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(54) Title: USES OF COMBINATIONS OF A GH SECRETAGOGUE AND A GROWTH HORMONE

(57) Abstract: The present invention relates to the use of a secretagogue, such as a ghrelin-like compound, in combination with a growth hormone, such as somatotropin or a variant thereof, for the production of a medicament for the treatment of an individual in need thereof, and to a method of treating or preventing cachexia, stimulating appetite, food intake and/or weight gain in an individual in need thereof by administering a ghrelin-like compound. Furthermore, the invention relates to pharmaceutical compositions comprising at least one secretagogue and at least one growth hormone, and medical packagings comprising one or more of said pharmaceutical composition. In another aspect, the present invention relates to a method of treating an individual in need thereof, such as an individual suffering from cachexia, by administering at least one secretagogue, such as a ghrelin-like compound, in combination with at least one growth hormone.

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Uses of combinations of a GH secretagogue and a growth hormone

All patent and non-patent references cited in the application, or in the present application, are also hereby incorporated by reference in their entirety.

Field of invention

The present invention relates to the use of a secretagogue, such as a ghrelin-like compound, in combination with a growth hormone, such as somatotropin or a variant thereof, for the production of a medicament for the treatment of an individual in need thereof, and to a method of treating or preventing cachexia, stimulating appetite, food intake and/or weight gain in an individual in need thereof by administering a ghrelin-like compound. Furthermore, the invention relates to pharmaceutical compositions comprising at least one secretagogue and at least one growth hormone, and medical packagings comprising one or more of said pharmaceutical composition.

Furthermore, the present invention relates to a method of treating an individual in need thereof, such as an individual suffering from cachexia, by administering at least one secretagogue, such as a ghrelin-like compound, in combination with at least one growth hormone.

Background of invention

Cachexia

Cachexia is found as the terminal state of many different clinical conditions or in chronic diseases such as cancer, infections, AIDS, congestive heart failure, rheumatoid arthritis, tuberculosis, cystic fibrosis and Crohn diseases. It can also occur in elderly people who do not have any obvious symptoms of disease. Although cachexia represents the complex metabolic syndrome that is seen in such patients it is commonly recognized as a progressive weight loss with depletion of host reserves of adipose tissue and skeletal muscle.

The core of cancer cachexia syndrome relates to the problem of progressive tumor growth and the catabolic side effects of conventional anti-neoplastic therapy. These two phenomena give rise to alterations in the neuro-endocrine system, to the

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production of a variety of pro-inflammatory cytokines and to the release of cancer-specific cachectic factors. In turn, these mediators cause either a reduction in food intake, abnormality in the metabolism or a combination of these two.

Cachexia is associated with particular types of cancer eg. cancer of the upper gastrointestinal (GI) tract and lung cancer. At the moment of diagnosis, 80% of all patients with cancer in upper GI tract and 60 % of all patients with lung cancer have already experienced substantial weight loss (Bruera, E., BMJ 315.7117 (1997): 1219-22). Cachexia is more common in children and in elderly patients and becomes more pronounced as the cancer progresses. On average, the prevalence of cachexia increases from 50 % to more than 80% percent before death, and in more than 20 % of the patients cachexia is the main cause of death (Bruera 1219-22).

To detect cachexia, the individual's nutritional state is evaluated with a combination of clinical assessment, anthropometric tests (body weight, skin fold thickness and mid arm circumference) and imaging (DEXA scan, MR scan, CT scan and bioelectric impedance meassuring). Cachexia is generally suspected if an involuntary weight loss of greater than 5% of the premorbid weight is observed within a six-month period – especially when combined with muscle wasting. The most commonly used laboratory parameter for detection of cachexia is serum albumin. It is however an unspecific parameter. Other markers include transferrrin and transthyretin and other proteins with a short half life. Further markers of canchexia include IGF-1, and IGFBP-3) and testosterone.

Anorexia

Energy intake has been shown to be substantially reduced among weight-losing cancer patients. Cancer patients may frequently suffer from physical obstruction of the GI tract, pain, depression, constipation, malabsorption, debility or the side effects of treatment such as opiates, radiotherapy or chemotherapy, which all may decrease food intake Barber, M. D.et al. Surg.Oncol. 8.3 (1999): 133-41.. Cancer associated hypercalcemia may also induce nausea, vomiting and appetite loss. However there remain a large number of patients with cancer in whom there is no obvious clinical cause of reduced food intake. It is important to make this distinction,

as only the latter small subset of patients may benefit from medication that acts centrally to stimulate appetite.

The central mechanism of cancer induced anorexia and cachexia is complex and includes many different cytokines, hormones and other factors produced by the cancer cells:

Leptin: In normal physiological situations leptin plays an important role in triggering the adaptive response to starvation since weight loss causes leptin level to fall in proportion to the loss of body fat. However in cancer patients an increased level of cytokines (IL-1, IL-6, TNF-α, INF-γ) produced by the cancer cells may stimulate the expression and/or the release of leptin. Another possible mechanism of the cytokines is that they mimic the hypothalamic effect of excessive negative feedback signalling from leptin, leading to the prevention of the normal compensatory mechanism regarding food intake and body weight.

NPY (Neuropeptide Y): The hypothalamic NPY system is one of the key neural pathways disrupted in anorexia induced by IL-1 or other cytokines. The cytokines decreases the sensitivity for NPY (Inui review).

Melanocortins: Abberant melanocortin signalling may be a contributing factor in both anorexia and cachexia. Despite marked loss of body weight which would normally be expected to down-regulate the anorexigenic melanocortin signaling system as a way to conserve energy stores, the melanocortin system remains active during cancer induced cachexia. Central malanocortin blockade by AgRP or other antagonists reversed anorexia and cachexia in the animal models suggesting a pathogenic role of this system (Wisse, B. E., et al., "Melanocortin signaling and anorexia in chronic disease states." Ann.N.Y.Acad.Sci. 994 (2003): 275-81).

Metabolism

Hyper metabolism is defined as an elevation of the resting energy expenditure (REE), and is a feature of cachexia. Total energy expenditure involves REE (approximately 70%) and voluntary energy expenditure (approximately 25%) and energy expenditure in digestion (5%). Voluntary energy expenditure may be decreased in cachexia which may manifest clinically as apathy, fatigue and depression.

The orexigenic and the anorexigenic signals are known to respectively decrease and increase sympathetic nervous activity, which regulate REE by activating thermogenesis in brown adipose tissue in rodents and possibly in muscle in humans, through induction of the mitochondrial uncoupling protein (UCP). It has been suggested that activation of UCP in muscle in white adipose tissue by cytokines might be a molecular mechanism underlying the increase in the heat production and muscle wasting (Fearon, K. C. et al.,Int.J.Cardiol. 85.1 (2002): 73-81; Inui, A., CA Cancer J.Clin. 52.2 (2002): 72-91). Altered nutrition metabolism has been described in patients with cancer. Solid tumors produce large amounts of lactate, which is converted back into glucose through a process that uses large amount of ATPand is very energy inefficient. One of the major factors in the increase in fat metabolism may be lipid mobilizing factor (LMF) that acts directly on the adipocyte to release free fatty acid and glycerol. Cytokines may induce muscle protein catabolism indirectly by affecting the muscle repair processes.

Control of Growth

Growth hormones are involved in regulating an individual's growth patterns. Some act directly on target organs, while others act by triggering the production of other hormones, which activate specific organ functions necessary for growth. This finely tuned system can malfunction in several ways, causing abnormal growth.

Somatotropin (also termed human growth hormone) is an anterior pituitary hormone whose main effect is to promote growth of body tissues, and is vital for normal growth. Somatotropin has direct effects on intermediate metabolism, such as decreased glucose uptake, increased lipolysis, increased amino acid uptake and protein synthesis. Somatotropin is also known to have effects on adipose tissue (decreasing liposity), the liver (increased gluconeogenesis and IGF-1) and muscle (increased lean body mass). It is furthermore involved in stimulating linear growth, which is mediated by IGF-1. Somatotropin is derived from a prohormone which has an additional 26 AA attached to the N-terminal end. This tail is cleaved by an endopeptidase to generate active hormone. Fragments of somatotropin have been tested for somatotropin activity, and the results indicated that only the C-terminal region of somatotropin binds the somatotropin receptor.

Several isoforms of human growth hormones (hGH) have been identified. This heterogenity is caused by the existence of two different genes, differences in the splicing and differences in posttranslational modification. At the genetic level two different genes encodes for hGH: hGH-N is expressed in pituitary and hGH-V is expressed in the placenta. At mRNA level GH-N undergoes splicing into 20K and 22K isoforms. Posttranslationally 22K GH undergoes modifications such as acylation at its amino terminus, deamination and oligomerization . Some of the different isoforms of growth hormone are shown in table 1:

Table 1. Isoforms of human GH in pituitary extracts, placenta and plasma

Monomeric forms
hGH-22K or 22K
hGH-20K or 20K
N_{α}-acetylated hGH-22K or 'fast hGH'
Asn^{152}-desamido-hGH-22K
Gln^{137}-desamido-hGH-22K
hGH-V or placental GH
Glyco-hGH-V or glycosylated placental GH
Oligomeric forms
Dimeric, trimeric, tetrameric and pentameric hGH, composed of the above monomers in homopolymeric and heteropolymeric combinations
• non-covalent oligomers (~ 65%)
• disulfide oligomers (~ 33%)
• other covalently linked oligomers (~ 2%)
Circulating complexes
22K-GHBP complex
22K-α_2-macroglobulin complex
20K-GHBP complex
20K-α_2-marcoglobulin complex
hGH-V-GHBP complex
Others
Fragments and/or small molecular weight hGH immunoreactivity
16 kDa, 12 kDa and 30 kDa components

Other anterior pituitary hormones affect growth indirectly by working through other glands. These other growth hormones include:

- Thyroid Stimulating Hormone (TSH) - causes the thyroid gland to produce thyroid hormone, which regulates body metabolism and is essential for normal growth.
- Adrenocorticotropic Hormone (ACTH) - causes the adrenal glands to produce cortisol (stress hormone) and other hormones that enable the body to respond to stress.
- Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) - cause the sex glands (ovaries or testes) to produce sex hormones, which are necessary for adolescent sexual development and the growth spurt that accompanies puberty.

Ghrelin

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 act 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 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 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 be a member of the 7TM G protein coupled receptor family (Howard AD et al., 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.

Summary of invention

The present invention relates to use of a secretagogue, such as a ghrelin-like compound, more preferably human ghrelin, or a pharmaceutically acceptable salt thereof, in combination with a growth hormone, such as human somatotropin, or pharmaceutically acceptable salt thereof, for the preparation of a medicament for an individual in need thereof, such as for one or more of

a) prophylaxis or treatment of cachexia, and/or

b) prophylaxis or treatment of lipodystrophy, and/or

c) stimulation of appetite, and/or

d) stimulation of food intake, and/or

e) stimulation of weight gain, and/or

f) increasing body fat mass and/or

g) increasing or maintaining lean body mass

including any combination of the above.

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

Furthermore, the individual treated will experience an increased quality of life and prolonged survival.

In one preferred embodiment, said individuals are suffering from, or at risk of suffering from, appetite or body weight irregularities, such as cachexia, such as associated with HIV or AIDS and/or cancer cachexia, for example AIDS wasting. In another embodiment, said individual is suffering from a growth deficiency such as is caused by a somatotropin deficiency or dwarfism. In another embodiment, said individual is suffering from, or at risk of suffering from, cardiac cachexia. In another embodiment, said individual is suffering from, or at risk of suffering from, the condition of frailty.

The present invention further relates to a pharmaceutical composition comprising one or more growth hormone and a secretagogue, such as a ghrelin-like compound or ghrelin, and/or one or more pharmaceutically acceptable salt(s) thereof and pharmaceutically acceptable carriers, vehicles and/or excipients.

In yet a further aspect the invention relates to a medical packaging comprising one or more dosage units of the pharmaceutical composition comprising the compounds as defined above or one or more pharmaceutically acceptable salt(s) thereof and pharmaceutically acceptable carriers, vehicles and/or excipients.

The present invention further relates to a method for treating and/or preventing a pathological condition, comprising administering to an individual in need thereof an

effective amount of a secretagogue, such as a ghrelin-like compound or ghrelin in combination with a growth hormone, such as human somatotropin. Said pathological condition is preferably cachexia. The cachexia may be cancer cachexia, cardiac cachexia, cachexia caused by chronic infection/inflamation, chronic obstructive pulmonary disease, cachexia associated to kidney diseases, AIDS wasting and long lasting hospitalization. More preferably, said cachexia is cancer cachexia. In particular, said cancer cachexia is associated with the sub-types of cancer that induce a high degree of cachexia with an increase of REE, such as lung cancer and pancreatic cancer. Equally preferaby, said individual may be suffering from, or at risk of suffering from, growth retardation or dwarfism, for example caused by somatotropin insufficiency. Said methods for treating an individual may comprise treatment with ghrelin or an analog thereof prior to, and/or during and/or after the individual is subjected to treatment with said growth hormone. Said growth hormone is preferably human somatotropin, (alternatively termed hGH). In yet another embodiment of this aspect of the invention, said method is for the

a) prophylaxis or treatment of cachexia, and/or
b) prophylaxis or treatment of lipodystrophy, and/or
c) stimulation of appetite, and/or
d) stimulation of food intake, and/or
e) stimulation of weight gain, and/or
f) increase of body fat mass,
g) increase in/maintencance of lean body mass,
including any combination thereof,

in an individual by administering an effective dosage of a a secretagogue in combination with a growth hormone. Preferred combinations are: a); b); c); d); e); and f) in isolation; as well as a) + c); a) + d); a) + e); a) + f); b) + c); b) + d); b) + e); b) + f); a) + c) + d); a) + c) + e); a) + c) + f); a) + d) + e); a) + d) + f); a) + e) + f); a) + c) + d) + e); a) + c) + d) + f); a) + d) + e) + f); a) + c) + d) + e) + f); b) + c) + d); b) + c) + e); b) + c) + f); b) + d) + e); b) + d) + f); b) + e) + f); b) + c) + d) + e); b) + c) + d) + f); b) + d) + e) + f); and b) + c) + d) + e) + f); c) + d) + g); a) + b) + e) + g).

The present invention significantly lowers the risk of developing all the pathological conditions mentioned herein, for example cachexia, independent of the cause, such

as independent of the particular therapeutic cause of, or therapeutic factor contributing to, the pathological condition.

The present invention further relates to a method for monitoring the effect of a treatment of an individual with a ghrelin-like compound in combination with a growth hormone, comprising measuring the blood level in said individual of IGF-1, IGFBP-3, and/or ALS (acid-labile subunit). Accordingly, in another aspect the invention relates to a method for monitoring the effect of the administration of the compounds of the invention, comprising measuring one or more markers, in particular markers selected from IGF-I, IGFBP-3, ALS (acid-labile subunit), thyroid hormones, sex hormones, and albumin, more preferably from IGF-I, IGFBP-3, ALS (acid-labile subunit), more preferably IGF-1. These markers are all low in patients suffering from pathological conditions such as cachexia, and are expected to increase after treatment with ghrelin.

Recombinant human somatotropin (also known as rhGH) is known and is used for treatment of syndromes such as HIV, AIDS and cancer. Clinical efficacy in triggering weight gain has been demonstrated, e.g. after up to 12 weeks of somatropin therapy in clinical trials, patients gained an average of 1.6. kg of lean body mass. Therapy with rhGH has been reported to increase the lean body mass in a small number of patients. Results have also shown that somatotropin causes reduction in the size of 'buffalo hump', truncal fat, and fat loss and lipid abnormalities were not improved after administration of hGH. Furthermore, blood glucose control worsened (DP Kotler and others. ("Transient Insulin Resistance during Recombinant Human Somatotropin (rhGH) Therapy for HIV-Associated Adipose Redistribution Syndrome (HARS)" 42nd Interscience Conference on Antimicrobial Agents and Chemotherpay (42nd ICAAC). September 27-30, 2002. San Diego, CA").

Without being bound by theory, it is envisaged that a secretagogue such as ghrelin may be used, in combination with a growth hormone, to increase an individual's levels of the anabolic factor IGF-1, and also to optionally increase appetite and/or food intake, and that the combination of these two hormones lead to improvements in the body composition by prevention of loss of body weight and body fat,. Thus, it is believed that a combination treatment with ghrelin or a analog thereof would maintain and/or increase body fat in patients being treated with the growth hormone,

thereby effectively counteracting or at least reducing symptoms such as lipodystrophy caused by growth hormones such as hGH. Thus, it is believed that the administration of ghrelin with growth hormone prevents fat loss and fat distribution within the individual's body. Furthermore, without being bound by theory, it is further believed that the administration of growth hormone, leading to an increase in an individual's lean body mass. Thus, the positive effects of administration of a secretagogue and growth hormone synergistically lead to positive effects on the individual thus treated, such as one or more of

a) prophylaxis or treatment of cachexia, and/or
b) prophylaxis or treatment of lipodystrophy, and/or
c) stimulation of appetite, and/or
d) stimulation of food intake, and/or
e) stimulation of weight gain, and/or
f) increasing body fat mass, and/or
g) increasing/maintaining lean body mass

or any combination thereof.

Furthermore, it is believed, without being bound by theory, that administration of a growth hormone to an individual need thereof, in combination with a secretagogue, such as ghrelin or a ghrelin-like compound, will allow a lower quantity of growth hormone to be administered to successfully treat the patient or alleviate their symptoms than would be the case with administration of the growth hormone alone.

Detailed description of the invention

Definitions

Affinity: the strength of binding between receptors and their ligands, for example between an ghrelin and its cognate receptor.

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

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. NH_2 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 keeping with standard polypeptide, abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

SYMBOL		
1-Letter	3-Letter	AMINO ACID
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
B	Asx	Asn and/or Asp
C	Cys	cysteine

X	Xaa	Unknown or other

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 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 NH_2 or acetyl or to a carboxy-terminal group such as COOH.

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 19-22.

Chemotherapy: herein, the term “chemotherapy” refers to any treatment of an individual with a cytotoxic drug, said treatment causing a reduction in bone marrow content. By “cytotoxic drug” is meant a drug that kills or arrests the growth of cells, preferably by targeting specific parts of the cell growth cycle. Diseases that may be treated by chemotherapy include metastatic cancers

Concentration equivalent: A concentration equivalent is an Equivalents dosage being defined as the dosage of an analogue of a compound having in vitro and/or in vivo the same response as evaluated from a dosage-response curve as the wild-type form of said compound.

Dissociation constant, Kd: a measure to describe the strength of binding (or affinity or avidity) between receptors and their ligands, for example an antibody and its antigen.The smaller Kd 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.

Gastrectomy: Herein, "gastrectomy" and grammatical variations thereof, is defined as removal, loss or reduction in size of all, or part of, the stomach of an individual. Said removal or loss is preferably surgical removal. Preferably, the individual treated using the methods and uses of the present invention is not gastrectomised.

Ghrelin: a polypeptide as described by Kojima et al., Nature 402:656-660. Human 28 aa ghrelin has the amino acid of SEQ ID NO: 1.

GHS: growth hormone secretagogue a substance or chemiacal moity capable of binding and/or activating the GHS-1a receptor.

GHS-R 1a: the receptor for GHS. GHS-R 1a is also denoted GHS 1a. The receptor has GENBANK accession number NM_198407

HAART: Highly active antiretroviral therapy.

Immunologically distinct: The phrase immunologically distinct refers to the ability to distinguish between two polypeptides on the ability of an antibody to specifically bind one of the polypeptides and not specifically bind the other polypeptide.

Individual: A living animal or human. Said individual is preferably susceptible to a pathological condition condition, for example a cachectic condition as defined herein. 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.

Isolated: is used to describe the various compounds, polypeptides and nucleotides disclosed herein, that has 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.

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.

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

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

Sequence homology: In one embodiment, sequence homology refers to a comparison made between two molecules using standard algorithms well known in the art. The preferred algorithm for calculating sequence homology for the present invention is the Smith-Waterman algorithm, where e.g. one of SEQ ID NO:4-8 is used as the reference sequence to define the percentage identity of polypeptide homologs over its length. The choice of parameter values for matches, mismatches, and inserts or deletions is arbitrary, although some parameter values have been found to yield more biologically realistic results than others. One preferred set of parameter values for the Smith-Waterman algorithm is set forth in the "maximum similarity segments" approach, which uses values of 1 for a matched residue and -1/3 for a mismatched residue (a residue being either a single nucleotide or single amino acid) (Waterman, Bull. Math. Biol. 46, 473-500 (1984)). Insertions and deletions (indels), x, are weighted as

$$x_k = 1 + k/3,$$

where k is the number of residues in a given insert or deletion (Id.).

Specificity: The term specificity refers to the number of potential antigen binding sites which immunoreact with (specifically bind to) a given polypeptide. The polypeptide may be a single polypeptide or may be two or more polypeptides joined by disulfide bonding.

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.

In one aspect, the present invention relates to the use of a ghrelin-like compound or a pharmaceutically acceptable salt thereof and a growth hormone or pharmaceutically acceptable salt thereof for the preparation of a medicament treatment of an individual in need thereof. In one preferred embodiment, said medicament is for one or more of:

prophylaxis or treatment of cachexia, and/or

prophylaxis or treatment of lipodystrophy, and/or

stimulation of appetite, and/or

stimulation of food intake, and/or

stimulation of weight gain, and/or

increasing body fat mass and/or

increasing/maintenance lean body mass and/or

improving quality of life and survival.

in an individual in need thereof,

Growth hormone

By "growth hormone" is meant any hormone that is capable of stimulating growth of an individual during at least one period of said individual's life, for example during development. By "growth" is meant for example one or more of:

- increase in cellular size of one or more of an individual's constituent cells

- increase in the total cell number making up an individual
- growth of a particular area, tissue or organ of an individual, such as muscle and/or bone mass.

In one preferred embodiment of the present invention, the growth hormone is a growth hormone of a domestic animal, or a homologue, variant or functional equivalent thereof. By domestic animal is meant for example a farm animal or a meat-producing animal, such as a pig, cow, horse, sheep or fish. More preferably, said growth hormone is somatotropin (also termed growth hormone, GH) or a homologue, variant or functional equivalent thereof, most preferably human somatotropin (hGH), pig somatotropin or bovine somatotropin. In another preferred embodiment of the present invention, said growth hormone is selected from Somatotropin or any isoform thereof, Thyroid Stimulating Hormone (TSH), Adrenocorticotropic Hormone (ACTH), Luteinizing Hormone (LH) and/or Follicle Stimulating Hormone (FSH) or a homologue, variant or functional equivalent thereof. More preferably, said growth hormone is selected from the group consisting of:

- Monomeric hGH
- Dimeric hGH
- Trimeric hGH
- Tetrameric hGH
- Pentameric hGH

In another preferred embodiment, said growth hormone is selected from the group consisting of:

- Non-covalent oligomers of hGH
- Disulfide oligomers of hGH
- Covalently linked hGH
- 22K-GHBP complex
- 22K-alpha2-macroglobulin complex
- 20K-GHBP complex
- 20K-alpha2-macroglobulin complex
- hGH-V_GHBP complex
- hGH-22K
- hGH-20K

- N-alpha-acetylated hGH-22K
- Asn152-desamido-hGH-22K
- Gln-137-desamido-hGH-22K
- hGH-V or placental GH
- Glyco-hGH-V or glycosylated placental GH

It is preferred that the growth hormone comprises a recombinant polypeptide. In another preferred embodiment, said hormone is purified or substantially pure.

Particularly preferred growth hormones for use in the present invention are any of the growth hormones selected from the list consisting of: Saizen/serostim (Serono), Nutropin (Ipsen/Genentech), NutropinAq (Ipsen/Genentech), Norditropin simplex (Novo Nordisk), Humatrope (Eli Lilly), Genotropin (Pfizer) or Zomacton (Ferring).

It is envisaged that the term "growth hormone" as used herein also refers to growth hormone-like compounds, such as variants, analogues, functional equivalents and homologues of growth hormones. One preferred growth hormone for use in the present invention is the naturally occurring human growth hormone somatotropin, and isoforms thereof, the amino acid sequences of which are shown in SEQ ID NO: 4-8, or a variant, homologue or functional equivalent thereof.

The present invention includes diastereomers as well as their racemic and resolved enantiomerically pure forms. Growth hormone-like compounds 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 growth hormone-like compound are the L-enantiomer.

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 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 Ave., Madison, Wis. 53705). This software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

A homologue of one or more of growth hormone specified herein may vary in one or more amino acids as compared to known growth hormone sequences, but is capable of 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 may comprise an amino acid sequence capable of being recognised by an antibody, said antibody also recognising a known growth hormone or growth hormone-like compound, preferably somatotropin. Examples of homologues comprises one or more conservative amino acid 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.

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 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 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 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 aspargines (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.

The following table lists preferred, but non-limiting, conservative amino acid substitutions.

ORIGINAL RESIDUE	EXEMPLARY SUBSTITUTIONS
ALA	SER, THR, VAL, GLY
ARG	LYS
ASN	HIS, SER
ASP	GLU, ASN
CYS	SER
GLN	ASN, HIS
GLU	ASP, GLU
GLY	ALA, SER
HIS	ASN, GLN
ILE	LEU, VAL, THR
LEU	ILE, VAL
LYS	ARG, GLN, GLU, THR
MET	LEU, ILE, VAL
PHE	LEU, TYR
SER	THR, ALA, ASN
THR	SER, ALA
TRP	ARG, SER
TYR	PHE
VAL	ILE, LEU, ALA
PRO	ALA

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).

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.

In a preferred embodiment the growth hormone comprises a homologue having an amino acid sequence at least 60 % homologous to at least one of SEQ ID NO 4, 5, 6, 7, or 8.

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 96 %, such as at least 97 %, such as at least 98 %, such as at least 99 % homologous to at least one of SEQ ID NO 4, 5, 6, 7 or 8.

In a more preferred embodiment the percentages mentioned above relate to the identity of the sequence of a homologue as compared to SEQ ID NO 4. In another, equally preferred embodiment, the percentages mentioned above relate to the identity of the sequence of a homologue as compared to SEQ ID NO: 5. In another, equally preferred embodiment, the percentages mentioned above relate to the identity of the sequence of a homologue as compared to SEQ ID NO: 6. In another, equally preferred embodiment, the percentages mentioned above relate to the identity of the sequence of a homologue as compared to SEQ ID NO: 7. In another, equally preferred embodiment, the percentages mentioned above relate to the identity of the sequence of a homologue as compared to SEQ ID NO: 8.

The growth hormone-like compound may also be administered in a form, wherein the growth hormone-like compound is conjugated to another entity.

For example the compound may be a conjugate of growth hormone-like or a derivative or homologue thereof and another peptide, such as a secretagogue as defined herein. Examples of peptides in the conjugate may also be found in US patent application 2003040472

In another preferred embodiment, said growth hormone-like compound may be a functional homologue of a growth hormone, i.e may cause one or more of the following, for example in an in vitro or in vivo test (e.g. an effect on a cell line or animal model, assayed using methods known to those skilled in the art):

- increase in cellular size
- increase in the total cell number
- growth of a particular area, tissue or organ of an individual, such as muscle or bone.

In another preferred embodiment, a functional equivalent of a growth hormone, such as somatotropin, is capable of causing known chemical changes associated with said growth hormone activity, such as stimulating the liver and other tissues to secrete IGF-1, measurable using methods known in the art, for example ELISA methods.

Uses of bovine somatotropin (bST) in combination with a secretagogue

Milk yield and persistency responses to bST have been observed for all dairy breeds examined. The mechanism of action of bST involves a series of orchestrated changes in the metabolism of body tissues so that more nutrients can be used for milk synthesis. It is these coordinated changes that allow the animal to achieve an increased milk yield while remaining normal and healthy. Bioenergetic studies demonstrated that bST-supplemented animals are not stressed. Similarly, there are no adverse health effects from bST even under poor management conditions. Composition of milk (fat, protein, lactose, cholesterol, minerals, and vitamins) is not substantially altered when bST is used and does not differ in manufacturing characteristics. With bST use, a unit of milk is produced with less feed and protein

supplement and with a reduction in animal excreta (manure, urine, and methane). (See Bauman DE, "Bovine somatotropin: review of an emerging animal technology", J Dairy Sci. 1992 Dec;75(12):3432-51.)

Ghrelin can of course be administered by itself to animals to caused benfeficial effects such as appetite increase. However, without being bound by theory, it is envisaged that a treatment of a milk-producing animal, such as a ruminent, such as a bovine individual, with a combination of a secretagogue and a growth hormone, such a bST, will lead to both increased milk yield and beneficial effects such as increased appetite and BMI of the individual. The secretagogue will also itself cause growth hormone release, thus prolonging the effects of the composition.

Use of porcine somatotropin (pST) in combination with a secretagogue

Porcine somatotropin (pST) also affects growth and nutrient partitioning in growing pigs (see Etherton TD, "The biology of somatotropin in adipose tissue growth and nutrient partitioning", J Nutr. 2000 Nov;130(11):2623-5). The development of techniques to produce large quantities of recombinantly derived pST enabled numerous long-term studies to be conducted in which the effects of daily pST administration could be evaluated. Collectively, these studies established that treatment of growing pigs with pST markedly stimulated muscle growth and, concurrently, reduced fat deposition. In growing pigs, maximally effective doses of pST increase average daily gain as much as 10-20%, improve feed efficiency 15-30%, decrease adipose tissue mass and lipid accretion rates by as much as 50-80% and concurrently increase protein deposition by 50%. These effects are associated with a decrease in feed intake of approximately 10-15%. These responses occur because pST has a wide array of biological effects that modulate nutrient partitioning between adipose tissue and skeletal muscle. The decrease in adipose tissue growth is due to a reduction in lipogenesis that is the consequence of pST blunting the effects of many insulin-dependent events. With respect to fatty acid synthase (FAS), a pace-setting enzyme in the lipogenic pathway, enzyme activity is markedly reduced by pST. This is the result of a pST-mediated decrease in FAS mRNA levels that occurs because FAS gene transcription is decreased. The consequence of the decrease in lipid synthesis is that adipocyte hypertrophy is impaired and, hence, tissue growth.

Without being bound by theroy, it is thus envisaged that a treatment of a meat-producing animal, such as a pig, boar or hog, with a combination of a secretagogue and a growth hormone, such a pST, will lead to both increased lean meat yield and beneficial effects such as increased appetite and BMI of the animal. The secretagogue will also itself cause growth hormone release, thus prolonging the effects of the composition.

Secretagogues

The term "secretagogue" is used herein in its normal meaning, i.e. a substance or chemical moiety capable of binding and, preferably activating the GHS 1a receptor. For example, a secretagogue according to the invention may in one embodiment be selected from the group of:
L-692-429, L-692-585 (Benzoelactam compounds)
MK677 (Spiroindaner)
G-7203, G-7039, G-7502 (Isonipecotic acid peptidomimetic)
NN703, ipamorelin
Exemplery secretagogues include ghrelin, such as human ghrelin, and ghrelin-like compounds, such as analogues and homologues of ghrelin. It is meant herein that the term "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.

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

Ghrelin-like compound

Any GHS-R1A 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:

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 occurring 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 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

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

Accordingly, the term "secretagogue" or "growth hormone secretagogue", or "GHS-R1a 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.

The present invention includes diastereomers as well as their racemic and resolved enantiomerically pure forms. GHS-R1a 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.

Further suitable GHS-R1a 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.

Methods for production of GHS-R1a secretagogues are well known to thoese skilled in the art, for example in Example 2 of PCT patent application PCT/DK2004/000519 (Gastrotech Pharma), incorporated herein by reference.

Functionality

The GHS-R1A ligands described herein 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 stimulate, partially stimulate, or inhibit receptor activity. Furthermore, the compounds may be able to modulate the activity of other GHS-R1A ligands, such as ghrelin, by for instance blocking the action of ghrelin – i.e. antagonize the effects of agonists.

Agonists of the GHS-R1A may be either full agonists, i.e. be able to fully stimulate the receptor and the signalling cascades, equal to the activities of ghrelin, or partial agonists, i.e. ligands that are only able to partially stimulate the receptor and the signalling cascade, measured as described below. Such partial agonists may also be able to fully or partially antagonize the actions of full agonists such as ghrelin.

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

One simple measure of the ability of a ligand to activate the ghrelin receptor is to measure its EC50, i.e. the dose at which the compound activates the receptor to half of the maximal obtainable effect using same compound. The receptor can either be

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

As the receptor is generally believed to be primarily coupled to the Gq signalling pathway, any suitable assay which monitors 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 GTPgS binding combined with, e.g., anti-G-alpha-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 phopholipase 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 (ERK ½, p38, junK, etc.). NF-κ-B translocation and CRE driven gene transcription may also be measured.

5) Binding of fluorescently tagged arrestin to the activated ghrelin receptor

Examples of suitable protocols for use in determining GHS-R1A ligand functionality are given in Example 5 of PCT application publication no. WO2005014032 (Gastrotech Pharma A/S).

In one embodiment the binding of a compound to the receptor GHS-R 1A is measured by the use of any of the assays described herein above.

A GHS-R1A ligand according to 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 acylated human ghrelin as determined using the assay described herein above. Greater refers to potency and thus indicates a lesser amount is needed to achieve binding inhibition.

In one embodiment of the invention, the GHS-R1A ligand 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.

In a further embodiment the dissociation constant (Kd) of the GHS-R1A ligand is less than 500 nM. In a still further embodiment the dissociation constant (Kd) 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.

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.

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.

It is to be understood that the the term "secretagogue" as used herein also encompasses variants, homologues and functional equivalents of secretagogues, such as ghrelin.

The 28 aa human ghrelin has the sequence shown in SEQ ID NO: 1, and when acylated is acylated in position 3. Homologues of ghrelin and other secretagogues will include, for example, conservative or non-conservative substitutions, as defined earlier herein. 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).

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.

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

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 96 %, such as at least 97 %, such as at least 98 %, such as at least 98 % homologous to SEQ ID NO 1.
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, rat ghrelin SEQ ID NO: 3. Other homologues are the variants described in EP 1197496 (Kangawa) incorporated herein by reference.

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 $-NH_2$ 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 oroxyproline, 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.

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.

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.

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).

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.

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.

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 a-carbonyl group of the last amino acid. Carboxy terminus protecting groups include amide, methylamide, and ethylamide.

Conjugates

The ghrelin-like compound may also be administered in a form, wherein the ghrelin-like compound is conjugated to another entity.

For example the compound may be a conjugate of ghrelin or a derivative or homologue thereof and another peptide, such as a peptide having effect on nociceptin receptor ORL1. In one embodiment the conjugate is a a conjugate of ghrelin or a derivative or homologue thereof and Ac-RYY(RK)(WI)RK)-NH_2, where the brackets show allowable variation of amino acid residues. Examples peptides in the conjugate may also be found in US patent application 2003040472

Method for production

Ghrelin-like compounds and growth hormones 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, 2 d Edition, Cold Spring Harbor Laboratory Press, 1989.

One suitable method for synthetic production of the secretagogue for use in the present invention is described in Example 2 of WO2005014032 (Gastrotech Pharma A/S).

Indications

In one embodiment of the present invention, the present invention relates to the use of a secretagogue compound in combination with a growth hormone, for the preparation of a medicament for the treatment or prophylaxis of one or more pathological conditions. These pathological conditions may relate to pathological weight or fat loss, and it is envisaged that said medicament may be for one or more of:

a) prophylaxis or treatment of cachexia, and/or
b) prophylaxis or treatment of lipodystrophy, and/or
c) stimulation of appetite, and/or
d) stimulation of food intake, and/or
e) stimulation of weight gain, and/or
f) increase of body fat mass, and/or
g) increase/maintain lean body mass

In one preferred embodiment, said medicament is to increase and/or maintain lean body mass. Other preferred combinations include, but are not restricted to,

g)+e);
c) + d);
g) + c) + d)
e) + a).

Frailty

In one embodiment of the present invention, the individual treated is classified as suffering from the condition of frailty.

Frailty is a condition characterised by impaired strength, reduced endurance, and increased vulnerability. Recently an objective definition of frailty has been proposed and validated (Fried LP et al., J Gerontol A Biol Sci Med Sci. 2001;56:M146-56). According to this definition individuals are considered frail if they meet at least three of the following five criteria:

- Unintentional weight loss.
- General feeling of exhaustion.
- Weakness.
- Slow walking speed.
- Low levels of physical activity.

These five criteria are defined herein as follows:

- Unintentional weight loss: subjects with an unintentional weight loss of more than 10 lb in the previous year are considered positive for this criterion.

- General feeling of exhaustion. Two items from the CES-D Scale are used to characterize exhaustion 1) "I felt that everything I do is an effort" and 2) "I cannot get going". Individuals are asked to indicate if they felt that way 0 (none of the time), 1 (some of the time [1-2 days a week]), 2 (a moderate amount of time [3-4 days], or 3 (most of the time). Subjects answering 2 or 3 to either of these questions are positive for the exhaustion criterion.

- Weakness. Weakness is assessed by measurement of grip strength in the dominant hand using a JAMAR handheld dynamometer set at level 2. Three attempts at maximal squeeze are recorded. The average value is adjusted

by the individual's body mass index (BMI). Those with grip strength in the bottom 20% are considered positive for the weakness criterion.

- Slow walking speed. Individuals are asked to walk 15 ft (4.5m) at their usual pace, following a standardized protocol; and time is measured by a trained examiner. Height- and sex-adjusted time points are used, with the slowest 20% being considered positive for this criterion.

- Low levels of physical activity. This criterion is based on the Modified Minnesota Leisure Time Activities questionnaire and involves self-report regarding whether a person performed any of 18 activities in the prior week, along with the frequency and duration of these activities. Kilocalories of energy expended in a week on leisure time activity are calculated. Those in the bottom quartile of physical activity are deemed positive for this criterion.

Using the above definition of frailty and excluding individuals with acute or chronic medical conditions, about 7% of the population older than 65 years of age and 20% of the population older than 80 years are frail. Frailty is considered a distinct pathophysiological condition separate from the normal aging process. Frailty is an independent predictor for deteriorating mobility, disability, hospitalization and death with hazard ratios ranging from 1.82 to 4.46, unadjusted, and 1.29-2.24, adjusted for a number of risk factors ((Fried LP; Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci. 2001;56:M146-56).

The main cause of unintentional weight loss in elderly subjects is reduced appetite and food intake. On average energy intake decreases by approximately 30% between 20 and 80 years of age (Chapman IM. Endocrinology of anorexia of ageing. Best Pract Res Clin Endocrin Metab 2004;18:437-52). In many elderly the decline in energy intake exceeds the decline in energy expenditure leading to weight loss. Aging also results in an impaired homeostatic regulation of food intake. Consequently, elderly are less likely to regain weight following an illness or an event leading to loss of body weight, such as surgery or trauma.

Weight loss in elderly subjects is characterised by a disproportionally high loss of muscle mass leading to sarcopenia, a relative deficiency of skeletal muscle. Sarcopenia reduces strength and contributes to impaired functional capacity. Sarcopenia may also occur in the absence of weight loss. Sarcopenic obese subjects have a low skeletal muscle mass with a high percent body fat. Sarcopenic obesity is associated with a particularly high risk of developing disability and dependence.

Endocrine factors may be causally involved in the development of frailty (Wilson JF. Frailty – and its dangerous effects – might be preventable. Ann Intern Med 2004;141:489-492). With increasing age growth hormone (GH) and IGF-1 levels decrease, and in males testosterone levels decreases. These changes may aggravate the physiological loss of muscle mass and strength in elderly resulting in impaired physical capacity. In addition, circulating ghrelin levels are 20-35% lower in elderly than in young subjects and a reduced GH-response to exogenous ghrelin administration has been observed in older subjects suggesting a decline in ghrelin sensitivity with increasing age ((Chapman IM. Endocrinology of anorexia of ageing. Best Pract Res Clin Endocrin Metab 2004;18:437-52). Hypoghrelinemia and ghrelin resistance may play a role in the loss of appetite and the loss of body weight seen in frail elderly.

The combined orexigenic and anabolic effects of ghrelin and GH will be beneficial in frail elderly subjects. Thus ghrelin and GH combination therapy will stimulate appetite and food intake, reverse or limit body weight loss and loss of muscle mass, reduce weakness and improve physical capacity.

For treatment of individuals suffering from the condition of frailty, it is preferred that ghrelin, or a ghrelin-like compound, is administered once or twice daily and dosage levels will vary between about 0.01 µg/kg body weight to 10 g/kg body weight daily, preferably between about 0.01 µg/kg body weight to 1 mg/kg body weight, most preferably between 0.01 to 100 µg /kg body weight. The growth hormone, preferably GH, will be administered once daily, preferable in the evening, and dosage levels will vary between 0.001 mg/kg and 10 mg/kg, preferably between 0.01 mg/kg and 1 mg/kg.

More preferably, said growth hormone is selected from the group consisting of:

- Monomeric hGH
- Dimeric hGH
- Trimeric hGH
- Tetrameric hGH
- Pentameric hGH

In another preferred embodiment, said growth hormone is selected from the group consisting of:

- Non-covalent oligomers of hGH
- Disulfide oligomers of hGH
- Covalently linked hGH
- 22K-GHBP complex
- 22K-alpha2-macroglobulin complex
- 20K-GHBP complex
- 20K-alpha2-macroglobulin complex
- hGH-V_GHBP complex
- hGH-22K
- hGH-20K
- N-alpha-acetylated hGH-22K
- Asn152-desamido-hGH-22K
- Gln-137-desamido-hGH-22K
- hGH-V or placental GH
- Glyco-hGH-V or glycosylated placental GH

In another preferred embodiment, said growth hormone has SEQ ID NO: 4-8 or is a homologue or functional equivalent thereof.

Cachexia

The word cachexia comes from the Greek kakos for "bad" and hexis for "condition." Cachexia is one of the most distressing and devastating symptoms of several severe diseases, such as cancer, robbing people of their energy, sense of well-being, and quality of life, and increasing their dependence on others. Cachexia often

accompanies malignancies of the pancreas, stomach, esophagus, lung, and intestines.

The foremost sign of cachexia is weight loss, not only of fatty tissue but also of muscle tissue and even bone. This non-fatty tissue is also known as "lean body mass." In addition, there is loss of appetite (anorexia), weakness (asthenia), and a drop in hemoglobin level (anemia).

Treatment of cachexia is not simply a matter of eating more. Even if the person wants to eat, even if he or she tries to eat, even if the person is given nutrients through a stomach tube or intravenously, the condition will normally not be reversed.

Recent research has revealed that the condition is now regarded as part of the body's reaction to the presence of the underlying disease. Recent research also indicates that, in some cases, tumors themselves produce substances that induce cachexia.

Cachexia, or wasting, as it may also be called is seen with several diseases, such as AIDS, cancer, chronic heart failure, chronic lung disease, such as COLD, COPD, liver cirrhosis, renal failure, and autoimmune diseases such as rheumatoid arthritis and systemic lupus, sepsis and severe infection. Furthermore, wasting is also seen in aging. All these indications are envisaged as being treatable using the pharmaceutical compositions and methods of the present invention.

Preferably, the growth hormone administered for treatment of cachexia is selected from the group consisting of:

- Monomeric hGH
- Dimeric hGH
- Trimeric hGH
- Tetrameric hGH
- Pentameric hGH

In another preferred embodiment, said growth hormone is selected from the group consisting of:

- Non-covalent oligomers of hGH
- Disulfide oligomers of hGH
- Covalently linked hGH
- 22K-GHBP complex

- 22K-alpha2-macroglobulin complex
- 20K-GHBP complex
- 20K-alpha2-macroglobulin complex
- hGH-V_GHBP complex
- hGH-22K
- hGH-20K
- N-alpha-acetylated hGH-22K
- Asn152-desamido-hGH-22K
- Gln-137-desamido-hGH-22K
- hGH-V or placental GH
- Glyco-hGH-V or glycosylated placental GH

In another preferred embodiment, said growth hormone has SEQ ID NO: 4-8 or is a homologue or functional equivalent thereof.

Thus, in one preferred embodiment of the present invention, the individual treated is suffering from AIDS wasting. Patients with advanced AIDS frequently experience weight loss and loss of muscle mass leading to impaired functional performance and quality of life. In AIDS patients with an unintentional weight loss of at least 10% or a body weight below 90% of the lower limit of ideal weight 12 week GH monotherapy (0.1 mg/kg/day) has been shown to induce a significant and sustained increase in lean body mass (mean gain 2.5 kg in the GH-group vs. 0.1 kg in the placebo group, $p<001$) and a reduction in fat mass (mean loss 1.7 kg in the GH-group vs. 0.3 kg in the placebo group, $p<0.001$). Physical performance as assessed by determination of work output during treadmill exercise also improved significantly by GH-therapy and was correlated with the increase in lean body mass (Schambelan M, Ann Intern Med. 1996;125:873-82).

Without being bound by theory, it is believed that the combined orexigenic and anabolic effects of ghrelin and GH combination therapy are superior to GH monotherapy in AIDS patients with unintentional weight loss.

For treatment of wasting, such as AIDS wasting or cancer cachexia, it is preferred that a ghrelin-like compound, such as ghrelin, is administered once or twice daily. Preferably, dosage levels will vary between about 0.01 µg/kg body weight to 10 g/kg body weight daily, preferably between about 0.01 µg/kg body weight to 1 mg/kg

body weight, most preferably between 0.01 to 100 µg /kg body weight. The growth hormone, preferably GH, will be administered once daily, preferably in the evening, and dosage levels will vary between 0.001 mg/kg and 10 mg/kg, preferably between 0.01 mg/kg and 1 mg/kg.

Cardiac Cachexia

In another preferred embodiment of the present invention, the individual treated is suffering from cardiac cachexia, such as due to congestive heart failure (CHF), preferably in the absence of growth hormone deficiency.

Growth hormone (GH) profoundly affects the developing and adult myocardium. Adult patients with GH deficiency (GHD) and GH excess (acromegaly) provide important models to understand the effects of GH in adult cardiac physiology (Meyers DE et al., Mayo Clin Proc 78:1521-1526). Both clinical and experimental evidence illustrates the specific physiological abnormalities that are likely associated with the excess cardiovascular mortality observed in both acromegaly and GHD. GH excess in humans is accompanied by myocardial hypertrophy, which initially enhances cardiac output but ultimately leads to disminished cardiac performance. Conversely, GH deficiency leads to reduced cardiac muscle mass and consequently to impaired cardiac function (Isgaard J 2004, Horm Res 62 Suppl 4:31-38).

GH therapy for congestive heart failure in the absence of preexisting GHD in a clinical trial has proved ineffective. The duration of treatment was 3 month and the dose was 2 IU/day (0.68 mg/day). This is a much lower dose than most other indications require – but high doses may lead to fluid retention, which are a heavy load for the heart. On the other hand CHF patients have been described to have a lower sensitivity for GH, which argue for treatment with higher doses of GH in these patients.

The combination of ghrelin and GH is an optimisation of the treatment of CHF patients, preferably under catabolic conditions. This combination will prevent the adverse events from GH treatment and result in a positive effect because of the positive effects exerted by ghrelin.

For treatment of cardiac cachexia or congestive heart failure, the dose of GH should be preferably be in the range of 0,01-0,001mg/kg/day; more preferably 0.01-0.05mg/kg/day.

More preferably, said GH is selected from the group consisting of:

- Monomeric hGH
- Dimeric hGH
- Trimeric hGH
- Tetrameric hGH
- Pentameric hGH

In another preferred embodiment, said growth hormone is selected from the group consisting of:

- Non-covalent oligomers of hGH
- Disulfide oligomers of hGH
- Covalently linked hGH
- 22K-GHBP complex
- 22K-alpha2-macroglobulin complex
- 20K-GHBP complex
- 20K-alpha2-macroglobulin complex
- hGH-V_GHBP complex
- hGH-22K
- hGH-20K
- N-alpha-acetylated hGH-22K
- Asn152-desamido-hGH-22K
- Gln-137-desamido-hGH-22K
- hGH-V or placental GH
- Glyco-hGH-V or glycosylated placental GH

In another preferred embodiment, said growth hormone has SEQ ID NO: 4-8 or is a homologue or functional equivalent thereof.

Cancer cachexia

In one embodiment of the invention the treatment with a secretagogue, such as a ghrelin-like compound, in combination with a growth hormone, is for the treatment or

prevention of cancer cachexia caused by a catabolic disorder. This is particularly experienced when the cancer is a lung cancer, a pancreatic cancer, liver cancer, other GI tract cancers,and in particular lung cancer and upper GI tract cancers.

In another embodiment of the invention the treatment with a secretagogue, such as a ghrelin-like compound, in combination with a growth hormone, is for the treatment or prevention of cancer cachexia caused by an anorectic disorder.

In yet another embodiment the treatment with a secretagogue, such as a ghrelin-like compound, in combination with a growth hormone, is for the treatment or prevention of cancer cachexia independent of the cause of cachexia, as well as for cachexia caused by a combination of the catabolic disorder and the anorectic disorder.

Another sub-group of cancer are those with anorexia caused by dysregulation of the central appetite regulatory centre in hypothalamus, where other possible reasons to eat less are excluded. In particular individuals in terminal cancer states where further cancer treatment is impossible would benefit from treatment with growth hormone and a secretagogue as a palliative treatment to increase food intake, improve the digestion and metabolism.

Accordingly, a third aspect of the invention relates to the palliative treatment of terminal cancer states in an individual in need thereof.

Without being bound by theory the rationale for the treatment with a secretagogue, in particular a ghrelin-like compound is based on the following:

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. Locally, ghrelin may act as an initiator of afferent activity in for example afferent vagal neurons. Such neurons will relay the ghrelin stimulus to centers in the CNS such as the nucleus tractus solitarirus (NTS) which further communicate with appetite and energy homeostasis regulatory centers such as the paraventricular nucleus and arcuate nucleus in the hypothalamus. As a hormone, ghrelin is believed to act on central appetite regulating POMC and NPY/AGRP neurons, which express ghrelin receptors. Most of these neurones in the arcuate nucleus as such are located inside

the blood brain barrier and is consequently not accessible to blood born messengers such as ghrelin. However, some POMC and NPY/AGRP neurons are found in the nearby median eminence a cicumventricular organ, which is clearly outside the blood brain barrier and are therefore target for hormonally transmitted ghrelin signaling from the GI tract. However, recently it has been described that ghrelin is transported across the blood brain barrier Banks, W. A. et al. J.Pharmacol.Exp.Ther. 302.2 (2002): 822-27.. It is important to note that at the central appetite regulatory center for example at the NPY / AGRP neurons – i.e. the first level neurons in the stimulatory branch of appetite control – ghrelin acting through stimulatory ghrelin receptors is the only stimulatory input known from the periphery. All other hormones and neurotransmitters: leptin, insulin, PYY3-36, a-MSH etc. act as inhibitors on the NPY/AGRP neurons in this important "appetite gate-keeping" center. Since the NPY system in down-regulated during cancer induced cachexia, Ghrelin stimulation of this system may be able to normalize the condition. Similarly the melanocortin that is active during cancer induced cachexia, may be inhibited by Ghrelin through stimulation of AgRP.

Increase in ghrelin has also been shown to increase ATCH and the following cortisol level. This action may have important beneficial implication for the treatment of cachexia as cortisol decreases the level of cytokines (IL-1β, IL-6, TNF-α, IFN-α). Administration of glucocorticoids is already widely used in the palliative setting for symptoms associated with cancer (Inui 72-91)

Furthermore, it is known that ICV injection of ghrelin has been shown to decrease core body temperature in rodents, which indicates a decrease in the REE (Lawrence CB, Endocrinology. 2002 Jan;143(1):155-62). Without being bound by theory, it is expected that ghrelin will revert the increase in REE, which is an important feature of the cachexia. The secretogogue, in particular a ghrelin-like compound, may be administered in combination with the growth hormone using any suitable regime taking into account the knowledge of the expected cancer progress as well as the anti-neoplastic therapy regime.

In one embodiment it is preferred that the secretogogue, in particular a ghrelin-like compound, and the growth hormone, are administered prophylactically for preventing the cachectic state from starting. In this embodiment, the treatment may

be started before any anti-neoplastic treatment initiates. It may be administered continuously during the anti-neoplastic treatment or it may be administered at intervals, for example between periods with anti-neoplastic therapy. By administering during and in particular between the periods of anti-neoplastic therapy, the risk that the treated individual acquires infections and other complications may be reduced due to the better health conditions.

Lipodystrophy

Lipodystrophic syndromes encompass a heterogeneous group of rare disorders characterized by partial or generalized loss of adipose tissue depots [Am J Med 2000 108, 143-152]. There are several different types of lipodystrophies and the degree of fat loss may vary from very small depressed areas to near complete absence of adipose tissue Some patients may have only cosmetic problems while others may also have severe metabolic complications such as dyslipidemia, hepatic steatosis, and severe insulin resistance [Trend Endo Meta 200011:410-416]. These disorders can either be inherited (familial or genetic lipodystrophies) or can occur secondary to various types of illnesses or drugs (acquired lipodystrophies). All these indications are envisaged as being treatable using the pharmaceutical compositions and methods of the present invention.

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

As discussed above, facilitating a weight gain and facilitating maintenance of weight, in particular in individuals suffering from a pathologically weight loss, is not only a matter of stimulating appetite and/or food intake.

Accordingly, in one aspect the present invention relates to the stimulation of appetite by administering a secretagogue in combination with a growth hormone. Stimulation of appetite does not necessarily lead to an increase in food intake, and accordingly, the present invention further relates in another aspect to the stimulation of food intake by administering a secretagogue in combination with a growth hormone.

And in a third aspect stimulation of weight gain by administering a secretagogue in combination with a growth hormone.

Preferably the combination of growth hormone and secretagogue compound is useful for stimulating food intake and weight gain, more preferably for stimulating weight gain.

As discussed below it is preferred that the combination is administered prior to a meal, such as within 45 minutes of a meal.

Furthermore, the secretagogue compound in combination with a growth hormone may be administered to facilitate maintenance of physical functioning, and/or facilitate recovery of physical function, for example in individuals recovering from major surgeries, such as insertion of a hip prosthesis, amputations, and bone fractures.

In particular the present invention is useful for treatment of under weight subjects, or for preventing loss of weight to a stage of being medically under-weight. Under-weight subjects include those having a body weight about 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.

Increasing weight or appetite can be useful for maintaining weight or producing a weight or appetite gain in an under weight subject, or in a patient having a disease or undergoing treatment that affects weight or appetite. In addition, animals, for example farm animals, such as pigs, cows, sheep and chickens, can be treated to gain weight.

Somatotropin Deficiency

The compounds and compositions of the present invention may be used for the the treatment of growth hormone deficiency syndromes, such as defined herein. Without being bound by theory, it is believed that administration of growth hormone leads to a beneficial increase in the amount of growth hormone present in the individual treated, and administraion of a secretagogue in combination further potentiates by triggering one or more effects such as appetite and food intake increases, thereby

supplying the individual with sufficient energy to respond appropriately to the growth hormone thus administered. Administration of said secretagogue will also trigger further somatostation release, thus further beneficially increasing the levels of growth hormone present within the individual.

Somatotropin deficiency exists when somatotropin is absent or produced in inadequate amounts. If other pituitary hormones are lacking, the condition is called hypopituitarism. When all the pituitary hormones are missing, the condition is termed panhypopituitarism. Somatotropin deficiency may be total (no somatotropin is produced) or partial (some somatotropin is produced, but not enough to support normal growth). Hypopituitadism may be congenital, resulting from abnormal formation of the pituitary or hypothalamus before an individual is born, or acquired, for example stemming from damage to the pituitary or hypothalamus during or after birth. Congenital hypopituitaism is present at birth, although it may not be apparent for many months. Acquired hypopituitarism may become evident any time during infancy or childhood, and may occur after severe head injury or a serious illness such as meningitis or encephalitis. Many cases of acquired hypopituitadsm result from a tumor called craniopharyngioma. This tumor may press on the hypothalamus or pituitary, causing one or more hormone deficiencies. Treatment consists of surgical removal of the tumor, which usually results in permanent hypopituitarism.

The child with somatotropin deficiency is often small, with an immature face and chubby body build. The rate of growth of all body parts is slow, so that the child's proportions remain normal. Intelligence is normal. If the child's height has been plotted on a growth chart, it will appear to be leveling off and falling away from the child's established growth curve.

If the doctor suspects that a pituitary problem may exist, blood tests can measure concentrations of hormones in the blood and the ability of the pituitary gland to respond to various stimuli. Somatotropin deficiency is moderately difficult to diagnose because the pituitary gland produces somatotropin in bursts. This means that the level of somatotropin in a single random blood sample is likely to be very low. One way of testing for somatotropin deficiency is to give the child a substance that causes the release of a somatotropin burst in normal children and measure the amount of somatotropin present in several blood samples obtained over a period of time. Since any child may not respond to any given test on a given day, more than

one stimulus may be needed to evaluate the child's ability to produce somatotropin. Several somatotropin stimulators have been identified. These include vigorous exercise and several chemicals and drugs (insulin, arginine, glucagon, L-dopa, clonidine).

Another way of testing somatotropin secretion involves hospitalizing the child and measuring the amount of somatotropin present in blood samples obtained overnight during sleep or even during an entire 24 hour period. Since about two thirds of total somatotropin production occurs during deep sleep, this test provides a better reflection of how much somatotropin the child's pituitary gland normally produces.

Somatotropin Deficiency Syndrome may also occur in adults (termed "adult somatotropin deficiency syndrome"), and a lack of response to standard somatotropin stimulation tests is required for accurate diagnosis. The diagnosis of Adult Somatotropin Deficiency may also require a prior history of childhood somatotropin deficiency or a history of organic pituitary disease. One way of diagnosing somatotropin deficiency is by measuring IGF-1 levels. This may be done using any method known to one skilled in the art. For example, IGF-1 levels can be measured using the methods of Juel et al. (Juul A, Bang P, Hertel NT, Main K, Dalggard P, Jørgensen K, Müller J, Hall K, Skakkebæk NE. Serum insulin-like growth factor-I in 1030 healthy children, adolsecents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab 1994; 78: 744-752). For a guide on "normal levels" using this method for measuring leels, see the following table:

Total IGF-I levels in healthy adults
units: ng/ml

	MEN		
Age	Mean	2SD	-2SD
20	413	677	213
25	301	496	154
25-35	209	353	103
35-45	196	327	100
45-55	164	276	81
55-65	141	251	63
65-75	129	239	52

	WOMEN		
Age	Mean	2SD	-2SD
20	385	628	202
25	291	478	149
25-35	246	404	127
35-45	189	313	96
45-55	152	262	73
55-65	156	268	74
65-75	139	252	60

Herein, it is preferred that somatotropin deficiency is defined as at least 10 % lower IGF-1 levels for a given age, sex and build, such as at least 15 % lower, such as at least 20 % lower, such as at least 25 % lower, such as at least 30 % lower, such as at least 35 % lower, such as at least 40 % lower, such as at least 50 % lower, such as at least 65 % lower, such as at least 70 % lower, such as at least 75 % lower, such as at least 80 % lower.

A decrease in the level of somatotropin in adults may result in:

- weakened heart muscle contraction and heart rate
- increased arterial plaque and blood pressure
- elevated lipids or fats in the blood:
    - cholesterol
    - low density lipoproteins (LDL)
    - triglycerides
- decreased exercise capacity due to decreased cardiac output
- decreased energy due to decreased metabolic rate
- abnormal body composition:
    - increased abdominal obesity (waist to hip ratio)
    - decreased bone density due to decreased synthesis of bone
    - increase in fractures and osteoporosis
    - decreased muscle strength and muscle size
    - decreased lean body mass
    - increased fat mass
- problem with sleep quality
- decreased social contact
- psychological changes, such as nervousness and/or shyness, sadness and/or depression
- symptoms of low blood sugar:

- weakness or tiredness
- headaches
- poor concentration or memory

One cause of low somatotropin levels is hypopituitarism, a medical condition in which the pituitary gland fails to release its hormones. The anterior lobe of the pituitary releases the hormones that control the thyroid (TSH), the adrenal glands(ACTH), and the gonads (ovaries or testes) through FSH and LH. It also releases human somatotropin. The other hormones of the pituitary are prolactin inhibiting hormone (reteards release of breast milk) and anti-diuretic hormone (which allows the concentration of urine). Hypopituitarism has many possible causes, including:

- Congenital or acquired
- Inflammation
- Surgery
- Radiation
- Autoimmune disease
- Tumour
- infection

All these indications are envisaged as being treatable using the pharmaceutical compositions and methods of the present invention.

In another preferred embodiment, the methods of the invention are used to treat individuals suffering from, or at risk of suffering from, chronic renal insufficiency. It is believed that the methods of the present invention would act to encourage growth promotion.

For the treatment or prophylaxis of Growth Hormone deficiency in children or Turner Syndrome according to the present invention, it is preferred that the maximal dose is usually 0.05 mg/kg/day GH or a functionally equivalent dose of another growth hormone. For the treatment of children short for their gestational age, it is preferred

that the treatment according to the invention comprises a 0.035mg/kg/day dose of GH or a functionally equivalent dose of another growth hormone.

For treatment of adults with growth hormone deficiency according to the present invention, it is preferred the adults who are beginning replacement therapy receive a dosage of not more than 0.006 mg/kg/d GH or a functionally equivalent dose of another growth hormone. For adult patients under 35 years of age, the preferred dose is up to 0.025 mg/kg/d GH or a functionally equivalent dose of another growth hormone, and for patients over 35 years of age, the preferred dose is up to 0.0125 mg/kg/d GH or a functionally equivalent dose of another growth hormone.

Small bowel syndrome

In another preferred embodiment of the present invention, the combination of a growth hormone with a ghrelin-like compound is used for treatment of an individual suffering from small bowel syndrome, preferably being treated one month every year with high doses of GH (0.1 mg/kg/day) or another growth growth hormone to increase absorption.

Somatotropin and ageing

Similarities have been observed between the changes associated with ageing and the symptoms of somatotropin deficiency (see Toogood AA, "Somatotropin (GH) status and body composition in normal ageing and in elderly adults with GH deficiency", Horm Res. 2003;60(Suppl 1):105-11). Changes such as increased fat mass and decreased muscle mass occur both in somatotropin -deficient patients and in otherwise healthy elderly individuals. Moreover, ageing is associated with decreasing somatotropin and insulin-like growth factor I (IGF-I) levels. Studies suggest that healthy elderly individuals have normal pituitary reserves of somatotropin, but that spontaneous somatotropin secretion falls by around 14% per decade of adult life, leading to a state of functional somatotropin insufficiency. Despite this, elderly patients with somatotropin deficiency experience reductions in somatotropin secretion and IGF-I levels, compared with controls, which are of similar magnitude to those seen in younger somatotropin -deficient adults. The metabolic changes associated with somatotropin deficiency are also seen in elderly patients compared with healthy elderly controls. Fat mass, particularly in the abdominal region, is significantly increased, with a strong correlation between fat

mass and body mass index. Markers of bone formation and resorption are significantly reduced in the somatotropin -deficient patients. Elderly adults who have hypothalamic-pituitary disease have a degree of somatotropin deficiency that can be distinguished from the decline in somatotropin and IGF-I levels that is seen with normal ageing. Somatotropin deficiency in elderly patients leads to significant changes in body composition and bone. Thus, these patients are likely to benefit from the compositions and methods of the present invention, with further beneficial effects resulting from administration of a secretagogue, such as increasing appetite and BMI in this vulnerable group of individuals .

Other pathological conditions currently treated using somatotropin

Somatotropin is known for treatment of girls suffering from Turner's syndrome, and is administered during growth years. Turner's syndrome occurs when one or a part of one X chromosome is lost from either the ovum or sperm during incorrect cell division. The incidence is 1 in 2,500 to 7,000 births. Somatotropin, androgens, and some estrogens are needed to minimize the disabilities associated with the syndrome. Somatotropin is also indicated for stunted growth caused by kidney problems. The treatment of growth failure in children caused by renal insufficiency is a stop-gap measure, since most of these children are awaiting kidney transplantation.

Somatotropin, or another growth hormone, in combination with a secretagogue, is also envisaged in one embodiment of the present invention as being beneficial for the treatment of cardiac insufficiency. Without being bound by theory, it is envisaged that the reasons for this may include the following: The interaction of somatotropin on IGF-1 (Insulin-like growth factor I) helps control heart morphology and function in adults (Nature Medicine 1996, 2, 1, 29-31 January). Somatotropin, or agonists and antagonists, may improve deteriorated cardiac performance caused by ventricular wall thinning or cardiac hypertrophy (nonproductive myocardial growth, a major risk factor for heart attacks) by encouraging useful muscle tissue growth. Without being bound by theory, it is further envisaged that the secretagogue will further trigger somatotropin release, thus prolonging any beneficial effects and having other beneficial effects such as increasing appetite, thus allowing the individual to respond fully to the released growth hormone.

Somatotropin is known for the treatment of children with idiopathic (of unknown origin) short stature, also called non-somatotropin deficient short stature. "Short stature" has been defined by the American Association of Clinical Endocrinologists and the Growth Hormone Research Society as height more than 2 standard deviations (SD) below the mean for age and sex. This corresponds to the shortest 2.3 percent of children. More preferably, the children treated are even shorter, such as more than 2.25 SD below the mean for age and sex, or the shortest 1.2% of children. In randomized, double-blind study in 71 children aged 9-15 years, patients received injections of either Humatrope (manufactured by Elli Lilly Co.) or placebo three times weekly until adult height was reached. Thirty-three patients contributed final height measurements after a mean treatment duration of 4.4 years. Mean final height of the Humatrope patients exceeded that of the placebo patients by approximately 1.5 inches. In a second study, patients received one of three increasing doses of Humatrope, in divided doses six times weekly. The average duration of treatment to final height was 6.5 years. Final height exceeded that predicted at the time of enrollment in the majority of patients, and by up to nearly four inches in some. In the high-dose group, mean final height exceeded mean height predicted at baseline by nearly three inches. Various somatotropin products are also currently indicated in children for short stature associated with growth hormone deficiency, dwarfism, growth retardation, chronic renal insufficiency, Turner syndrome, Prader-Willi syndrome, and in children born small for gestational age. All the above indications are envisaged as being treatable with the compounds and methods of the present invention.

Combination therapy

The compounds according to the invention may be administered in combination with at least one other compound. By administering compounds "in combination" is meant herein that the different compounds may be administered simultaneously, either as separate compositions or combined in a unit dosage form, or administered sequentially. Said other compound(s) may also be adminstered with the compounds of the present invention in a "kit-of-parts" system.

It is further envisaged that the compounds and/or methods of the present invention may be used in combination with at least one other therapeutic method. By carrying out this other therapeutic method "in combination" is meant herein that said other therapeutic method may be carried out before, during or after treatment with the compounds and/or methods of the present invention.

In one preferred embodiment of the present invention, the growth hormone and secretagogue may be administered to an individual in need thereof in combination with one or more other growth hormone and/or secretagogue. Without being bound by theory, it is believed that increasing the variety of these compounds will increase therapeutic efficacy.

In one preferred embodiment of the present invention, it is envisaged that the secretagogue and growth hormone may be administered in combination with any suitable therapeutic method. In one embodiment of the present invention, said therapeutic method is an anti-neoplastic therapy, including chemotherapy, radiotherapy and surgical treatment. In particular it is used in combination with chemotherapy and radiotherapy. Thus, in one embodiment the present invention relates to a method of treating cancer comprising administering an effective amount of radiotherapy and an effective amount of the pharmaceutical composition according to the invention. The treatment with the pharmaceutical composition of the present invention may be started before the radiotherapy treatment initiates. It may be administered continuously during the radiotherapy or it may be administered at intervals, for example between periods with radiotherapy therapy.

In another embodiment the present invention relates to a method of treating cancer comprising administering an effective amount of chemotherapy and an effective amount of the pharmaceutical composition according to the invention. The treatment with the pharmaceutical composition may be started before the chemotherapy treatment initiates. It may be administered continuously during the chemotherapy or it may be administered at intervals, for example between periods with chemotherapy therapy.

Furthermore, the combination treatment may be co-formulations of the secretagogue and/or growth hormone and the chemotherapy.

In another embodiment the secretagogue compound in combination with a growth hormone 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 conditions such as cachexia.

In yet another embodiment the secretagogue is administered in combination with a NSAID, such as indomethacin, and COX1 inhibitors or COX2 inhibitors. Another combination may be with erythropoietin/EPO. Another combination may be with one or more of leptin, agonists of the renin-angiotensin system, opioid receptor agonists or peroxisome proliferator-activated receptor gamma agonists.

In one preferred embodiment, in any of the treatments described herein, ghrelin or an analogue thereof may be used in combination with one or more other stomach-derived factor. This other stomach-derived factor may include any hormone, acylated or nonacylated peptide, amino acid derivative, nucleotide, fatty acid derivative, carbohydrate or other substance derived or secreted from the stomach, and may preferably (but not exclusively) be selected from the following list: pacreastatin, gastrin, resistine, prostaglandins such as prostaglandin E2 and intrinsic factor.

In addition to "stomach derived factors", ghrelin can also be used in combination with any synthetic low or high molecular weight agonist acting on the the same receptor as a "stomach derived factor", such as another secretagogue.

In addition, ghrelin and/or its analogues may be used in combination with another body weight and/or body fat inducing factor. Exemplarily mentioned factors are melanin-concentrating hormone (MCH), MCH receptors agonists, especially MCH receptor 1 agonists, neuropeptide Y (NPY), NPY receptor 1 agonists, NPY receptor 5 agonists, and NPY receptor 2 antagonists including peptide YY (PYY) and PYY (3-36), alpha-melanocyte stimulating hormone (alpha-MSH, alpha-melanocortin), melanocortin-3 receptor (MC3R) antagonists, melanocortin-4 receptor (MC4R) antagonists, agouti-related peptide (Agrp), Agrp- agonists, cocaine- and amphetamine-regulated transcript (CART) antagonists, orexin receptor 1 and

receptor 2 agonists, growth hormone (GH), GH receptor agonists, insulin-like growth factor-1 (IGF-1), and IGF-1 receptor 1 agonists, hypercaloric feeding, glucocorticoids, progestational drugs, cyproheptadine and other antiserotonergic drugs, branched-chain amino acids, prokinetic Agents (Motilin, metoclopramide, 10 mg), eicosapentanoic acid, cannabinoids, 5'-Deoxy-5-Fluorouridine, melatonin, thalidomide, ACE inhibitors and/or beta-receptor antagonists. Further ghrelin may be combined with agents used in the treatment of an underlying disease including antithyroid agents such as iodine, ^{131} Iodine, propylthiouracil, thiamazole, carbimazole, methimazole, antidiabetic agents such as insulin, sulfonylureas, metformin, acarbose, thiazolidinediones, meglitinides, antacids, H2-blockers or proton pump inhibitors.

Pharmaceutical compositions

Whilst it is possible for the compounds or salts of the present invention to be administered as the raw chemical, it is preferred to present them in the form of a pharmaceutical composition. Accordingly, the present invention further provides a pharmaceutical composition, for medicinal application, which comprises ghrelin or an analogue or pharmaceutically acceptable salt thereof, and a growth hormone or analogue and/or pharmaceutically acceptable salt thereof as herein defined, and a pharmaceutically acceptable carrier therefor. Said compositions of the present invention may preferably be delivered to an individual in any way so as to achieve a beneficial effect, preferably by stimulating appetite and/or preventing malnutrition, and/or improving the individual's sense of well-being or quality of life. In one preferred embodiment, a composition according to the present invention is administered via an oral, nasal, pulmonary, transdermal or parenteral route. More preferably, the composition is administered via the oral or pulmonary route. Other drug-administration methods, which are effective to deliver the drug to a target site or to introduce the drug into the bloodstream, are also contemplated.

In one aspect the invention relates to a pharmaceutical composition comprising a ghrelin-like compound and growth hormone as defined herein, such as wherein the bulky hydrophobic group of the ghrelin-like compound is an acyl group 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.

In another embodiment the pharmaceutical composition comprising a mixture of at least 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. In another embodiment, the pharmaceutical compositions comprises a mixture of at least two different growth hormones. Without being bound by theory it is believed that such mixtures will have a longer half-life in plasma, or will more closely resemble the natural situation.

In yet another embodiment, the pharmaceutical composition comprises acylated ghrelin-like compounds, 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 Ghrelin-like compound.

In another aspect the invention relates to a pharmaceutical composition comprising any 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 increase the half-life of the acylated secretagogue, 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.

Any suitable transport molecules known to the skilled person may be used. Examples of transport molecules may be liposomes, micelles, and/or microspheres.

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 powderlike 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 α-tocopherol and water-soluble iron-specific chelators, such as ferrioxianine, are preferred.

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".

The shape of micelles formed in dilute surfactant solutions is approximately spherical. The polar head groups of the surfactant molecules are arranged in an outer spherical shell whereas their hydrocarbon chains are oriented toward the center, forming a spherical core for the micelle. The hydrocarbon chains are randomly coiled and entangled and the micellar interior has a nonpolar, liquid-like character. In the micelles of polyoxyethylated nonionic detergents, the polyoxyethlene moieties are oriented outward and permeated by water. This arrangement is energetically favorable since the hydrophilic head groups are in contact with water and the hydrocarbon moieties are removed from the aqueous medium and partly shielded from contact with water by the polar head groups. The hydrocarbon tails of the surfactant molecules, located in the interior of the micelle, interact with one another by weak van der Waals forces.

The size of a micelle or its aggregation number is governed largely by geometric factors. The radius of the hydrocarbon core cannot exceed the length of the

extended hydrocarbon chain of the surfactant molecule. Therefore, increasing the chain length or ascending homologous series increases the aggregation number of spherical micelles. If the surfactant concentration is increased beyond a few percent and if electrolytes are added (in the case of ionic surfactants) or the temperature is raised (in the case of nonionic surfactants), the micelles increase in size. Under these conditions, the micelles are too large to remain spherical and become ellipsoidal, cylindrical or finally lamellar in shape.

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 laureate, 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.

In a preferred embodiment, the pharmaceutical composition is not immunogenic when administered to a individual for therapeutic purposes, unless that purpose is to induce an immune response.

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 an individual without the production of undesirable physiological effects such as nausea, dizziness, gastric upset and the like.

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.

The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. 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.

The pharmaceutical composition of the present invention can include pharmaceutically acceptable salts of the compounds therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide).

Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, ammonium salts and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydriodic, phosphoric, sulpfuric and nitric acids and the like. Representative examples of 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, pamoic, bismethylene salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, ethylenediaminetetraacetic (EDTA), p-aminobenzoic, glutamic, benzenesulfonic and ptoluenesulfonic 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. 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 also 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 compounds or pharmaceutical acceptable acid addition salts thereof in the context of the present invention are any hydrates (hydrated forms) thereof.

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.

Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solution and various organic solvents. 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 amines, polyoxyethylene or water.

The pharmaceutical compositions formed by combining the compounds of the invention and the pharmaceutical acceptable carriers are then readily administered in a variety of dosage forms suitable for the disclosed routes of administration. The compositions may conveniently be presented in unit dosage form by methods known in the art of pharmacy.

In a preferred embodiment of the invention the composition comprises the secretagogue or a salt thereof as a lyophilisate and the composition further comprises a solvent. In another embodiment the composition is a solution of the secretagogue or a salt thereof. Preferably, the solvent may be any suitable solvents, such as described herein, and preferably the solvent is saline or a physiological buffer like phosphate buffer.

The invention also relates to a method for preparing a medicament or pharmaceutical composition comprising an compound of the invention, comprising admixing at least one secretagogue as defined above with a physiologically acceptable carrier.

In a still further aspect, the invention relates to a pharmaceutical composition comprising, as an active ingredient, a compound as defined above or a pharmaceutical acceptable salt thereof together with a pharmaceutical acceptable carrier.

Accordingly, the composition may further include the transport molecules as described above.

Compositions for parenteral administration

The compounds of the present invention 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. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol. Examples of oily or nonaqueous carriers, diluents, solvents or vehicles include propylene glycol, polyethylene glycol, vegetable oils (e.g., olive oil), and injectable organic esters (e.g., ethyl oleate), and may contain formulatory agents such as preserving, wetting, emulsifying or suspending, stabilizing and/or dispersing agents. Alternatively, 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 the compounds of the present invention or an analogue or pharmaceutically acceptable salt thereof, (and for example antigenic epitopes and

protease inhibitors) 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.

Oils useful in parenteral compositions include petroleum, animal, vegetable, or synthetic oils. 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. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

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 detergents such as, for example, alkyl-.beta.-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

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 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 injections, immediately prior to use. Extemporaneous injection solutions and

suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

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.

Sterile injectable solutions are prepared by incorporating the compounds of the present invention , 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.

Saizen/serostim (Serono) may be administered by use of cool.click, which is a needle-free device that delivers Saizen through the skin in less than one second. The drug is delivered subcutaneously and provides the equivalent biological dose of Saizen as do needle injections. Nutropin and NutropinAq (Ipsen/Genentech) are both recombinant human growth hormone administered using is either a reconstitution system or liquid preparation.

Subcutaneous administration

It is important to note that ghrelin receptors are found in a number of places in the organism besides on the GH producing cells and in the hypothalamic centers for appetite etc. control. It the CNS these receptors are tuned to receiving signals from local ghrelin containing neurons. Peripherally secreted or artificially administered ghrelin will not reach such sites due to the blood brain barrier, however currently available so-called GH secretagouges, which are small organic compounds such as MK-0677, will pass the blood brain barrier and also reach these sites – and consequently have the danger of causing unwanted side effects. Thus such compounds which do have the advantage of being for example orally active will not be optimal for mimicking the natural pre-meal, appetite inducing surge of ghrelin, since they will reach basically all ghrelin receptors in the body. In contrast, by using the natural peptide, ghrelin itself or homologues thereof, and administering it

peripherally – as in the present invention – it is ensured that only the relevant, appetite regulating ghrelin receptors are reached and stimulated.

Furthermore, 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 belongs to the class of receptors, so-called G protein coupled receptors or 7TM receptors, that upon continued exposure to an agonist will be desentizised, 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) binding of inhibitory proteins such as arrestin (which sterically block the binding of signal transduction molecules such as G proteins). Other 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 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 ensures that the target cell is adjusted in its signal transduction system etc. to this situation.

The present invention provides a procedure for an optimal administration of ghrelin to patients in order to obtain a maximal response and avoid for example desensitization mechanisms.

Accordingly, the present invention relates in one aspect to administration of the secretagogue compound in boluses, preferably a bolus prior to each main meal. It has been found, in contrary to the prolonged administration processes in the prior art, that a bolus administration leads to not only stimulation of appetite, but also to stimulation of feed intake and more important to stimulation of weight gain.

Without being bound by theory, it is believed that premeal subcutaneous injection, intravenous injection or short-term infusions of appropriate doses of ghrelin will ensure that a robust stimulation of appetite inducing ghrelin receptors will be obtained with minimal constraint to the mobility etc. of the patient. Thus for example patients with hip fractures can in the post operative situation be treated in the premeal period and if required during the meal as such, but will be free to move around and participate in the important post operative physicotherapeutic regimens.

Suitable dosing regimens 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 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, equilibrium, and elimination of a drug.

As described above, in one aspect of the invention, the secretagogue compound is administered subcutaneously.

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

In a preferred embodiment the secretagogue compound is administered subcutaneously in a premeal bolus.

The secretagogue compound can also be administered during a meal as a bolus. The mode of administration during a meal includes subcoutaneous administration, such as a subcoutaneously administered bolus.
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 ghrelin to ensure robust and appropriate appetite stimulation without causing 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.

Accordingly, it is preferred that the secretagogue according to the invention 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.

The present invention preferably deals with methods for administering a secretagogue 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 fragile elderly, post operative patients, patients with lost appetite as part of cachexia for example precipitated by cancer, cardiac disease etc. with a sufficient extra stimulatory input to their appetite regulating ghrelin receptors, which normally are reached by ghrelin in the pre-meal situation.

Compositions for administration as suppositories

The compounds of the present invention may 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.

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%].

Compositions for aerosol, nasal or inhalation delivery

It is contemplated that the compounds may be formulated for administration 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.

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

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.

Pharmaceutically acceptable salts

Pharmaceutically acceptable salts of the instant compounds, where they can be prepared, are also intended to be covered by this invention. These salts will be ones which are acceptable in their application to a pharmaceutical use. By that it is meant that the salt will retain the biological activity of the parent compound and the salt will not have untoward or deleterious effects in its application and use in treating diseases.

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

The compounds of the invention may be administered in the form of an alkali metal or earth alkali metal salt thereof, concurrently, simultaneously, or together with a pharmaceutically acceptable carrier or diluent, especially and preferably in the form of a pharmaceutical composition thereof, whether by oral, rectal, or parenteral (including subcutaneous) route, in an effective amount.

Examples of pharmaceutically acceptable acid addition salts for use in the present inventive pharmaceutical composition include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, p-toluenesulphonic acids, and arylsulphonic, for example.

Administration – dosing regimes

The pharmaceutical preparations described herein are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. When desired, compositions can be prepared with enteric coatings adapted for sustained or controlled release administration of the active ingredient.

In one aspect of the present invention, a suitable dose of the compositions described herein is administered in pharmaceutically effective amounts to an individual in need of such treatment. Herein, “pharmaceutically effective amounts”, is defined as 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. The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of a compound, alone or in combination with other agents, calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier, or vehicle. The specifications for the unit dosage forms of the present invention depend on the particular compound or compounds employed and the effect to be achieved, as well as the pharmacodynamics associated with each compound in the host. The dose administered should be an " effective amount" or an amount necessary to achieve an "effective level" in the individual patient.

The dosage requirements will vary with the particular drug composition employed, the route of administration and the particular subject being treated. Ideally, a patient to be treated by the present method will receive a pharmaceutically effective amount of the compound in the maximum tolerated dose, generally no higher than that required before drug resistance develops. Suitable dosing regimens 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 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, equilibrium, and elimination of a drug.

The dosage must be adjusted to the need of the individual patient. A preferred dosage of a composition employed according to the invention is in a concentration equivalent to from about 0.1 mg to about 10 mg ghrelin per kg bodyweight, which is preferably administered daily. More preferably, the medicament comprises a unit dosage form of from about 5 to about 250 mg of ghrelin or an analogue thereof, more preferably from about 20 mg to about 200 mg, more preferably from about 20 mg to about 100 mg. The secretagogues of the present invention may be administered admixed with a pharmaceutically acceptable carrier or diluent.

A preferred dosage of a composition employed according to the invention is in a concentration equivalent of the secretagogue from about 0.1 mg to about 10 mg ghrelin per kg bodyweight, which is preferably administered daily. More preferably, the medicament comprises a unit dosage form of from about 5 to about 250 mg of ghrelin or an analogue thereof, more preferably from about 20 mg to about 200 mg, more preferably from about 20 mg to about 100 mg. The secretagogues of the present invention may be administered admixed with a pharmaceutically acceptable carrier or diluent.

A further preferred dosage of a composition employed according to the invention is in a concentration equivalent of the growth hormone from about 0.1 µg to about 10 mg growth hormone per kg bodyweight, which is preferably administered daily. More preferably, the medicament comprises a unit dosage form of from about 5 to about 250 mg of growth hormone or an analogue thereof, more preferably from about 20 mg to about 200 mg, more preferably from about 20 mg to about 100 mg. The growth hormones of the present invention may be administered admixed with a pharmaceutically acceptable carrier or diluent.

It is recommended to start treatment with a low dose 0.15-0.3 mg/day. It is recommended to increase the dosage gradually at monthly intervals based on the clinical response and the patient's experience of adverse events and IGF-I level.

For treatment of a cachetic condition, it is preferred in one embodiment that the dosage of a growth hormone is high, to provide maximum effect. Said dosage is preferably 0.1 µm/kg/day- 1 g/kg/day, such as 1 µm/kg/day- 10 mg/kg/day, such as 5 µm/kg/day- 1 mg/kg/day, most preferably around 0.1 mg/kg/day.

It should be noted that the normal ghrelin response which occurs before a meal is a short-lived surge in plasma concentrations of ghrelin and that due to the relative short half life of the peptide an i.v. injection of ghrelin will ensure that a similar short-lived peak on ghrelin concentrations can be obtained. The administration route must ensure that the non-degraded, bioactive form of the peptide will be the dominating form in the circulation, which will reach the ghrelin receptors and stimulate these. Thus, in order to obtain the maximum effect of the medicament it is preferably administered from one to three times daily, each administration being within 90 minutes of a meal, such as within 85 minutes of a meal, such as within 80 minutes of a meal, such as within 75 minutes of a meal, such as within 70 minutes of a meal, such as within 65 minutes of a meal, such as within 60 minutes of a meal, such as within 55 minutes of a meal, such as within 50 minutes of a meal, such as within 45 minutes of a meal, such as within 40 minutes of a meal, such as within 35 minutes of a meal, such as within 30 minutes of a meal, such as within 25 minutes of a meal, such as within 20 minutes of a meal, such as within 15 minutes of a meal, such as within 10 minutes of a meal, such as within 5 minutes of a meal. More preferred the

medicament is administered prior to each main meal, such as administered three times daily.

For the present invention the dosage will vary depending on the compound employed and the mode of administration. Dosage levels of the growth hormone may for example vary between about 0.01 µg/kg body weight to 10 mg/kg body weight daily, preferably between about 0.01 µg/kg body weight to 1 mg/kg body weight, more preferably between 0.1 to 10 µg /kg body weight. For all methods of use disclosed herein for the compounds, the daily oral growth hormone dosage regimen will preferably be from about 0.01 µg to about 80 mg/kg of total body weight. The daily parenteral growth hormone dosage regimen about 0.01 µg to about 80 mg/kg of total body weight. The daily topical growth hormone dosage regimen will preferably be from 0.01 µg to 150 mg, administered one to four, preferably two or three times daily. The daily inhalation growth hormone dosage regimen will preferably be from about 0.01 µg /kg to about 1 mg/kg per day.

It will also be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound or a pharmaceutically acceptable salt thereof will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a compound or a pharmaceutically acceptable salt thereof given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

Furthermore, since the "effective level" is used as the preferred endpoint for dosing, the actual dose and schedule can vary, depending on interindividual differences in pharmacokinetics, drug distribution, and metabolism. The "effective level" can be defined, for example, as the blood or tissue level desired in the patient that corresponds to a concentration of one or more compounds according to the invention.

Medical packaging

In one embodiment of the present invention, a medical packaging is provided, said medical packaging comprising one or more predefined dosage units of the pharmaceutical composition described above. In one preferred embodiment of the present invention, said packaging comprises from one to three dosage units, such as one, two or three dosage units. In another, equally preferred, embodiment of the present invention, said packaging has from 7 to 21 dosage units, such as 7, 14, or 21 dosage units. In another preferred embodiment of the present invention said dosage unit comprises an amount of the secretagogue, such as a ghrelin-like compound 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, 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 preferred embodiment of the present invention, said dosage unit comprises an amount of growth hormone or a salt thereof equivalent to from 0.3 μg to 600 mg growth hormone, such as of from 2.0 μg to 200 mg growth hormone, such as from 5.0 μg to 100 mg growth hormone, such as from 10 μg to 50 mg growth hormone, such as from 10 μg to 5 mg growth hormone, such as from 10 μg to 1.0 mg growth hormoneIt is further preferred that the medical packaging ot the present invention comprises instructions for administering the pharmaceutical composition. Said instructions preferably include instructions referring to administration of said pharmaceutical composition during a meal or at the most 90 minutes prior to a meal, such as at the most 45 minutes prior to a meal, such as at 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 of a meal, such as immediately prior to a meal. In another preferred embodiment of the present invention, said packaging is in the form of a cartridge, such as a cartridge for an injection pen.

Methods for treatment and monitoring effect of treatment

In another aspect of the present invention is provided a method for treating and/or preventing a pathological condition, comprising administering to an individual in need thereof an effective amount of a ghrelin-like compound as defined herein in combination with a growth hormone as defined herein. In one preferred

embodiment, said said pathological condition is selected from cachexia, HIV, AIDS, cancer cachexia, anorectic disorder. In another embodiment, said pathological condition is caused by a growth hormone deficiency and/or a growth disorder. Said administration may be in combination with an effective amount of another medicament, such as an NSAID medicament. Furthermore, in another aspect of the present invention is provided a method for monitoring the effect of a treatment of an individual with a ghrelin-like compound in combination with a growth hormone, comprising measuring the blood level in said individual of IGF-1, IGFBP-3, and/or ALS.

Claims

1. Use of a secretagogue, or pharmaceutically acceptable salt thereof, in combination with a growth hormone, or pharmaceutically acceptable salt thereof, for the preparation of a medicament for treatment of an individual in need thereof.

2. Use according to claim 1, wherein said medicament is for one or more of

prophylaxis or treatment of cachexia, and/or

prophylaxis or treatment of lipodystrophy, and/or

stimulation of appetite, and/or

stimulation of food intake, and/or

stimulation of weight gain, and/or

increasing body fat mass and/or

increasing or maintaining lean body mass

in an individual in need thereof,

3. Use according to any of claims 1-2, wherein said medicament is for the treatment of an individual suffering from cachexia.

4. Use according to claim 3, wherein said cachexia is associated with HIV or AIDS, such as AIDS wasting.

5. Use according to claim 3, wherein said cachexia is cardiac cachexia.

6. Use according to claim 3, wherein said cachexia is cancer cachexia.

7. Use according to any of claims 5-6, wherein said individual is suffering from a cancer selected from lung cancer, pancreatic cancer, liver cancer, and a gastrointestinal tract cancers.

8. Use according to any of claims 6 or 7, in combination with an anti-neoplastic treatment.

9. Use according to claim 8, wherein said anti-neoplastic treatment is a chemotherapy medicament and/or radiotherapy.

10. Use according to any of claims 1-2, wherein said medicament is for the treatment of dwarfism and/or growth retardation.

11. Use according to claim 10, wherein said dwarfism and/or growth retardation is caused by the individual having insufficient physiological levels of growth hormone.

12. Use according to any of the preceding claims, wherein said growth hormone is a mammalian growth hormone, or a homologue, variant or functional equivalent thereof.

13. Use according to any of the preceding claims, wherein said growth hormone is a growth hormone of a domestic animal, or a homologue, variant or functional equivalent of said growth hormone.

14. Use according to any of claims 1-13, wherein said growth hormone is human growth hormone, or a homologue, variant or functional equivalent thereof.

15. Use according to any of the preceding claims, wherein said growth hormone is selected from Somatotropin or any isoform thereof, Thyroid Stimulating Hormone (TSH), Adrenocorticotropic Hormone (ACTH), Luteinizing Hormone (LH) and/or Follicle Stimulating Hormone (FSH)or a homologue, variant or functional equivalent thereof.

16. Use according to any of the preceding claims, wherein said growth hormone comprises a recombinant polypeptide.

17. Use according to any of preceding claims, wherein said growth hormone is selected from the group consisting of:

- Monomeric hGH
- Dimeric hGH
- Trimeric hGH
- Tetrameric hGH
- Pentameric hGH

18. Use according to any of the preceding claims, wherein said growth hormone is selected from the group consisting of:

- Non-covalent oligomers of hGH
- Disulfide oligomers of hGH
- Covalently linked hGH
- 22K-GHBP complex
- 22K-alpha2-macroglobulin complex
- 20K-GHBP complex
- 20K-alpha2-macroglobulin complex
- hGH-V_GHBP complex
- hGH-22K
- hGH-20K
- N-alpha-acetylated hGH-22K
- Asn152-desamido-hGH-22K
- Gln-137-desamido-hGH-22K
- hGH-V or placental GH
- Glyco-hGH-V or glycosylated placental GH

19. Use according to any of the preceding claims, wherein said growth hormone is selected from the group consisting of SEQ ID NO: 4-8 or a homologue thereof.

20. Use according to any of the preceding claims, wherein said secretagogue is ghrelin or a pharmaceutically acceptable salt thereof.

21. Use according to claim 20, wherein said ghrelin is human ghrelin, or a pharmaceutically acceptable salt thereof.

22. Use according to any of claims 1-19, wherein said secretagogue is a ghrelin-like compound or pharmaceutically acceptable salt thereof.

23. Use according to claim 22, wherein said 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.

24. Use according to claim 23, 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.

25. Use according to any of claims 23-24, wherein X^2 is selected from the group of modified Ser, modified Cys and modified Lys, such as wherein X^2 is modified Ser.

26. Use according to any of claims 23-25, wherein the ghrelin-like compound is selected from a compound of

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

formula III Z^1 – Gly- Ser – (X^2) – $(X^3)_n$- Z^2, and

formula IV Z^1 – Gly – (X^2) – $(X^3)_n$- Z^2.

27. Use according to claim 26, wherein the ghrelin-like compound is having formula III.

28. Use according to any of claims 23-27, wherein $(X^3)_n$ comprises a sequence selected from one or more of the sequences shown below:

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

Phe Leu Ser Pro Glu

Phe Leu Ser Pro

Phe Leu Ser

Phe Leu

Phe

29. Use according to any of claims 23-27, 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.

30. Use according to any of claims 23-29, wherein the acyl group is a C1-C35 acyl group.

31. Use according to any of claims 23-30, wherein the medicament is in a formulation for subcutaneous administration.

32. Use according to any of claims 23-30, wherein the medicament is in a formulation for parenteral administration.

33. Use according to any of claims 23-30, wherein the medicament is in a formulation for nasal administration.

34. Use according to any of the preceding claims, wherein the medicament is in a formulation for pulmonary administration.

35. Use according to any of the preceding claims, wherein the formulation comprises the secretagogue compound or a pharmaceutically acceptable salt thereof.

36. Use according to any of the preceding claims, wherein the formulation comprises the secretagogue compound, and/or
the growth hormone, and/or one
or more salt(s) thereof,
as a lyophilisate, and the formulation further comprises a solvent, said lyophilisate and said solvent being in separate compartments until administration.

37. Use according to any of the preceding claims, wherein the formulation is a solution of the secretagogue compound or a salt thereof and/or the growth hormone or a salt thereof.

38. Use according to any of claims 36-37, wherein the solvent is saline.

39. Use according to any of the preceding claims, wherein the medicament is administered before a meal or during the intake of a meal.

40. Use according to any of the preceding claims, wherein the medicament is administered in a concentration equivalent of the secretagogue of from 10 ng to 10 mg ghrelin per kg body weight.

41. Use according to any of the preceding claims, wherein the medicament is administered in a concentration equivalent of the secretagogue of 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.

42. Use according to any of the preceding claims, wherein the medicament is administered as a bolus injection prior to or during the intake of a meal, said bolus injection comprising an amount of the secretagogue compound or a salt thereof equivalent to from 0.3 μg to 600 mg ghrelin.

43. Use according to any of the preceding claims, wherein the medicament is administered as a bolus injection prior to or during the intake of a meal, said bolus injection comprising an amount of the secretagogue compound 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.

44. Use according to any of the preceding claims, wherein the medicament is administered as a bolus injection prior to or during the intake of a meal, said

bolus injection comprising an amount of the growth hormone or a salt thereof equivalent to from 2.0 μg to 200 mg somatotropin, such as from 5.0 μg to 100 mg somatotropin, such as from 10 μg to 50 mg somatotropin, such as from 10 μg to 5 mg somatotropin, such as from 10 μg to 1.0 mg somatotropin.

45. Use according to any of the preceding claims, wherein the medicament is ad ministered from one to three times daily, each administration being 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, such as 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.

46. Use according to any of the preceding claims, 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.

47. Use according to any of the preceding claims, wherein the medicament is administered in a dosage comprising an amount of the growth hormone or a salt thereof equivalent to from 0.3 μg to 600 mg somatotropin.

48. Use according to claim any of the preceding claims, wherein the medicament is administered in a dosage comprising an amount of the secretagogue or a salt thereof equivalent to from 0.3 μg to 600 mg ghrelin.

49. A pharmaceutical composition comprising a growth hormone as defined according to any of claims 12 to 19, and a secretagogue compound as defined

according to any of claims 20-30 and/or one or more pharmaceutically acceptable salt(s) thereof and pharmaceutically acceptable carriers, vehicles and/or excipients.

50. The pharmaceutical composition according to claim 49, wherein said composition further comprises transport molecules, such as liposomes, micelles, iscoms, and/or microspheres.

51. A medical packaging comprising one or more dosage units of the pharmaceutical composition described in any of claims 49-50.

52. The medical packaging according to claim 51, said packaging comprising from one to three dosage units, such as three dosage units.

53. The medical packaging according to claim 51, said packaging having from 7 to 21 dosage units.

54. The medical packaging according to claim 51, having 7, 14, or 21 dosage units.

55. The medical packaging according to any of the claims 51 to 54, wherein said dosage unit comprises an amount of the secretagogue compound 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, 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.

56. The medical packaging according to any of the claims 51 to 55, wherein said dosage unit comprises an amount of the growth hormone or a salt thereof equivalent to from 0.3 μg to 600 mg growth hormone, such as of from 2.0 μg to 200 mg growth hormone, such as from 5.0 μg to 100 mg growth hormone, such as from 10 μg to 50 mg growth hormone, such as from 10 μg to 5 mg growth hormone, such as from 10 μg to 1.0 mg growth hormone.

57. The medical packaging according to any of the claims 51 to 56, comprising instructions for administering the pharmaceutical composition.

58. The medical packaging according to claim 57, wherein said instructions include instructions referring to administration of said pharmaceutical composition during a meal or at the most 90 minutes prior to a meal, such as at the most 45 minutes prior to a meal, such as at 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 of a meal, such as immediately prior to a meal.

59. The medical packaging according to any of claims 51-58, wherein the packaging is in the form of a cartridge, such as a cartridge for an injection pen.

60. A method for treating and/or preventing a pathological condition, comprising administering to an individual in need thereof an effective amount of a secretagogue compound as defined according to any of claims 20-30 in combination with a growth hormone as defined according to any of claims 12-19.

61. Method according to claim 60, wherein said pathological condition is selected from AIDS wasting, cardiac cachexia or the condition or frailty.

62. Method according to claim 60, wherein said pathological condition is caused by a growth hormone deficiency and/or a growth disorder.

63. Method of claim 60, comprising administering to an individual in need thereof an effective amount of the pharmaceutical composition described in any of claims 49-50 in combination with an effective amount of a NSAID medicament.

64. A method for monitoring the effect of a treatment of an individual with a secretagogue compound in combination with a growth hormone as defined in any of claims 12-19, comprising measuring the blood level in said individual of IGF-1, IGFBP-3, and/or ALS.

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SEQ ID NO: 8

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