptor can be expressed in a cell line such as HEK 293, COS 7, and CHO not normally expressing the receptor by an expression vector, wherein the same cell line without the expression vector can act as a control.

25

Functionality of a GH secretagogue may be demonstrated in mammals using e.g. the test for the absolute bioavailability of iv administered Ghrelin and sc administered Ghrelin described in Example 3 of PCT patent application PCT/DK2004/000519 (Gastrotech Pharma), or the method in Example 6 "Efficacy of subcutaneous administration of Ghrelin" from PCT patent application PCT/DK2004/000519 (Gastrotech Pharma), or the trial described in Example 11 "Administering ghrelin to cancer cachexia patients" from PCT patent application

30

PCT/DK2004/000519 (Gastrotech Pharma). All these Examples are hereby incorporated herein by reference

Identity and homology

5 The term "identity" or "homology" shall be 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
10 sequence identity. Neither N- or C-terminal extensions nor insertions shall 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
15 Ave., Madison, Wis. 53705). This software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

A ghrelin homologue of one or more of the sequences specified herein may vary in one or more amino acids as compared to the sequences defined, but is capable of
20 performing the same function, i.e. a homologue may be envisaged as a functional equivalent of a predetermined sequence. A ghrelin homologue is preferably a ghrelin-like compound as defined above.

As described above a homologue of any of the predetermined sequences herein
25 may be defined as:

- i) homologues comprising an amino acid sequence capable of being recognised by an antibody, said antibody also recognising the 28 aa human ghrelin, preferably the acylated 28 aa human ghrelin, and/or
30
- ii) homologues comprising an amino acid sequence capable of binding selectively to GHS-R 1a, and/or

- iii) homologues having a substantially similar or higher binding affinity to GHS-R 1a than the 28 aa human ghrelin, preferably the acylated 28 aa human ghrelin.

5 In the above examples, the 28 aa human ghrelin has the sequence shown in SEQ ID NO: 1, and when acylated is acylated in position 3.

The antibodies used herein may be antibodies binding the N-terminal part of ghrelin or the C-terminal part of ghrelin, preferably the N-terminal part of ghrelin. The
10 antibodies may be antibodies as described in Ariyasu et al. "Delayed short-term secretory regulation of ghrelin in obese animals: Evidenced by a specific RIA for the active form of ghrelin, *Endocrinology* 143(9):3341-3350, 2002.

Examples of homologues comprises one or more conservative amino acid
15 substitutions including one or more conservative amino acid substitutions within the same group of predetermined amino acids, or a plurality of conservative amino acid substitutions, wherein each conservative substitution is generated by substitution within a different group of predetermined amino acids.

20 Homologues may thus comprise conservative substitutions independently of one another, wherein at least one glycine (Gly) of said homologue is substituted with an amino acid selected from the group of amino acids consisting of Ala, Val, Leu, and Ile, and independently thereof, homologues, wherein at least one of said alanines (Ala) of said homologue thereof is substituted with an amino acid selected from the
25 group of amino acids consisting of Gly, Val, Leu, and Ile, and independently thereof, homologues, wherein at least one valine (Val) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Leu, and Ile, and independently thereof, homologues thereof, wherein at least one of said leucines (Leu) of said homologue thereof is substituted with an
30 amino acid selected from the group of amino acids consisting of Gly, Ala, Val, and Ile, and independently thereof, homologues thereof, wherein at least one isoleucine (Ile) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val and Leu, and independently thereof, homologues thereof wherein at least one of said aspartic acids (Asp) of said
35 homologue thereof is substituted with an amino acid selected from the group of

amino acids consisting of Glu, Asn, and Gln, and independently thereof, homologues thereof, wherein at least one of said phenylalanines (Phe) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Tyr, Trp, His, Pro, and preferably selected from the group of amino acids consisting of Tyr and Trp, and independently thereof, homologues thereof, wherein at least one of said tyrosines (Tyr) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Trp, His, Pro, preferably an amino acid selected from the group of amino acids consisting of Phe and Trp, and independently thereof, homologues thereof, wherein at least one of said arginines (Arg) of said fragment is substituted with an amino acid selected from the group of amino acids consisting of Lys and His, and independently thereof, homologues thereof, wherein at least one lysine (Lys) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Arg and His, and independently thereof, homologues thereof, wherein at least one of said asparagines (Asn) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Gln, and independently thereof, homologues thereof, wherein at least one glutamine (Gln) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Asn, and independently thereof, homologues thereof, wherein at least one proline (Pro) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Tyr, Trp, and His, and independently thereof, homologues thereof, wherein at least one of said cysteines (Cys) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, and Tyr.

Conservative substitutions may be introduced in any position of a preferred predetermined sequence. It may however also be desirable to introduce non-conservative substitutions, particularly, but not limited to, a non-conservative substitution in any one or more positions.

A non-conservative substitution leading to the formation of a functionally equivalent homologue of the sequences herein would for example i) differ substantially in polarity, for example a residue with a non-polar side chain (Ala, Leu, Pro, Trp, Val, Ile, Leu, Phe or Met) substituted for a residue with a polar side chain such as Gly,

Ser, Thr, Cys, Tyr, Asn, or Gln or a charged amino acid such as Asp, Glu, Arg, or Lys, or substituting a charged or a polar residue for a non-polar one; and/or ii) differ substantially in its effect on polypeptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

10

Substitution of amino acids may in one embodiment be made based upon their hydrophobicity and hydrophilicity values and the relative similarity of the amino acid side-chain substituents, including charge, size, and the like. Exemplary amino acid substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

15

In a preferred embodiment the binding domain comprises a homologue having an amino acid sequence at least 60 % homologous to SEQ ID NO 1.

20

More preferably the homology is at least 65 %, such as at least 70 % homologous, such as at least 75 % homologous, such as at least 80 % homologous, such as at least 85 % homologous, such as at least 90 % homologous, such as at least 95 % homologous, such as at least 98 % homologous to SEQ ID NO:1.

25

In a more preferred embodiment the percentages mentioned above relates to the identity of the sequence of a homologue as compared to SEQ ID NO:1.

Homologues to SEQ ID NO:1 may be 27 aa human ghrelin SEQ ID NO: 2, or rat ghrelin SEQ ID NO:3. Other homologues are the variants described in EP 1197496 (Kangawa) and WO 01/92292 (Merck) and WO 01/56592 (Novo Nordisk) incorporated herein by reference.

30

Bulky hydrophobic group

The bulky hydrophobic group of the secretagogue according to the invention is any bulky hydrophobic group capable of providing the des-acylated 28 aa human ghrelin, or an analogue thereof, with binding affinity to GHS-R 1a. Any suitable amino acid may be modified with any suitable bulky hydrophobic group; in a preferred embodiment, a Ser residue (preferably amino acid number 3 in the amino acid chain) is modified with the bulky hydrophobic group.

When the amino acid being modified contains e.g. -OH, -SH, -NH or -NH₂ as a substituent group in a side chain thereof, a group formed by acylating such a substituent group is preferred. The mode of linkage may thus be selected from the group consisting of ester, ether, thioester, thioether, amide and carbamide.

For example, if the modified amino acid is serine, threonine, tyrosine or oxyproline, the amino acid has a hydroxyl group in the side chain. If the modified amino acid is cysteine, the amino acid has a mercapto group in the side chain. If the modified amino acid is lysine, arginine, histidine, tryptophan, proline or oxyproline, it has an amino group or imino group in the side chain.

The hydroxyl group, mercapto group, amino group and imino group described above may thus have been chemically modified. That is, the hydroxyl group or mercapto group may be etherized, esterified, thioetherified or thioesterified. The imino group may have been iminoetherified, iminothioetherified or alkylated. The amino group may have been amidated, thioamidated or carbamidated.

Further, the mercapto group may have been disulfidated, the imino group may have been amidated or thioamidated, and the amino group may have been alkylated or thiocarbamidated.

In a preferred embodiment the modified amino acid is Ser coupled through an ester linkage to the hydrophobic group.

The hydrophobic group may be any group with a saturated or unsaturated alkyl or acyl group containing one or more carbon atoms. In one embodiment the bulky hydrophobic group is an acyl group, including groups formed by removing a hydroxyl

group from an organic carboxylic acid, organic sulfonic acid or organic phosphoric acid. The organic carboxylic acid includes e.g. fatty acids, and the number of carbon atoms thereof is preferably 1 to 35. In the organic sulfonic acid or organic phosphoric acid, the number of carbon atoms thereof is preferably 1 to 35.

5

Accordingly, the acyl group is preferably selected from a C1-C35 acyl group, such as a C1 – C20 acyl group, such as a C1 – C15 acyl group, such as a C6 – C15 acyl group, such as a C6 – C12 acyl group, such as a C8 – C12 acyl group.

10

More preferably the acyl group is selected from the group of C7 acyl group, C8 acyl group, C9 acyl group, C10 acyl group, C11 acyl group, and C12 acyl group. Such acyl group may be formed from octanoic acid (preferably caprylic acid), decanoic acid (preferably capric acid), or dodecanoic acid (preferably lauric acid), as well as monoene or polyene fatty acids thereof.

15

In one embodiment the acyl group is selected from the group of C8 acyl group, and C10 acyl group. Such acyl groups may be formed from octanoic acid (preferably caprylic acid), or decanoic acid (preferably capric acid).

20

In another embodiment the acyl group is selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.

25

Further preferred bulky hydrophobic groups are disclosed in PCT patent application PCT/DK2004/000519 (Gastrotech Pharma) and Danish patent application no. PA 200401875, both incorporated herein by reference.

30

Furthermore, the modified amino acid may be any amino acid wherein a group is modified as described in EP 1 197 496 (Kangawa), which is hereby incorporated by reference.

Protecting group

The ghrelin-like compound according to the invention may comprise a protecting group at the N-terminus or the C-terminus or at both.

35

A protecting group covalently joined to the N-terminal amino group reduces the reactivity of the amino terminus under in vivo conditions. Amino protecting groups include - C1-10 alkyl, -C1-10 substituted alkyl, -C2-10 alkenyl, -C2-10 substituted alkenyl, aryl, -C1-6 alkyl aryl, -C(O)- (CH₂)¹⁻⁶-COOH, -C(O)-C1-6 alkyl, -C(O)-aryl, -C (O)-O-C1-6 alkyl, or-C (O)-O-aryl. Preferably, the amino terminus protecting group is acetyl, propyl, succinyl, benzyl, benzyloxycarbonyl or tbutyloxycarbonyl.

A protecting group covalently joined to the C-terminal carboxy group reduces the reactivity of the carboxy terminus under in vivo conditions. The carboxy terminus protecting group is preferably attached to the α -carbonyl group of the last amino acid. Carboxy terminus protecting groups include amide, methylamide, and ethylamide.

Conjugates

The secretagogue, such as a ghrelin-like compound, to be used in the present invention may be provided in the form of a secretagogue conjugate, i.e. a molecule comprising the secretagogue conjugated to another entity.

The other entity may be any substance that is capable of conferring improved properties to the secretagogue, e.g. in terms of improved stability, half-life, etc. Examples of suitable entities are described in PCT patent application PCT/DK2004/000519 (Gastrotech Pharma) and Danish patent application no. PA 200401875, both incorporated herein by reference

Methods for production

GH-secretagogues, such as ghrelin-like compounds, can be produced using techniques well known in the art. For example, a polypeptide region of a ghrelin-like compound can be chemically or biochemically synthesized and modified. Techniques for chemical synthesis of polypeptides are well known in the art. (See e.g., Vincent in Peptide and Protein Drug Delivery, New York, N. Y., Dekker, 1990.). Examples of techniques for biochemical synthesis involving the introduction of a nucleic acid into a cell and expression of nucleic acids are provided in Ausubel, Current Protocols in Molecular Biology, John Wiley, 1987-1998, and Sambrook et al., in Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989. Other methods for production of GH-secretagogues are

disclosed in Example 2 of PCT/DK2004/000519 (Gastrotech Pharma), incorporated herein by reference thereto.

Pharmaceutical compositions

5

While it is possible for a GH secretagogue or salt thereof to be administered as the raw chemical, it is preferred to present it in the form of a pharmaceutical composition. Accordingly, in one aspect the present invention relates to a pharmaceutical composition comprising a GH secretagogue (or pharmaceutically acceptable salt thereof) suitable for use in the present invention. The pharmaceutical composition preferably comprises a pharmaceutically acceptable carrier, vehicle and/or excipient. The carrier, vehicle and/or excipient should be compatible with the GH secretagogue or salt thereof. In a preferred embodiment, the pharmaceutical composition is not immunogenic when administered to a human in accordance with the present invention.

15

As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a human without the production of undesirable physiological effects such as nausea, dizziness, gastric upset and the like.

20

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art. Typically such compositions are prepared as sterile injectables either as liquid solutions or suspensions, aqueous or non-aqueous, however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified.

25

Suitable pharmaceutical carriers include sterile aqueous solution and various organic solvents and inert solid diluents or fillers. Examples of solid carriers are lactose, terra alba, sucrose, cyclodextrin, talc, gelatine, agar, pectin, acacia, magnesium stearate, stearic acid or lower alkyl ethers of cellulose. Examples of liquid carriers are syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid

30

amines, polyoxyethylene or water. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof.

5 In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient. It is preferred that the formulation has a pH within the range of 3.5-8, such as in the range 4.5-7.5, such as in the range 5.5-7, such as in the range 6-7.5, most preferably around 7.3. However, as is understood by one skilled in the art, the pH range may be adjusted according to the individual treated and the administration procedure. For example, some GH secretagogues may be easily stabilised at a lower pH, so in another preferred embodiment of the the invention the formulation has a pH within the range 3.5-7, such as 4-6, such as 5-6, such as 5.3-5.7, such as 5.5.

15 Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, organic esters such as ethyl oleate, and water-oil emulsions.

20 The pharmaceutical composition can include a pharmaceutically acceptable salt of the GH secretagogue therein. The salt will be one which is acceptable in its therapeutic use. By that it is meant that the salt will retain the biological activity of the GH secretagogue and the salt will not have untoward or deleterious effects in its application and use in treating diseases.

25 Pharmaceutically acceptable salts are prepared in a standard manner. If the GH secretagogue is a base it is treated with an excess of an organic or inorganic acid in a suitable solvent. If the GH secretagogue is an acid, it is treated with an inorganic or organic base in a suitable solvent.

30 The pharmaceutically acceptable salt may be an acid addition salts including salts of inorganic acids as well as organic acids. Acid addition salts are formed with free amino groups of the GH secretagogue. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydriodic, metaphosphoric, phosphoric, sulphuric and nitric acids and the like. Representative examples of

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suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, glycolic, lactic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methanesulfonic, ethanesulfonic, tartaric, ascorbic, pantoic, bismethylene salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, ethylenediaminetetraacetic (EDTA), p-aminobenzoic, glutamic, benzenesulfonic and p-toluenesulfonic acids and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutical acceptable salts listed in J. Pharm. Sci. 1977,66,2, which is incorporated herein by reference. The metal salt may be an alkali metal or earth alkali metal salt. Examples of metal salts include lithium, sodium, potassium and magnesium salts and the like. Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium and tetramethylammonium salts and the like.

Salts formed with the free carboxyl groups can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

Also included within the scope of pharmaceutical acceptable acid addition salts of a GH secretagogue is any hydrate (hydrated form) thereof.

In a preferred embodiment of the invention the composition comprises a GH secretagogue or a salt thereof, as a lyophilisate and a solvent, said lyophilisate and said solvent being in separate compartments until administration. In another embodiment the composition is a solution of the GH secretagogue or a salt thereof. In both embodiments the solvent may be any suitable solvent, such as described herein, and preferably the solvent is saline.

The invention also relates to a method for preparing a medicament or pharmaceutical composition comprising a compound for use in accordance with the invention, comprising admixing at least one GH secretagogue or a salt thereof with a physiologically acceptable carrier.

The pharmaceutical composition comprising at least one GH secretagogue, or a pharmaceutically acceptable salt thereof, may comprise one GH secretagogue species, e.g. wildtype human ghrelin or a salt or hydrate thereof. Alternatively, the pharmaceutical composition comprises at least two different GH secretagogues or pharmaceutically acceptable salt(s) thereof, of which one e.g. is wildtype human ghrelin. The difference may for example be compounds having different anchor groups. For example, the pharmaceutical composition may comprise both wild-type human ghrelin and L-692-429, produced by Merck.

10 In another embodiment, the pharmaceutical composition comprises at least one acylated GH secretagogue, or a pharmaceutically acceptable salt thereof, in combination with a desacylated Ghrelin-like compound, or a pharmaceutically acceptable salt thereof, such as any of the desacylated ghrelin-like compounds described in WO03051389 (Theratechnologies: "Pharmaceutical compositions comprising unacylated ghrelin and therapeutical uses thereof"), incorporated herein by reference.

In one particular embodiment the invention relates to the use of a pharmaceutical composition comprising a mixture of at least two different GH-secretagogues (such as e.g. two different ghrelin-like compounds), such as a mixture of a ghrelin-like compound being acylated with a C8 acyl and a ghrelin-like compound being acylated with a C10 acyl. Without being bound by theory it is believed that such a mixture will have a longer half-life in plasma.

25 In yet another embodiment, the pharmaceutical composition used comprises one or more acylated GH secretagogue(s) (preferably ghrelin-like compound(s)), optionally compounds having different acyl chain lengths preferably selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group, further optionally in combination with a desacylated secretagogue, such as a desacylated Ghrelin-like compound.

In a preferred aspect of the invention the GH-secretagogue (such as a ghrelin-like compound) is administered with a substance capable of increasing the half-life of the GH-secretagogue, for example by incorporating the secretagogue into liposomes, micelles, iscoms, and/or microspheres or other transport molecules. This

is of particular interest when an amino acid residue of the ghrelin-like compound is modified with a bulky hydrophobic group and it is desirable to protect the modified amino acid from degradation.

5 Thus, in one aspect, the invention relates to the use of a pharmaceutical composition comprising any secretagogue, such as any ghrelin-like compound as defined above or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients said composition further comprising transport molecules. The transport molecules are primarily added in order to
10 increase the half-life of the acylated compound, preventing premature des-acylation, since the des-acylated ghrelin is not active at the GHS-R 1a. Transport molecules act by having incorporated into or anchored to it the compound according to the invention.

15 Any suitable transport molecules known to the skilled person may be used. Examples of transport molecules are those described in the conjugate section. Other preferred examples are liposomes, micelles, and/or microspheres.

Conventional liposomes are typically composed of phospholipids (neutral or
20 negatively charged) and/or cholesterol. The liposomes are vesicular structures based on lipid bilayers surrounding aqueous compartments. They can vary in their physiochemical properties such as size, lipid composition, surface charge and number and fluidity of the phospholipids bilayers. The most frequently used lipid for liposome formation are: 1,2-Dilauroyl-*sn*-Glycero-3-Phosphocholine (DLPC), 1,2-
25 Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC), 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine (DPPC), 1,2-Distearoyl-*sn*-Glycero-3-Phosphocholine (DSPC), 1,2-Dioleoyl-*sn*-Glycero-3-Phosphocholine (DOPC), 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphoethanolamine (DMPE), 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphoethanolamine (DPPE), 1,2-Dioleoyl-*sn*-Glycero-3-Phosphoethanolamine (DOPE), 1,2-Dimyristoyl-
30 *sn*-Glycero-3-Phosphate (Monosodium Salt) (DMPA), 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphate (Monosodium Salt) (DPPA), 1,2-Dioleoyl-*sn*-Glycero-3-Phosphate (Monosodium Salt) (DOPA), 1,2-Dimyristoyl-*sn*-Glycero-3-[Phospho-*rac*-(1-glycerol)] (Sodium Salt) (DMPG), 1,2-Dipalmitoyl-*sn*-Glycero-3-[Phospho-*rac*-(1-glycerol)] (Sodium Salt) (DPPG), 1,2-Dioleoyl-*sn*-Glycero-3-[Phospho-*rac*-(1-glycerol)]
35 (Sodium Salt) (DOPG), 1,2-Dimyristoyl-*sn*-Glycero-3-[Phospho-L-Serine] (Sodium

Salt) (DMPS), 1,2-Dipalmitoyl-*sn*-Glycerol-3-[Phospho-L-Serine] (Sodium Salt) (DPPS), 1,2-Dioleoyl-*sn*-Glycerol-3-[Phospho-L-Serine] (Sodium Salt) (DOPS), 1,2-Dioleoyl-*sn*-Glycerol-3-Phosphoethanolamine-N-(glutaryl) (Sodium Salt) and 1,1',2,2'-Tetramyristoyl Cardiolipin (Ammonium Salt). Formulations composed of DPPC in combination with other lipid or modifiers of liposomes are preferred e.g. in combination with cholesterol and/or phosphatidylcholine.

Long-circulating liposomes are characterized by their ability to extravasate at body sites where the permeability of the vascular wall is increased. The most popular way to produce long circulating liposomes is to attach hydrophilic polymer polyethylene glycol (PEG) covalently to the outer surface of the liposome. Some of the preferred lipids are: 1,2-Dipalmitoyl-*sn*-Glycerol-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (Ammonium Salt), 1,2-Dipalmitoyl-*sn*-Glycerol-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-5000] (Ammonium Salt), 1,2-Dioleoyl-3-Trimethylammonium-Propane (Chloride Salt) (DOTAP).

Possible lipid applicable for liposomes are supplied by Avanti, Polar lipids, Inc, Alabaster, AL. Additionally, the liposome suspension may include lipid-protective agents which protect lipids against free-radical and lipid-peroxidative damages on storage. Lipophilic free-radical quenchers, such as alpha-tocopherol and water-soluble iron-specific chelators, such as ferrioxanine, are preferred.

A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Pat. Nos. 4, 235,871, 4,501,728 and 4,837,028, all of which are incorporated herein by reference. One method produces multilamellar vesicles of heterogeneous sizes. In this method, the vesicle-forming lipids are dissolved in a suitable organic solvent or solvent system and dried under vacuum or an inert gas to form a thin lipid film. If desired, the film may be redissolved in a suitable solvent, such as tertiary butanol, and then lyophilized to form a more homogeneous lipid mixture which is in a more easily hydrated powder like form. This film is covered with an aqueous solution of the targeted drug and the targeting component and allowed to hydrate, typically over a 15-60 minute period with agitation. The size distribution of the resulting multilamellar vesicles can be shifted toward smaller sizes by hydrating the lipids under more vigorous agitation conditions or by adding solubilizing detergents such as

deoxycholate. Additionally, the liposome suspension may include lipid-protective agents which protect lipids against free-radical and lipid-peroxidative damages on storage. Lipophilic free-radical quenchers, such as alpha-tocopherol and water-soluble iron-specific chelators, such as ferrioxamine, are preferred.

5

Micelles are formed by surfactants (molecules that contain a hydrophobic portion and one or more ionic or otherwise strongly hydrophilic groups) in aqueous solution. As the concentration of a solid surfactant increases, its monolayers adsorbed at the air/water or glass/water interfaces become so tightly packed that further occupancy requires excessive compression of the surfactant molecules already in the two monolayers. Further increments in the amount of dissolved surfactant beyond that concentration cause amounts equivalent to the new molecules to aggregate into micelles. This process begins at a characteristic concentration called "critical micelle concentration".

15

Common surfactants well known to one of skill in the art can be used in the micelles of the present invention. Suitable surfactants include sodium laurate, sodium oleate, sodium lauryl sulfate, octaoxyethylene glycol monododecyl ether, octoxynol 9 and PLURONIC F-127 (Wyandotte Chemicals Corp.). Preferred surfactants are nonionic polyoxyethylene and polyoxypropylene detergents compatible with IV injection such as, TWEEN-80., PLURONIC F-68., n-octyl-beta-D-glucopyranoside, and the like. In addition, phospholipids, such as those described for use in the production of liposomes, may also be used for micelle formation.

25

One suitable formulation for preparing pharmaceutical compositions for use in the present invention is described in Example 9 of PCT patent application PCT/DK2004/000519 (Gastrotech Pharma). An example of how one skilled in the art may investigate the pharmacokinetics of different formulations is given in Example 10 of PCT patent application PCT/DK2004/000519 (Gastrotech Pharma). Both Examples are incorporated herein by reference.

30

In one preferred embodiment, the compounds of the present invention are formulated as described in the literature for an administration route selected from: buccal delivery, sublingual delivery, transdermal delivery, inhalation and needle-free injection, such as using the methods developed by Powderjet.

35

For inhalation, the compounds of the present invention can be formulated as using methods known to those skilled in the art, for example an aerosol, dry powder or solubilized such as in micro droplets, preferably in a device intended for such delivery (such as commercially available from Aradigm, Alkermes or Nektar).

Compositions for parenteral administration

The GH secretagogue or a salt thereof may be formulated for parenteral administration (e.g., by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. A pharmaceutical composition for parenteral administration may include sterile aqueous and non-aqueous injectable solutions, dispersions, suspensions or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol, as well as sterile powders to be reconstituted in sterile injectable solutions or dispersions prior to use.

The active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution for constitution before use with a suitable vehicle, e.g., sterile, pyrogen-free water. Aqueous solutions should be suitably buffered if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. The aqueous solutions are particularly suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. The sterile aqueous media employed are all readily available by standard techniques known to those skilled in the art.

Solutions of GH secretagogues or pharmaceutically acceptable salts thereof can be prepared in water or saline, and optionally mixed with a nontoxic surfactant. Compositions for intravenous or intra-arterial administration may include sterile aqueous solutions that may also contain buffers, liposomes, diluents and other suitable additives.

Examples of oily or nonaqueous carriers, diluents, solvents or vehicles for parental use include propylene glycol, polyethylene glycol, animal, synthetic or vegetable oils, and injectable organic esters, and may contain formulatory agents such as

preserving, wetting, emulsifying or suspending, stabilizing and/or dispersing agents. Specific examples of oils useful in such compositions include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral compositions include oleic acid, stearic acid, and isostearic acid.

5 Suitable organic esters include fatty acid esters such as ethyl oleate and isopropyl myristate.

10 Suitable soaps for use in parenteral compositions include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides; (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric
15 detergents such as, for example, alkyl-beta-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

20 The parenteral compositions typically will contain from about 0.5 to about 25% by weight of the active ingredient in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such compositions will typically range from about 5 to about 15% by weight. Suitable surfactants include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the
25 high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral compositions can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for
30 injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

35 The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions comprising the active ingredient that are

adapted for administration by encapsulation in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage.

- 5 Sterile injectable solutions are prepared by incorporating the compound(s) or pharmaceutically acceptable salt(s) thereof in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization.

10 **Compositions for oral delivery**

Those GH secretagogue types capable of remaining biologically active in an individual after oral administration (such as short peptides) can be formulated in a wide range of oral administration dosage forms. The pharmaceutical compositions
15 and dosage forms may comprise the compounds of the invention or its pharmaceutically acceptable salt or a crystal form thereof as the active component. The pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, and dispersible granules. A solid carrier can be one or more substances which may also act as
20 diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, wetting agents, tablet disintegrating agents, or an encapsulating material.

Preferably, the composition will be about 0.5% to 75% by weight of a compound or
25 compounds of the invention, with the remainder consisting of suitable pharmaceutical excipients. For oral administration, such excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like.

30 In powders, the carrier is a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain 1-70% of the
35 active compound. Suitable carriers are magnesium carbonate, magnesium stearate,

5 talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term "preparation" is intended to include the composition of the active compound with encapsulating material as carrier providing a capsule in which the active component, with or without carriers, is surrounded by a carrier, which is in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be as solid forms suitable for oral administration.

10 Drops can be used according to the present invention and may comprise sterile or non-sterile aqueous or oil solutions or suspensions, and may be prepared by dissolving the active ingredient in a suitable aqueous solution, optionally including a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be
15 clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100 °C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container aseptically. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride
20 (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Other forms suitable for oral administration include toothpaste, gel dentrifice or chewing gum. Emulsions may be prepared in solutions in aqueous propylene glycol solutions or may contain emulsifying agents such as lecithin, sorbitan monooleate, or acacia. Aqueous solutions can be prepared by dissolving the active
25 component in water and adding suitable colorants, flavors, stabilizing and thickening agents. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well known
30 suspending agents. Solid form preparations include solutions, suspensions, emulsions, syrups and elixirs and may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

Compositions for topical administration

It is contemplated that the compounds of the invention can be delivered topically. Regions for topical administration include the skin surface. Compositions for topical
5 administration via the skin and mucous membranes should not give rise to signs of irritation, such as swelling or redness.

The compounds described herein can be administered transdermally. Transdermal
10 administration typically involves the delivery of a pharmaceutical agent for percutaneous passage of the drug into the systemic circulation of the patient. The skin sites include anatomic regions for transdermally administering the drug and include the forearm, abdomen, chest, back, buttock, mastoidal area, and the like.

The GH secretagogues may be formulated for topical administration to the
15 epidermis as ointments, creamse, gels or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also containing one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents,
20 thickening agents, or coloring agents. Compositions suitable for topical administration in the mouth include lozenges comprising active agents in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

25

Compositions for aerosol, nasal or inhalation delivery

It is contemplated that the GH secretagogues may be formulated for administration
30 to the respiratory tract and including intranasal administration, and for nasal administration. The solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The compositions may be provided in a single or multidose form. In the latter case of a dropper or pipette this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may

be achieved for example by means of a metering atomizing spray pump. A suitable formulation for nasal administration is described in EP 1 466 610.

5 For inhalation, the compounds can be formulated as using methods known to those skilled in the art, for example an aerosol, dry powder or solubilized such as in microdroplets, preferably in a device intended for such delivery (such as commercially available from Aradigm, Alkermes or Nektar).

10 Compositions administered by aerosols 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 in accordance with methods known in the art.

Administration as suppositories

15 It is contemplated that the GH secretagogues to be used herein may also be formulated for administration as suppositories. A low melting wax, such as a mixture of fatty acid glycerides or cocoa butter is first melted and the active component is dispersed homogeneously, for example, by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and to solidify.

20 The active compound may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%]).

25

Compositions for other types of delivery

Other types of delivery of the compounds in accordance with the present invention are also foreseen, such as implants.

30 Subcutaneous administration

Any parenteral administration form that will ensure that the ghrelin receptors which normally are the target for peripherally produced ghrelin in the premeal situation will be exposed to sufficient levels of the bioactive form of a GH secretagogue or a salt thereof to ensure robust and appropriate appetite stimulation, without causing
35 desensitization of the system, may be part of the present invention. However, taken

into consideration that the individuals to be treated possibly will have to receive treatment for a longer period, such as weeks or months, it is preferred that the administration form is well suited herefor.

- 5 Accordingly, it is preferred that the GH secretagogue or a salt thereof is administered subcutaneously in an amount sufficient to allow sufficient levels of the bioactive form, e.g. an acylated or anchorgroup containing form, to reach the receptors in time, such as prior to the forthcoming meal. Dose and frequency of administration by the subcutaneous route are as described above.

10

Bolus administration

- From a molecular pharmacological point-of-view it is important to note that it has been found that the ghrelin receptor normally is exposed to short-lived surges in the concentrations of the natural agonist ligand, ghrelin. The GHS-R 1a receptor
15 belongs to the class of receptors, so-called G protein coupled receptors or 7TM receptors, that upon continued exposure to an agonist will be desensitized, internalized and down-regulated. These mechanisms, which are inherent to the overall signal transduction system, involve processes such as receptor phosphorylation (which in itself decreases the affinity of the receptor for the agonist)
20 binding of inhibitory proteins such as arrestin (which sterically block the binding of signal transduction molecules such as G proteins). Another part of the agonist mediated desensitization process is receptor internalization (i.e. physical removal of the receptor from the cell surface where it could bind the agonist) as well as receptor down regulation (i.e. decreased production / expression of the receptor). Receptor
25 internalization could after short-lived exposure of the receptor to agonist be followed by a re-sensitization process, where the receptor is dephosphorylated and recycled to the cell surface to be used again. Without being bound by theory, it is believed that, upon prolonged stimulation, which would occur for example during a long-lasting continuous infusion of the agonist, the receptor down-regulation process
30 ensures that the target cell is adjusted in its signal transduction system etc. to this situation.

- Accordingly, the present invention relates in one aspect to administration of a secretagogue, such as a ghrelin-like compound, in boluses, preferably a bolus prior
35 to each main meal.

Administration

- 5 In one embodiment of the present invention, the medicament can be administered as a bolus injection or by fast running infusion, i.e. an infusion preferably lasting less than 120 minutes, such as less than 90 minutes, for example less than 60 minutes, such as less than 45 minutes, such as less than 30 minutes, for example less than 25 minutes, such as less than 20 minutes, such as less than 15 minutes, for
- 10 example less than 12 minutes, such as less than 10 minutes, such as less than 8 minutes, for example less than 6 minutes, such as less than 5 minutes, such as less than 4 minutes, for example less than 3 minutes, such as less than 2 minutes, such as less than 1 minute.
- 15 The bolus injection or the fast running infusion can be administered prior to a meal or during a meal as described in more detail herein below. In one preferred embodiment the medicament is administered as a bolus. The bolus is preferably administered subcutaneously.
- 20 Any parenteral administration form that will ensure that the ghrelin receptors which normally are the targets for peripherally produced ghrelin in the premeal situation will be exposed to sufficient levels of the bioactive form of ghrelin to ensure robust and appropriate appetite stimulation, without causing desensitization of the system,
- 25 may be part of the present invention. However, taken into consideration that the individuals to be treated possibly will have to receive treatment for a longer period, such as weeks or months, it is preferred that the administration form is well suited therefore.
- 30 Accordingly, it is preferred that the secretagogue, such as a ghrelin-like compound, is administered subcutaneously in an amount sufficient to allow sufficient levels of the bioactive form of ghrelin, i.e. the acylated form, to reach the receptors in time, such as prior to the forthcoming meal.
- 35 The present invention preferably deals with methods for administering a secretagogue, such as ghrelin, in a way which mimics the physiologically pre-meal

situation as closely as possible yet providing patients in need of increased food intake, for example post operative patients with a sufficient extra stimulatory input to their appetite regulating ghrelin receptors, which normally are reached by ghrelin in the pre-meal situation.

5

Suitable dosing regimens for the various compounds and methods of the present invention are preferably determined taking into account factors well known in the art including type of subject being dosed; age, weight, sex and medical condition of the subject; the route of administration; the renal and hepatic function of the subject; the
10 desired effect; and the particular compound employed.

Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution,
15 equilibrium, and elimination of a drug.

As described above, in one aspect of the invention, the secretagogue, such as ghrelin or a ghrelin-like compound, is administered subcutaneously.

20

In another aspect the secretagogue, such as ghrelin or a ghrelin-like compound, is administered as a premeal bolus, wherein the administration form may be any suitable parenteral form.

In a preferred embodiment the secretagogue, such as ghrelin or a ghrelin-like
25 compound, is administered subcutaneously in a premeal bolus.

The secretagogue, such as ghrelin or a ghrelin-like compound, can also be administered during a meal as a bolus. The mode of administration during a meal includes subcutaneous administration, such as a subcutaneously administered
30 bolus.

Pharmaceutical compositions for parenteral administration include sterile aqueous and non-aqueous injectable solutions, dispersions, suspensions or emulsions, as well as sterile powders to be reconstituted in sterile injectable solutions or
35 dispersions prior to use.

Other suitable administration forms include suppositories, sprays, ointments, cremes, gels, inhalants, dermal patches, implants, pills, tablets, lozenges and capsules.

5

Ghrelin is primarily cleared by the kidneys and ghrelin administration yields markedly higher plasma levels in patients with renal failure than in healthy subjects (Wynne et al. J Am Soc Nephrol 2005; Aug (54)(8):2390-5. Accordingly a dosing regimen will be developed based on ghrelin pharmacokinetics and pharmacodynamics in patients with renal impairment.

10

A typical dosage of a compound employed according to the invention is in a concentration equivalent to from 10 ng to 10 mg ghrelin per kg bodyweight. The concentrations and amounts herein are given in equivalents of amount ghrelin, wherein the ghrelin is the 28 aa human ghrelin. Equivalents may be tested as described in the section entitled "Functionality", above.

15

In a preferred embodiment the medicament is administered in a concentration equivalent to from 0.1 µg to 1 mg ghrelin per kg bodyweight, such as from 0.5 µg to 0.5 mg ghrelin per kg bodyweight, such as from 1.0 µg to 0.1 mg ghrelin per kg bodyweight, such as from 1.0 µg to 50 µg ghrelin per kg bodyweight, such as from 1.0 µg to 10 µg ghrelin per kg bodyweight.

20

As described above, the secretagogue, such as ghrelin or a ghrelin-like compound, is preferably administered as a bolus. Accordingly, in one embodiment the medicament is administered as a bolus prior to a meal, said bolus comprising an amount of the secretagogue or a salt thereof equivalent to from 0.3 µg to 150 mg ghrelin. More preferably, the medicament is administered as a bolus prior to a meal, said bolus comprising an amount of the secretagogue or a salt thereof equivalent to from 2.0 µg to 200 mg ghrelin, such as from 5.0 µg to 100 mg ghrelin, such as from 10 µg to 50 mg ghrelin, such as from 10 µg to 5 mg ghrelin, such as from 10 µg to 1.0 mg ghrelin.

30

In a preferred embodiment, the ghrelin-like compound is administered as a bolus in an amount equivalent to 10 µg ghrelin per kg bodyweight.

Combination treatments

In a further aspect of the invention the present compounds may be administered in combination with further pharmacologically active substances or therapeutic method or other pharmacologically active material. By the phrase "in combination" with another substance(s) and/or therapeutic method(s) is meant herein that said another substance(s) and/or therapeutic method(s) is administered to the individual thus treated before, during (such as concurrently with) and/or after treatment of an individual with a secretagogue. In all cases of combination treatment described herein, the combination may be in the form of kit-in-part systems, wherein the combined active substances may be used for simultaneous, sequential or separate administration. In all cases, it is preferred that any of the herein-mentioned medicaments are administered in pharmaceutically effective amounts, i.e. an administration involving a total amount of each active component of the medicament or pharmaceutical composition or method that is sufficient to show a meaningful patient benefit.

In a preferred embodiment, the secretagogue is administered to the organ transplantation patient in combination with one or more immunosuppressive agents and/or one or more antimicrobial agents.

Combinations wherein all active ingredients are appetite-regulating agents

The secretagogue(s) according to the invention can also be administered in combination with other appetite-regulating agents, including more than one type of growth hormone secretagogue, such as another ghrelin-like compound, such as a ghrelin-like compound comprising a structure defined by formula I, described herein. Other secretagogues suitable for combination administration with another secretagogue compound are any of the secretagogue compounds described herein. In one preferred embodiment of the present invention, wild type ghrelin (most preferably human wild type ghrelin) is administered in combination with a different, ghrelin-like compound – this combination is envisaged to enhance and/or prolong the effect of the secretagogues on the ghrelin receptor. In another preferred embodiment of the present invention, a ghrelin-like compound that is not wild type ghrelin is administered in combination with a different ghrelin-like compound that is not wild-type ghrelin– again, this combination is envisaged to enhance and/or

prolong the effect of the secretagogues on the ghrelin receptor. In a similar way, several different secretagogues may be administered to an individual to increase efficacy on the ghrelin receptor – such as greater than 2 different secretagogue types, such as 3, such as 4, such as 5, such as 6, such as 7, such as greater than 8
5 different secretagogue types. The secretagogue according to the invention, such as ghrelin or a ghrelin-like compound(s) can also be administered in combination with a pharmaceutically effective amount of a growth hormone, including hGH.

In one preferred embodiment of the present invention the secretagogue, such as
10 ghrelin or a ghrelin-like compound, may be administered in combination with IGF-1, IGFBP-3, or ALS, preferably with IGF-1. The rationale behind this combination treatment is to increase the level of IGF-1, IGFBP-3, and/or ALS found to be low in cachectic individuals. In a further embodiment of the invention, the secretagogues, such as ghrelin or a ghrelin-like compound, may be administered in combination
15 with compounds known to stimulate appetite, such as melanocortin receptor antagonists, neuropeptide Y receptor agonists including agonists selective for individual subtypes of the neuropeptide Y receptors, leptin or leptin receptor agonists, cannabinoids including marijuana and marijuana derivatives, antipsychotics, especially atypical antipsychotics such as sertindole, Sulpirid,
20 Clozapine, Risperidone, Quetiapin, Amisulpride, Ziprasidon, and Olanzapine.

The GH secretagogue may also be administered in combination with an ingredient or therapy useful in a supportive care regimen, such as one or more of the following:

- G-CSF (and analogues thereof)
- 25 - EPO (and analogues thereof)
- Cannabinoid(s)
- Progestagen(s)
- Androgen(s) (such as SARM and androgen receptor modulators).
- Analgetics such as opioids

30

Further suitable combinations are disclosed in PCT patent application
PCT/DK2004/000519 (Gastrotech Pharma), incorporated herein by reference.

35

Medical packaging

The compounds used in the invention may be administered alone or in combination with pharmaceutically acceptable carriers or excipients, in either single or multiple doses. The formulations may conveniently be presented in unit dosage form by methods known to those skilled in the art.

It is preferred that the compounds according to the invention are provided in a kit. Such a kit typically contains an active compound in dosage forms for administration. A dosage form contains a sufficient amount of active compound such that a desirable effect can be obtained when administered to a subject, preferably prior to at least one meal a day, more preferably prior to each main meal, such as three times a day, during the course of 1 or more days.

Thus, it is preferred that the medical packaging comprises an amount of dosage units corresponding to the relevant dosage regimen. Accordingly, in one embodiment, the medical packaging comprises a pharmaceutical composition comprising a compound as defined above or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients, said packaging having from 7 to 21 dosage units, or multiples thereof, thereby having dosage units for one week of administration or several weeks of administration.

The dosage units are as defined above, i.e. a dosage unit most preferably comprises an amount of the ghrelin-like compound or a salt thereof equivalent to from 0.3 µg to 150 mg ghrelin, such as of from 2.0 µg to 100 mg ghrelin, such as from 5.0 µg to 75 mg ghrelin, such as from 10 µg to 50 mg ghrelin, such as from 10 µg to 5 mg ghrelin, such as from 10 µg to 1.0 mg ghrelin. In another embodiment, said dosage unit comprises an amount of the GH secretagogue or a salt thereof equivalent to from 0.3 µg to 600 mg ghrelin, such as of from 2.0 µg to 200 mg ghrelin, such as from 5.0 µg to 100 mg ghrelin.

The medical packaging may be in any suitable form for parenteral, in particular subcutaneous administration. In a preferred embodiment the packaging is in the form of a cartridge, such as a cartridge for an injection pen, the injection pen being such as an injection pen known from insulin treatment.

When the medical packaging comprises more than one dosage unit, it is preferred that the medical packaging is provided with a mechanism to adjust each administration to one dosage unit only.

- 5 Preferably, a kit contains instructions indicating the use of the dosage form to achieve a desirable affect and the amount of dosage form to be taken over a specified time period. Accordingly, in one embodiment the medical packaging comprises instructions for administering the pharmaceutical composition. In particular said instructions may include instructions referring to administration of said
- 10 pharmaceutical composition either during a meal, or preferably at the most 45 minutes prior to a meal, such as at the most 30 minutes prior to a meal, such as at the most 25 minutes prior to a meal, such as at the most 20 minutes prior to a meal, such as at the most 15 minutes prior to a meal, such as at the most 10 minutes prior to a meal, such as at the most 5 minutes prior to a meal.

15

Examples

Example 1

Competition binding assays

5 Transfected COS-7 cells were transferred to culture plates one day after transfection at a density of 1×10^5 cells per well aiming at 5 - 8 % binding of the radioactive ligand. Two days after transfection competition binding experiments were performed for 3 hours at 4°C using 25 pM of ^{125}I -ghrelin (Amersham, Little Chalfont, UK). Binding assays were performed in 0.5 ml of a 50 mM Hepes buffer, pH
10 7.4, supplemented with 1 mM CaCl_2 , 5 mM MgCl_2 , and 0.1 % (w/v) bovine serum albumin, 40 microgram/ml bacitracin. Non-specific binding was determined as the binding in the presence of 1 micromole of unlabeled ghrelin. Cells were washed twice in 0.5 ml of ice-cold buffer and 0.5-1 ml of lysis buffer (8 M Urea, 2 % NP40 in 3 M acetic acid) was added and the bound radioactivity was counted.
15 Determinations were made in duplicate. Initial experiments showed that steady state binding was reached with the radioactive ligand under these conditions.

Example 2

Functional tests on the ghrelin receptor

20

Transfections and tissue culture - COS-7 cells were grown in Dulbecco's modified Eagle's medium 1885 supplemented with 10 % fetal calf serum, 2 mM glutamine and 0.01 mg/ml gentamicin. Cells were transfected using calcium phosphate precipitation method with chloroquine addition as previously described (Holst et al.
25 Mol. Pharm (1998); 53;1;p166-175, "Steric hindrance mutagenesis versus alanine scan in mapping of ligand binding sites in the tachykinin NK1 receptor"). For gene dose experiments variable amounts of DNA were used. The amount of cDNA ($20\mu\text{g}/75\text{cm}^2$) resulting in maximal signalling was the used for dose responds curves.. HEK-293 cells were grown in D-MEM, Dulbecco's modified Eagle's medium
30 31966 with high glucose supplemented with 10 % fetal calf serum, 2 mM glutamine and 0.01 mg/ml gentamicin. Cells were transfected with Lipofectamine 2000 (Life Technologies).

Phosphatidylinositol turnover - One day after transfection COS-7 cells were
35 incubated for 24 hours with 5 μCi of $[\text{}^3\text{H}]$ -myo-inositol (Amersham, PT6-271) in 1 ml

medium supplemented with 10% fetal calf serum, 2 mM glutamine and 0.01 mg/ml gentamicin per well. Cells were washed twice in buffer, 20 mM HEPES, pH 7.4, supplemented with 140 mM NaCl, 5 mM KCl, 1 mM MgSO₄, 1 mM CaCl₂, 10 mM glucose, 0.05 % (w/v) bovine serum; and were incubated in 0.5 ml buffer
5 supplemented with 10 mM LiCl at 37°C for 30 min. After stimulation with various concentrations of peptide for 45 min at 37°C, cells were extracted with 10 % ice-cold perchloric acid followed by incubation on ice for 30 min. The resulting supernatants were neutralized with KOH in HEPES buffer, and the generated [³H]-inositol phosphate was purified on Bio-Rad AG 1-X8 anion-exchange resin as described.
10 Determinations were made in duplicates.

CRE, SRE and NF-κ-B reporter assay. HEK293 cells (30 000 cells/well) seeded in 96-well plates were transiently transfected. In case of the CRE reporter assay the cells were transfected with a mixture of pFA2-CREB and pFR-Luc reporter plasmid
15 (PathDetect CREB trans-Reporting System, Stratagene) or SRE-Luc (PathDetect SRE Cis-Reporting System, Stratagene) and the indicated amounts of receptor DNA. Following transfection cells were maintained in low serum (2.5%) throughout the experiments and were treated with the respective inhibitor of intracellular signalling pathways. One day after transfection, cells were treated with the
20 respective ligands in an assay volume of 100μl medium for 5 hrs. The assay was terminated by washing the cells twice with PBS and addition of 100μl luciferase assay reagent (LucLite, Packard). Luminescence was measured in a TopCounter (Top Count NXT™, Packard) for 5 sec. Luminescence values are given as relative light units (RLU).

25
MAP Kinase assay: COS 7 cells (seeding density 150.000 cells/well) were transfected in the assay plates. Two days after transfection the indicated concentration of ligand were added to assay medium without any serum and incubated for 10 min at 37° C. The reaction were stopped by removing the medium
30 and two washing steps with ice cold PBS. The cells were lysed in sample buffer and separated on SDS/10 % PAGE according to Laemmli ("Cleavage of structural proteins during the assembly of the head of bacteriophage T4" Nature vol 227, p680-685). Proteins were transferred onto nitrocellulose and Western blot analysis carried out using 1:5000 dilution of mouse monoclonal antiphospho-ERK1/2 anti-body
35 (Santa Cruz Biotechnology). Total ERK protein was determined using a 1:10000

dilution of anti-ERK antibody (Santa Cruz Biotechnology). Blots were probed anti mouse horseradish peroxidase-conjugated secondary antibodies, visualised using enhanced chemiluminescence reagent (Amersham Bioscience, New Jersey, US) and quantified by densitometric analysis. ERK1/2 phosphorylation was normalized according to the loading of protein by expressing the data as a ratio of phospho-ERK1/2 over total ERK1/2. Results were expressed as percentage of the value obtained in non stimulated mock transfected cells.

Example 3

Pre-clinical model:

Randomised, placebo-controlled trial evaluating long-term ghrelin therapy in rats undergoing allogeneic, single lung transplantation.

Design: All surgical procedures will be performed under general anaesthesia. Left donor lungs will be harvested from forty donor rats and flushed with a standard preservation solution. Recipient rats will undergo left thoracotomy and following pneumonectomy the pulmonary artery, main bronchus, and pulmonary vein of the donor lung will be anastomosed with the corresponding recipient structures. Postoperatively the transplanted rats will receive subcutaneous injections of ghrelin at a dose of 100 mg/kg or placebo twice daily for 8 weeks.

Endpoints: 24-h food intake and body weight will be measured at baseline and at weekly intervals throughout the study. Lean body mass will be assessed by DEXA scanning at baseline, 1 week and 8 weeks after transplantation.

Similar examples can be performed with other types of transplantation models, as is known by one skilled in the art.

Example 4

Clinical proof-of-concept:

Randomised, placebo-controlled trial evaluating long-term ghrelin therapy in malnourished patients undergoing lung transplantation.

Design: Fifty patients with end-stage lung COPD and BMI < 19 who are awaiting single lung transplantation will receive subcutaneous ghrelin at a dose of 15 mg/kg or placebo injections twice daily while on the waiting list and for 8 weeks after transplantation.

- 5 Endpoints: Appetite, food intake, body weight, lean body mass as assessed by DEXA scanning and quality-of-life as assessed by SF-36 will be measured at baseline, 1 week and 8 weeks after surgery. The incidence of postoperative complications as well as the pre-and postoperative morbidity and mortality will be registered.

10

Similar examples can be performed with other types of transplantation patients, as is known by one skilled in the art.

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Claims

1. Use of a secretagogue compound for the preparation of a medicament for the stimulation of appetite, food intake and/or weight gain in a transplantation patient in need of such treatment.

2. The use according to claim 1, wherein the secretagogue is ghrelin or a pharmaceutically acceptable salt thereof.

3. The use according to claim 1, wherein the secretagogue is a ghrelin-like compound or a pharmaceutically acceptable salt thereof

wherein the ghrelin-like compound comprises a structure defined by formula I

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$, wherein

Z^1 is an optionally present protecting group

each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

wherein one or more of X^1 and X^3 optionally may be modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

Z^2 is an optionally present protecting group,

m is an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

4. The use according to claim 3, wherein m is an integer in the range of from 1-9, such as of from 1-8, such as of from 1-7, such as of from 1-6, such as of from 1-5, such as of from 1-4, such as of from 1-3, such as of from 1-2, such as 2.

5. The use according to any of claims 3 or 4, wherein X^2 is selected from the group of modified Ser, modified Cys and modified Lys, such as wherein X^2 is modified Ser.

10

6. The use according to any of claims 3 to 5, wherein the ghrelin-like compound is selected from a compound of

formula II $Z^1 - \text{Gly} - (X^1)_{m-1} - (X^2) - (X^3)_n - Z^2$,

15

formula III $Z^1 - \text{Gly} - \text{Ser} - (X^2) - (X^3)_n - Z^2$, and

formula IV $Z^1 - \text{Gly} - (X^2) - (X^3)_n - Z^2$.

7. The use according to claim 6, wherein the ghrelin-like compound is having formula III.

8. The use according to any of claims 6 or 7, wherein $(X^3)_n$ comprises a sequence selected from one or more of the sequences shown below:

25

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

30

Phe Leu Ser Pro Glu

Phe Leu Ser Pro

Phe Leu Ser

35

Phe Leu

Phe

- 5 9. The use according to any of claims 3 to 8, wherein n is an integer in the range of from 1-25, such as of from 1-24, such as from 1-15, such as of from 1-10, such as of from 10-25, such as of from 10-24, such as of from 15-25, such as of from 15-24.

- 10 10. The use according to any of claims 3 to 9, wherein $(X^3)_n$ is selected from one or more of the sequences shown below:

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

15

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln Pro

20

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu Gln

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys Leu

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Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Ala

30

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro

35

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser

5 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys

10 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln

Phe Leu Ser Pro Glu His Gln Arg Val Gln

15 Phe Leu Ser Pro Glu His Gln Arg Val

Phe Leu Ser Pro Glu His Gln Arg

20 Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

Phe Leu Ser Pro Glu

25 Phe Leu Ser Pro

Phe Leu Ser

30 Phe Leu

Phe

11. The use according to any of claims 3 to 10, wherein the acyl group is selected from a C1-C35 acyl group, such as a C1 – C20 acyl group, such as a C1 – C15 acyl group, such as a C6 – C15 acyl group, such as a C6 – C12 acyl group, such as a C8 – C12 acyl group.
- 5
12. The use according to any of claims 3 to 11, wherein the acyl group is selected from the group of C7 acyl group, C8 acyl group, C9 acyl group, C10 acyl group, C11 acyl group, and C12 acyl group.
- 10
13. The use according to any of claims 3 to 12, wherein the acyl group is selected from the group of C8 acyl group, and C10 acyl group.
14. The use according to any of claims 3 to 13, wherein the acyl group is selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.
- 15
15. The use according to any of preceding claims 1 to 14, wherein the medicament is in a formulation for parenteral, intravenous, intramuscular or subcutaneous administration.
- 20
16. The use according to claim 15, wherein the formulation comprises the secretagogue or a salt thereof as a lyophilisate and the formulation further comprises a solvent, said lyophilisate and said solvent being in separate compartments until administration.
- 25
17. The use according to any of claims 15, wherein the formulation is a solution of the secretagogue or a salt thereof.
18. The use according to claim 16 or 17, wherein the solvent is saline.
- 30
19. The use according to any of claims 1 to 18, wherein the medicament is administered in a concentration equivalent to from 10 ng to 10 mg ghrelin per kg bodyweight.

20. The use according to claim 19, wherein the medicament is administered in a concentration equivalent to from 0.1 μ g to 1 mg ghrelin per kg bodyweight, such as from 0.5 μ g to 0.5 mg ghrelin per kg bodyweight, such as from 1.0 μ g to 0.1 mg ghrelin per kg bodyweight, such as from 1.0 μ g to 50 μ g ghrelin per kg bodyweight, such as from 1.0 μ g to 10 μ g ghrelin per kg bodyweight.
21. The use according to any of claims 1 to 20, wherein the medicament is administered as a bolus prior to or during a meal, said bolus comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 0.3 μ g to 600 mg ghrelin
22. The use according to claim 21, wherein the medicament is administered as a bolus prior to or during a meal, said bolus comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 2.0 μ g to 200 mg ghrelin, such as from 5.0 μ g to 100 mg ghrelin, such as from 10 μ g to 50 mg ghrelin, such as from 10 μ g to 5 mg ghrelin, such as from 10 μ g to 1.0 mg ghrelin.
23. The use according to any of claims 1 to 22, wherein the medicament is administered prior to or during a meal, preferably less than 180 minutes prior to a meal, such as less than 90 minutes prior to a meal, for example less than 45 minutes prior to a meal, such as less than 30 minutes prior to a meal, for example less than 25 minutes prior to a meal, such as less than 20 minutes prior to a meal, for example less than 15 minutes prior to a meal, such as about 10 minutes prior to a meal, for example about 5 minutes prior to a meal, such as immediately prior to a meal, or during a meal.
24. The use according to any of claims 1 to 23, wherein the medicament is administered after commencing a meal, preferably less than 90 minutes after commencing a meal, for example less than 45 minutes after commencing a meal, such as less than 30 minutes after commencing a meal, for example less than 25 minutes after commencing a meal, such as less than 20 minutes after commencing a meal, for example less than 15 minutes after commencing a meal, such as less than 10 minutes after commencing a meal, for example less than 5 minutes after commencing a meal.

25. The use according to any of claims 1 to 24, wherein the medicament is administered from one to three times daily, preferably once prior to or during breakfast and/or once prior to or during lunch and/or once prior to or during dinner.
- 5
26. The use according to any of claims 1 to 25, wherein the organ transplantation patient is a lung transplantation patient.
27. The use according to any of claims 1 to 25, wherein the organ transplantation patient is a kidney transplantation patient.
- 10
28. The use according to any of claims 1 to 25, wherein the organ transplantation patient is a liver transplantation patient.
29. The use according to any of claims 1 to 25, wherein the organ transplantation patient is a heart transplantation patient.
- 15
30. The use according to any of claims 1 to 29, wherein the patient has a lean body mass of less than 80% of normal, such as less than 60% of normal and/or a body mass index below 17 kg/m².
- 20
31. The use according to any of claims 1 to 30, wherein the medicament is given during the pre-operative period.
32. The use according to any of claims 1 to 31, wherein the medicament is given during the peri-operative period.
- 25
33. The use according to any of claims 1 to 32, wherein the medicament is given during the post-operative period.
- 30
34. The use according to any of claims 1 to 33, wherein the medicament is given during the pre-operative, the peri-operative and the post-operative period.

35. The use according to any of claims 1 to 34, wherein the medicament is given until the lean body mass is more than 60% of normal, preferably more than 80% of normal, more preferably more than 90% of normal.
- 5 36. The use according to any of the preceding claims, wherein the transplant is a solid organ.
37. The use according to claim 36, wherein said solid organ is a lung.
- 10 38. The use according to claim 36, wherein said solid organ is a heart.
39. The use according to claim 36, wherein said solid organ is a liver.
40. The use according to claim 36, wherein said solid organ is a kidney.
- 15 41. The use according to claim 36, wherein said solid organ is a pancreas.
42. The use according to claim 36, wherein said solid organ is an intestine.
- 20 43. The use according to claim 36, wherein said solid organ is an extremity.
44. The use according to any of the claims 1-35, wherein the transplant is a Hematopoietic Stem Cell Transplantation
- 25 45. The use according to claim 44, wherein said stem cell transplantation is either Bone marrow transplantation or Peripheral blood stem cell transplantation.
46. The use according to any of claims 1-35, wherein the transplant is a reconstructive plastic surgery, such as reconstructive facial surgery, or
- 30 reconstructive surgery after burns.