Title: USES OF GROWTH HORMONE SECRETAGOGUES IN THE TREATMENT OF INDIVIDUALS SUFFERING FROM RENAL AND/OR LIVER FAILURE

Abstract: The invention relates to the use of a secretagogue compound for the preparation of a medicament for treatment of an individual suffering from renal failure and/or liver failure. Furthermore, the invention relates to a method for stimulating appetite, food intake and/or weight gain in an individual suffering from liver failure and/or renal failure, said method comprising administration of a secretagogue to said patient.
Uses of growth hormone secretagogues in the treatment of individuals suffering from renal and/or liver failure

This application claims priority from Danish patent application numbers PA 2004 01654 and PA 2004 01655, both filed 27th October 2004, which are hereby incorporated by reference in their entirety. All patent and non-patent references cited in these and the present application, are also hereby incorporated by reference in their entirety.

Field of the invention
The present invention relates to agents, compositions and methods for the stimulation of appetite, food intake and/or weight gain in individuals suffering from, or at risk of suffering from, renal failure and/or liver failure.

Background of invention
Ghrelin is a bioactive peptide which originally was described to be involved in the control of GH secretion but later found to be a major regulator of appetite, food intake and energy homeostasis (Kojima M et al., Trends Endocrinol Metab 12:118-122; Nakazato M et al., 2001, Nature 409:194-198). Similar to many other bioactive peptides, ghrelin probably acts both as a hormone, a paracrine substance and as a neurotransmitter. The story of ghrelin, its receptor and synthetic compounds acting through this receptor unraveled in a unique "reverse" order. In the eighties a synthetic hexa-peptide from a series of opioid-like peptides was found to be able to 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.

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

Renal failure

Patients with chronic renal failure often present severe symptoms from the upper
gastrointestinal tract such as vomiting, abdominal pain and early satiety. The
pathophysiological mechanisms behind these symptoms are unclear, but delayed
gastric emptying has been proposed as one of the contributing factors. Previous
studies have yielded conflicting results, although a single study has shown
correlation between GI symptoms and the delay in gastric emptying (Strid H,
Simren M, Stotzer PO, Abrahamsson H, Bjornsson ES. Delay in gastric emptying in
subgroup of patients with hypoalbuminemia who are unresponsive to dialysis and
adequate prescription of dietary intake may have delayed gastric emptying (Silang
R, Regalado M, Cheng TH, Wesson DE. Prokinetic agents increase plasma albumin
in these patients with delayed gastric emptying. Am.J.Kidney Dis. 2001;37(2):287-
93.).

The delay in gastric emptying is not correlated to any specific biochemical
parameter but is closely associated to the feeling of malaise and tiredness.
Liver Failure

Liver failure is a state in which the liver is unable to adequately perform its functions. Chronic liver failure may be caused by e.g. alcoholic liver disease, viral hepatitis, hemochromatosis, drug-induced hepatitis, Wilson's disease, sclerosing cholangitis, primary biliary cirrhosis, Budd-Chiari syndrome, venoocclusive disease, autoimmune hepatitis, steatohepatitis, partial hepectectomy, or Reye's syndrome.

Alcoholic liver failure, hepatitis C and drug induced liver failure constitute the three major groups of patients with liver failure. They all present a large number of gastrointestinal complaints including abdominal pain, early satiety, nausea, vomiting, abdominal distension and anorexia. In some cases these symptoms may be explained by the presence of organic disorders such as peptic ulcer disease or gastroesophagal reflux disease. However in many patients with cirrhosis no obvious cause is apparent for the gastrointestinal symptoms (Verne GN, Soldevia-Pico C, Robinson ME, Spicer KM, Reuben A. Autonomic dysfunction and gastroparesis in cirrhosis. J.Clin.Gastroenterol. 2004;38(1):72-6.).

Biliary atresia is the most common cause of liver failure in children. Other causes include hepatitis, Alagille's syndrome, familial intrahepatic cholestasis and metabolic diseases, such as α-1-antitrypsin deficiency, tyrosinaemia, type 1, Wilson's disease, cystic fibrosis, or glycogen storage disease type IV.

A person with liver failure may present with jaundice, malaise, fatigue, weakness, nausea, anorexia, pruritus, increased bleeding tendency, esophageal varices, ascites, hepatic encephalopathy, and generally failing health. In children liver failure is often complicated by failure to thrive and growth failure.

Liver failure is diagnosed by assessment of liver function tests, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, albumin, and bilirubin. Liver biopsy is the definitive test for determining the cause of liver failure.

The etiology of anorexia in chronic liver failure is not fully clarified, but may involve elevated cytokine levels, gastroparesis, malaise, and satiety caused by ascitis or hepatomegaly.
Prolonged gastrointestinal transit and delayed gastric emptying may be responsible for part of the feeling of satiety. Importantly, paracentesis to empty ascetic fluid does not alter the delayed gastric emptying but it decreases the feeling of satiety (Scolapio JS, Ukleja A, McGreevy K, Burnett OL, O'Brien PC. Nutritional problems in end-stage liver disease: contribution of impaired gastric emptying and ascites. J.Clin.Gastroenterol. 2002;34(1):89-93.). The presence of autonomic dysfunction is positively correlated to the delay in gastric emptying but it is probably not the only cause of the gastroparesis.

The initiation of delayed gastric emptying is not correlated to any specific biochemical parameter but is closely associated to the feeling of malaise and tiredness.

Treatment of liver failure is aimed at alleviating complications and preventing progressive liver damage. Commonly used therapies include colestyramine, ondansetron, rifampicin, variceal sclerotherapy, lactulose, metronidazole, ascitis paracentesis, sodium and fluid restriction, spironolactone. In patients with fulminant liver failure, transplantation remains the only therapeutic option.

Summary of the Invention
In a first aspect, the invention relates to the use of a growth hormone-(GH-) secretagogue compound for the preparation of a medicament for the stimulation of appetite in an individual suffering from renal failure and/or liver failure. Stimulation of appetite does not necessarily lead to an increase in food intake or stimulate weight gain and accordingly, the present invention further relates to another aspect: the stimulation of food intake and/or weight gain by administering a GH-secretagogue, such as a ghrelin-like compound.
Furthermore, the invention relates to a method for stimulating appetite, food intake and/or weight gain in an individual suffering from liver failure and/or renal failure, said method comprising administration of a GH-secretagogue to said patient.

Accordingly, the invention relates to the use of a GH-secretagogue compound for the preparation of a medicament for
a) stimulation of appetite, and/or
b) stimulation of food intake, and/or
c) stimulation of weight gain, and/or
d) increasing body fat mass, and/or
e) increasing lean body mass, and/or
f) increasing the muscle strength and/or
g) increasing the muscle perseverance, and/or
h) improving Quality of Life (QoL), and/or
i) decreasing nausea

including any combination of the above,

by administering a dosage of said medicament in an individual, wherein the individual is suffering from renal failure.

Preferred combinations are: a); b); c); d); e); f); g); h); and i); in isolation; as well as
a) + b); a) + c); a) + d); a) + e); b) + c); b) + d); b) + e); d) + e); c) + d) + e); a) + c) + d); a) + c) + e); a) + d) + e); a) + c) + d) + e); b) + c) + d); b) + c) + e); b) + d) + e); and b) + c) + d) + e); a) + b) + i); f) + g) + h); e) + f) + g) + h); c) + f); e) + f); c) + g); a) + h); d) + i); e) + i); h) + i). Particularly preferred combinations are a), b) and d); a) and d); and b) and d).

In another aspect, the invention relates to the use of a GH-secretagogue compound for the preparation of a medicament for the stimulation of gastric emptying in an individual suffering from liver failure and/or renal failure, in particular gastric emptying in relation to meals.

Without being bound by theory it is believed that ghrelin effects gastric emptying.

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

The orexigenic and metabolic effects of GH-secretagogues, such as ghrelin, reduce the morbidity and mortality in patients suffering from liver failure and/or renal failure.

Furthermore, these effects improve their quality of life.
Detailed Description of the Invention

Definitions

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

Amino Acid Residue: An amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are preferably in the "L" isomeric form. However, the amino acid encompasses every 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>
<thead>
<tr>
<th>1-Letter</th>
<th>3-Letter</th>
<th>AMINO ACID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>Tyr</td>
<td>tyrosine</td>
</tr>
<tr>
<td>G</td>
<td>Gly</td>
<td>glycine</td>
</tr>
<tr>
<td>F</td>
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<td>lysine</td>
</tr>
<tr>
<td>H</td>
<td>His</td>
<td>histidine</td>
</tr>
</tbody>
</table>
Q  Glu  glutamine
E  Glu  glutamic acid
Z  Glx  Glu and/or Gln
W  Trp  tryptophan
5  R  Arg  arginine
D  Asp  aspartic acid
N  Asn  asparagine
B  Asx  Asn and/or Asp
C  Cys  cysteine
10  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.

Appetite: Appetite in an individual is assessed by measuring the amount of food ingested and by assessing the individual's desire to eat. Appetite (for example hunger) is typically assessed with a short questionnaire given to individuals on a random basis several times a week. Typically, subjects rate their hunger, preoccupation with food, and desire to eat greater quantities and different types of food by answering the questions using analogue scales ranging from 1, not at all, to 5, extremely.

30  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 19-25.

Body fat mass: Body fat mass can be measured e.g. by the fat fold technique: In this technique, a pincer-type caliper is used to measure subcutaneous fat by determining
skin fold thickness at representative sites on the body. These skin fold measurements are then used to compute body fat by either adding the scores from the various measurements and using this value as an indication of the relative degree of fatness among individuals or by using the measurements in mathematical equations that have been developed to predict percent body fat. Body composition can also be assessed by Dual Energy X-ray Absorptiometry (DEXA) scanning, a non-invasive test which accurately quantifies the lean body mass, total fat mass and regional body fat (e.g. abdominal fat).

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

Dissociation constant, Kd: a measure to describe the strength of binding (or affinity or avidity) between receptors and their ligands, for example the GHR1a receptor and a ghrelin-like compound. The smaller 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.


Ghrelin-like compound: the term "ghrelin-like compound" as used herein refers to any compound which mimics the function of wild-type ghrelin, such as any of the compounds described herein, such as further described in the section below entitled "Functionality", for example wild-type human ghrelin. The ghrelin-like compound may be defined by the formula I:

\[ Z^1 \cdot (X^1)_m \cdot (X^2)_n \cdot Z^0, \] 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.

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

GHS: growth hormone secretagogue

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

Individual: A living animal or human. In preferred embodiments, the subject is a mammal, including humans and non-human mammals such as dogs, cats, pigs, cows, sheep, goats, horses, rats, and mice. In the most preferred embodiment, the subject is a human.
Isolated: is used to describe the various ghrelin-like compounds, polypeptides and nucleotides disclosed herein, that have been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified.

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

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

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

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

3) Computed Tomography (CT) provides unique direct information on body composition and permits quantification of all major tissue system level components (adipose tissue, skeletal muscle, visceral organs and bone) (Hemmingsson,A et al., 1982, Acta Radiol 23:149-151). CT moreover has the advantage of permitting the partition of the total adipose tissue (AT) mass into its subcutaneous and visceral components (Tokunaga,K., et al., INT.J.OBES. 7:437-445). This leaves CT with a significant advantage for body composition studies because of its potential for monitoring changes in the visceral AT and subcutaneous AT compartments.
separately, information, which is at present not available with any alternative in vivo technique except for MRI. CT scan for the assessment of body composition can be performed as either multi-slice covering the whole body and wherefrom calculations of whole body and regional AT can be derived. The CT method has been extensively validated. Animal and human cadaver studies have been used to verify the accuracy of the AT-estimate and its anatomical distribution showing a high correlation and therefore by some suggested as a "gold standard" against which to compare other non-invasive methods (Kvist, H., et al., 1988, INT.J.OBES. 12:249-266). At present, the most accurate in vivo methods of measuring body composition are multi-slice MRI and CT (Rossner, S., 1990, INT.J.OBES. 14:893-902). The highest precision is obtained using multi-slice CT scans (CV<1%) (Jensen, M.D., et al., 1995, American journal of clinical nutrition 61:274-278). However, due to radiation exposure, multi-slice CT is unsuitable for studies requiring repeated measurements on the same subject. A regional scan assessing the AT at the abdominal level is highly correlated with total AT mass in both men and women (r=0.92 and 0.97, respectively) (Despres, J.P., et al., American journal of clinical nutrition 54:471-477; Koester, R.S., et al., 1992, Int J Obes Relat Metab Disord 16:543-554; Lemieux, S., et al., 1993, American journal of clinical nutrition 58:463-467).

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

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

6) Dual energy X-ray Absorptiometry ("DXA" or "DEXA"): DXA is a direct, operator independent, non-invasive method to estimate body composition. Measurements are based on the differential attenuation of two X-rays as they pass through the body. It distinguishes bone mineral from soft tissue and subsequently divides the
latter into FM and FFM (Pietrobelli,A., et al., 1996, *Am J Physiol* 271:E941-E951). The analyzed data yield information about composition of the whole body but also permits regional body composition determination. DXA has been widely validated and appear to be a precise (CV~1% (bone), CV~3% (LBM and FM)) and simple way of measuring total and regional FM and LBM. Exposure to radiation is minimal (2-5 μSv) in most DXA machines.

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

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

Monoclonal Antibody: The phrase monoclonal antibody in its various grammatical forms refers to a population of antibody molecules that contains only one species of antibody combining site capable of immunoreacting with a particular antigen.

Muscle strength: Muscle strength is the maximal force that a muscle can exert. It is usually measured by determination of maximum voluntary isometric contraction, e.g. the handgrip strength test. Muscle strength can also be assessed by measurement of isokinetic strength of isolated muscles/muscle groups, such as the knee extension/flexor muscles.

Muscle perseverance: Muscle endurance is the ability of a muscle to exert repeated submaximal contractions over time. Muscular endurance can be assessed by a multitude of tests, such as a test consisting of dynamic contractions of the quadriceps muscle at a given percentage of maximal voluntary contraction at a regular pace until exhaustion. One method of measuring muscle endurance is to measure functional capacity. Functional capacity can be assessed by e.g. determination of the 6-minute walking distance or the minimum time the patient spends on walking 1 kilometer.

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

Individual suffering from renal failure: An individual having reduced kidney function, e.g. compared to a healthy individual.

Renal failure is for example the inability of the kidneys to excrete waste products leading to accumulation of these waste products above normal levels in the blood.

Patients with mild renal failure are often asymptomatic, whereas patients with moderate to severe chronic renal failure typically present with one or more of: fatigue, anaemia, itching, loss of appetite, nausea, vomiting, weight loss, dyspnoea, fluid retention, congestive heart failure, bleeding, hypertension, hyperkalemia,
acidosis, muscular cramps, nocturia, neuropathies, bleeding, stomatitis, pericarditis, osteodystrophy. The clinical presentation of renal failure not only depends on the degree of impairment but also on the underlying disease and superimposed complications.

In children renal failure is often complicated by growth failure which may be due to malnutrition, hyperparathyroidism or disturbances in the GH/IGF-1 axis.

Renal failure can e.g. be diagnosed by determination of the plasma concentrations of creatinine and urea and by measurement of the glomerular filtration rate (GFR). Assessment of the degree of renal impairment is based on GFR; a GFR of 60-89 ml/min indicates mild renal failure, a GFR of 30-59 ml/min moderate renal failure, a GFR of 15-29 ml/min severe renal failure and a GFR < 15 ml/min end-stage renal disease.

Chronic renal failure may be caused by primary glomerular diseases, such as IgA nephropathy, focal glomerulosclerosis, membranous nephropathy, membranoproliferative glomerulonephritis, idiopathic crescentic glomerulonephritis. Chronic renal failure may also be caused by glomerulopathies associated with systemic disease such as diabetes mellitus, post infectious glomerulonephritis, systemic lupus erythematosus, Wegener's granulomatosis, haemolytic ureic syndrome, amyloidosis, chronic interstitial nephropathies, hereditary nephropathies, such as polycystic kidney disease, Alport's syndrome, medullary cystic disease, nail-patella syndrome, hypertension, renal macrovascular disease (vasculopathy of the renal arteries), obstructive uropathy.

In children younger than 5 years, congenital renal diseases, such as renal hypoplasia, renal dysplasia, and obstructive uropathy, are the most common cause of chronic renal failure. In older children, hereditary diseases, metabolic diseases and acquired etiologies occur more frequently. Hereditary diseases include juvenile nephritis, cystic kidney, and Alport syndrome. The most frequent metabolic causes are cystinosis and oxalosis, and the principal acquired etiology is chronic glomerulonephritis.

The therapeutic options in chronic renal failure include fluid and potassium restriction, diuretics, high-energy low-protein diet, erythropoietin-α, iron, resin, sodium bicarbonate or calcium carbonate (for correction of acidosis), 1-
α-cholecalciferole and 1, 25-(OH)-D3, other prokinetics. Optimal control of underlying and/or concurrent diseases is essential to reduce the risk of progressive renal impairment and include antihypertensive agents (e.g. ACE inhibitors) and antidiabetic agents. Children with growth failure may benefit from growth hormone and/or IGF-1 therapy. Patients with end-stage renal failure require peritoneal- or hemodialysis, or renal transplantation for survival.

Anorexia in renal failure is assumed primarily to be caused by retension in the body fluid by one or more toxic substances as a consequence of reduced renal clearance (Mamoun. Nephrol Dial Transplant 1998;13:2460-63). Other factors including ascitis, increased CCK-plasma levels, alterations in taste and smell, delayed gastric emptying, dialytic loss of protein and amino acids, peritoneal dialysis induced hyperleptinaemia caused by glucose-based dialysis fluids, and the underlying disease may contribute to reduced nutritional intake. Furthermore, chronic acidosis may aggravate anorexia, stimulate catabolism and induce production of proinflammatory cytokines, which may lead to further protein degradation. Malnutrition associated inflammation is associated with increased morbidity and mortality in renal failure patients (Qureshi et al. J.Am.Soc.Nephrol. 13 Suppl 1 (2002);S28-S36;45; Ikizler et al. Kidney Int. 55.5 (1999):11945-51).

Individual suffering from liver failure: An individual having reduced liver function. The severity of liver failure can, for example, be assessed by the Child-Turcotte-Pugh classification or the MELD scoring system. The Child-Pugh classification (sometimes termed the Child-Turcotte-Pugh score) is based on a combination of clinical and biochemical parameters.

<table>
<thead>
<tr>
<th>Child-Pugh Classification</th>
<th>1 point</th>
<th>2 points</th>
<th>3 points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>&lt; 2</td>
<td>2 - 3</td>
<td>&gt; 3</td>
</tr>
<tr>
<td>- PBC and PSC patients</td>
<td>&lt; 4</td>
<td>4 - 10</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>&gt; 3.5</td>
<td>2.8 - 3.5</td>
<td>&lt; 2.8</td>
</tr>
<tr>
<td>PT: sec prolonged</td>
<td>1 - 3</td>
<td>4 - 6</td>
<td>&gt; 6</td>
</tr>
<tr>
<td>- INR</td>
<td>&lt; 1.7</td>
<td>1.8 - 2.3</td>
<td>&gt; 2.3</td>
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</tbody>
</table>
Thus, in one embodiment of the present invention, the patient group treated have a Child-Pugh classification of 1 point or 2 points or 3 points.

The MELD scoring system was initially evaluated as a predictor of 3-month survival following placement of a transjugular intrahepatic portosystemic shunt (TIPS), but the MELD model was later confirmed to be an accurate predictor of survival in a heterogenous population of patients with end-stage liver disease. This scoring system assesses severity of liver failure based on three objective variables: total bilirubin, international normalized ratio (INR) of prothrombin time, and serum creatinine. The MELD score can be calculated as follows: $0.957 \times \log (\text{creatinine}) + 0.378 \times \log (\text{bilirubin mg/dL}) + 1.12 \times \log (\text{INR}) + 0.643 \times 10.$

Polyclonal antibody: Polyclonal antibodies are a mixture of antibody molecules recognising a specific given antigen hence polyclonal antibodies may recognise different epitopes within said antigen.

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

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

GH-Secretagogue: a growth hormone secretagogue, i.e. a substance stimulating growth hormone release, such as ghrelin or a ghrelin-like compound. A GH secretagogue according to the invention may for example be selected from the group of:
L-692-429, L-692-585 (Benzoelactam compounds)
MK677 (Spiroindaner)
G-7203, G-7039, G-7502 (Isonipeptic acid peptidomimetic)
NN703, ipamorelin.

Ardana secretagogue analogue, EP01572

GHRP-1 (Bower)
GHRP-2 (Bower)
GHRP-6 (Bower)
Hexarelin (Europeptide)

Ipamorelin (Novo Nordisk)
NNC 26-0235 (Novo Nordisk)
G-7039 (Genentech)
G-7502 (Genentech)
NNC26-0323 (Genentech)

NN703 (Novo Nordisk)
NNC26-0722 (NN) (Novo Nordisk)
L-692,429 (Merck)
L-692,585 (Merck)
NNC26-0610 (NN) (Novo Nordisk)

MK-677 (Merck)
L-163,540 (Merck)
CP-424,391 (Pfizer)
EP51319 (Theratechnology)
RC-1291 (Rejuvenon)

BIM28125 (IPSEN)
BIM28131 (IPSEN)

In particular the secretagogue is a ghrelin-like compound, including 28 aa human ghrelin. The secretagogue may in one embodiment be non-acylated, for instance a non-acylated form of ghrelin or a non-acylated ghrelin-like compound. In one embodiment, another bulky group (such as e.g. a cholesterol or tryptophan moiety) replaces the usual acyl group on said non-acylated ghrelin-like compound.
Surfactant molecule: Molecule comprising a hydrophobic part and a hydrophilic part, i.e. molecule capable of being present in the interphase between a lipophilic phase and a hydrophilic phase.

Patients in need of treatment
The present invention is in one embodiment directed to the treatment of individuals suffering from renal failure. The renal failure may be caused by a variety of factors, such as infections or immunologic causes, as well as idiopathic. The invention is useful at any stage of renal failure, in particular useful in patients with end-stage renal failure and/or euemic individuals. One group of patients that can be treated with the present invention are those with hypoalbumineamia who are unresponsive to dialysis and adequate prescription of dietary intake.

One preferred patient group is patients suffering from chronic renal failure.

In another embodiment, the present invention is directed to the treatment of individuals suffering from liver failure. The liver failure may be caused by a variety of factors, such as infections, alcoholic or chemical causes, as well as idiopathic. Accordingly, the liver failure may be due to alcoholic liver failure, hepatitis, such as hepatitis C and drug induced liver failure.

In one embodiment, said liver failure is chronic liver failure. The liver failure can be caused by e.g. alcoholic liver disease, viral hepatitis, hemochromatosis, drug-induced hepatitis, Wilson’s disease, sclerosing cholangitis, primary biliary cirrhosis, Budd-Chiari syndrome, venoocclusive disease, autoimmune hepatitis, steatohepatitis, partial hepatectomy, or Reye’s syndrome.

In one preferred embodiment, the individual is suffering, or at risk of suffering from, alcoholic liver failure, hepatitis C or drug-induced liver failure constitute the three major groups of patients with liver failure.

In another embodiment of the present invention, the individual is suffering from biliary atresia, such as childhood biliary atresia. In another embodiment of the present invention, the individual is suffering from one or more of the following: hepatitis, Alagille’s syndrome, familial intrahepatic cholestasis and metabolic
diseases, such as α1-antitrypsin deficiency, tyrosinaemia, type 1, Wilson's disease, cystic fibrosis, or glycogen storage disease type IV.

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

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

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

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

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

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

In a third aspect the present invention relates to stimulation of weight gain, reducing a weight loss or maintaining a stable body-weight by administering a ghrelin-like compound to an individual suffering from renal failure and/or liver failure. Thus, preferably, the GH-secretagogue, such as a ghrelin-like compound, is useful for stimulating food intake and weight gain, more preferably the secretagogue, such as a ghrelin-like compound, is useful for stimulating weight gain, for reducing a weigh loss or for maintaining stable body weight.

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

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

An increase in the body fat mass of an individual can be readily assessed by the skilled person using a number of state of the art techniques. In one embodiment the invention relates to an increase in body fat mass without the individual gaining weight overall. A preferred embodiment of the invention leads to an increase in body fat of at least 1%, such as at least 2% compared to prior to the initiation of treatment, more preferably at least 4%, such as at least 5%, and at least 8% and at least 10%, even more preferably at least 20% or at least 40% above pre-treatment values.

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

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

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

In another preferred embodiment the invention leads to an increase in muscle
strength and/or muscle perseverance, e.g. measured as the maximal distance the
patient is capable to walk in 5 minutes, the minimum time the patient spends on
walking 1 kilometer, the length of time the patient can walk at maximum pace, grip
strength, knee extension power. When measured as walking capacity the increase
is preferably at least 5% compared to prior to initiation of treatment, such as at least
8% compared to prior to initiation of treatment, such as at least 12% compared to
prior to initiation of treatment, such as at least 20%, or at least 50% compared to
prior to initiation of treatment. When measured as grip strength, the increase is
preferably at least 10%, such as at least 20%, such as at least 30%, such as at least
50%, such as at least 75%, such as at least 100% compared to prior to initiation of
the treatment.

**Quality of Life**

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

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

(ii) EORTC QLQ-C30 (+3) questionnaire. The EORTC QLQ-C30 (version 1.0) is a 30 item core questionnaire intended for assessment of QOL among patients, the instrument is developed by the EORTC Quality of Life Study group. The first version has been validated in cancer patients and reference data from general populations have been published. The questionnaire comprises five functional scales; physical functioning (five questions), role functioning (two questions), emotional functioning (four questions), cognitive functioning (two questions) and social functioning (two questions). There are three symptom scales; fatigue (three questions), nausea and vomiting (two questions) and pain (two questions), and there are six single items on dyspnoea, insomnia, loss of appetite, constipation, diarrhea and financial difficulties. Two global questions are asking about the patient's health status and overall QOL. All scales and single-items measures range in score from 0 to 100. A high score for the functioning scales and the global health status and QOL represents a high level of functioning / health status and QOL. A high score for the symptom / item scales represents a high level of symptoms / problems. The QOL scores can be calculated according to the EORTC QLQ-C 30 scoring manual.

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

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

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

**Ghrelin-like compound**

Any GH-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:

\[ \text{Formula I: } Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2, \]

wherein

\[ Z^1 \text{ is an optionally present protecting group} \]
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 “GH-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. 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.

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

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

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


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

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

\[ \text{formula II} \quad Z^1 - \text{Gly-} (X^1)_{m-1} - (X^2) - (X^3)_h - Z^2, \]

\[ \text{formula III} \quad Z^1 - \text{Gly-} \text{Ser} - (X^2) - (X^3)_h - Z^2, \text{ and} \]

\[ \text{formula IV} \quad Z^1 - \text{Gly} - (X^2) - (X^3)_h - Z^2. \]

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

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

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

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

Phe Leu Ser Pro Glu His Gin

Phe Leu Ser Pro Glu His

Phe Leu Ser Pro Glu

Phe Leu Ser Pro

Phe Leu Ser

Phe Leu

Phe

In a preferred embodiment the length of the ghrelin-like compound is substantially similar to the length of human ghrelin, i.e. 27 or 28 amino acids. Accordingly, $n$ is preferably an integer in the range of from 1-25, such as of from 1-24, such as from 1-15, such as of from 1-10, or such as of from 10-25, such as of from 10-24, such as of from 15-25, such as of from 15-24. $n$ may equally be e.g. any of: 5-9, 5-24, 5-20, 9-25, 9-18, 9-12, 3-25, 3-22, 3-15, 3-10 etc.

Preferably, a ghrelin-like compound includes the naturally occurring 28 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 1, as well as the naturally occurring 27 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 2.

$(X^3)_n$ may be selected from any fragment of ghrelin, such as human ghrelin, and accordingly, $(X^3)_n$ may be selected from one or more of the sequences shown below or a homologue thereof:
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg

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

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

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

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

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

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

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

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

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

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

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

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

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

Phe Leu Ser Pro Glu His Gln Arg Val Gln

Phe Leu Ser Pro Glu His Gln Arg Val

Phe Leu Ser Pro Glu His Gln Arg

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

Or selected from

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

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

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

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

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

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

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

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

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

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

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

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

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln

25 Phe Leu Ser Pro Glu His Gln Lys Val Gln

Phe Leu Ser Pro Glu His Gln Lys Val

30 Phe Leu Ser Pro Glu His Gln Lys

Or selected from

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Or selected from

5

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

10

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

15

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

20

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

25

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

30

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

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

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

10 In another embodiment (X³)n comprises or consists of a sequence selected from the sequences
Phe Leu Ser Pro Glu His Gln
Phe Leu Ser Pro Glu His

15 Phe Leu Ser Pro Glu
Phe Leu Ser Pro
Phe Leu Ser
Phe Leu

20 Phe Leu
Phe

Further suitable GH secretagogues for use in the present invention are disclosed in PCT patent application no. PCT/DK2004/000529, Danish patent application no. PA 200401875, and PCT applications with publication numbers WO0192292 (Merck and Co. Inc), WO0134593 (Novo Nordisk AS) and WO0107475 ("Novel peptides", Kangawa et al.); said documents all being incorporated herein by reference.
Methods for production of GH secretagogues are well known to those skilled in the art, for example in Example 2 of PCT patent application PCT/DK2004/000519 (Gastrotech Pharma), incorporated herein by reference.

Functionality

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

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

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

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

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

1) an assay measuring the activation of Gq / G11 performed for example by measurement of 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 phospholipase C (PLC) one of the first down-stream effector molecules in the pathway, for example by measuring the accumulation of inositol phosphate which is one of the products of PLC.
3) More down stream in the signalling cascade is the mobilization of calcium from the intracellular stores

4) Further more down stream signalling molecules such as the activity of different kinds of MAP kinases (p38, jun, 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

Other examples of suitable protocols for use in determining secretagogue functionality are given in Example 5 of published patent application WO 2005/014032 (Gastrotech Pharma: “Use of secretagogues like ghrelin in cancer cachexia and for stimulating appetite”): Functional tests on the ghrelin receptor

A ghrelin-like compound used in the invention preferably has at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, functional activity relative to 28 aa human ghrelin as determined using the assay described herein above, and/or an EC50 greater than about 1,000 nM, greater than about 100 nM, or greater than about 50 nM, or greater than about 10 nM or greater than 1 nM. Greater refers to potency and thus indicates a lesser amount is needed to achieve binding inhibition.

In one embodiment of the use of the invention, the compound has a potency (EC50) on the GHS-R 1A of less than 500 nM. In another embodiment the compound has a potency (EC50) on the GHS-R 1A of less than 100 nM, such as less than 80 nM, for example less than 60 nM, such as less than 40 nM, for example less than 20 nM, such as less than 10 nM, for example less than 5 nM, such as less than 1 nM, for example less than 0.5 nM, such as less than 0.1 nM, for example less than 0.05 nM, such as less than 0.01 nM.

In a further embodiment the dissociation constant (Kd) of the compound 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.

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

**Identity and homology**

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
constituted 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 ghrelin homologue of one or more of the sequences specified herein may vary in
one or more amino acids as compared to the sequences defined, but is capable of
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 be defined as:

i) homologues comprising an amino acid sequence capable of being
recognised by an antibody, said antibody also recognising the 28 aa human
ghrelin, preferably the acylated 28 aa human ghrelin, and/or

ii) homologues comprising an amino acid sequence capable of binding
selectively to GHS-R 1a, and/or

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

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

The antibodies used herein may be antibodies binding the N-terminal part of ghrelin
or the C-terminal part of ghrelin, preferably the N-terminal part of ghrelin. The
antibodies may be antibodies as described in Ariyasu et al. "Delayed short-term
secretory regulation of ghrelin in obese animals: Evidenced by a specific RIA for the
active form of ghrelin, Endocrinology 143(9):3341-3350, 2002."
Examples of homologues comprises one or more conservative amino acid 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 asparagines (Asn) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Gln, and independently thereof, homologues thereof, wherein at least one glutamine (Gln) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Asn, and independently thereof, homologues thereof, wherein at least one proline (Pro) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Tyr, Trp, and His, and independently thereof, homologues thereof, wherein at least one of said cysteines (Cys) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, and Tyr.

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

A non-conservative substitution leading to the formation of a functionally equivalent homologue of the sequences herein would for example i) differ substantially in polarity, for example a residue with a non-polar side chain (Ala, Leu, Pro, Trp, Val, Ile, Leu, Phe or Met) substituted for a residue with a polar side chain such as Gly, Ser, Thr, Cys, Tyr, Asn, or Gln or a charged amino acid such as Asp, Glu, Arg, or Lys, or substituting a charged or a polar residue for a non-polar one; and/or ii) differ substantially in its effect on polypeptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

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 binding domain 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 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, or rat ghrelin SEQ ID NO:3. Other homologues are the variants described in EP 1197496 (Kangawa) and WO 01/92292 (Merck) and WO 01/56592 (Novo Nordisk) incorporated herein by reference.

**Bulky hydrophobic group**

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

When the amino acid being modified contains e.g. - OH, -SH, -NH or -NH₂ as a substituent group in a side chain thereof, a group formed by acylating such a substituent group is preferred. The mode of linkage may thus be selected from the group consisting of ester, ether, thioester, thioether, amide and carbamide.
For example, if the modified amino acid is serine, threonine, tyrosine or oxyproline, the amino acid has a hydroxyl group in the side chain. If the modified amino acid is cysteine, the amino acid has a mercapto group in the side chain. If the modified amino acid is lysine, arginine, histidine, tryptophan, proline or oxyproline, it has an amino group or imino group in the side chain.

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

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

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

The hydrophobic group may be any group with a saturated or unsaturated alkyl or acyl group containing one or more carbon atoms. In one embodiment the bulky hydrophobic group is an acyl group, including groups formed by removing a hydroxyl group from an organic carboxylic acid, organic sulfonic acid or organic phosphoric acid. The organic carboxylic acid includes e.g. fatty acids, and the number of carbon atoms thereof is preferably 1 to 35. In the organic sulfonic acid or organic phosphoric acid, the number of carbon atoms thereof is preferably 1 to 35.

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.

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

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

The other entity may be any substance that is capable of conferring improved properties to the secretagogue, e.g. in terms of improved stability, half-life, etc.

Examples of suitable entities are described in the following.

For example the secretagogue may be conjugated to a peptide, such as a peptide having effect on nociceptin receptor ORL1. In one embodiment the conjugate is a conjugate of ghrelin or a derivative or homologue thereof and a peptide having effect on ORL1, e.g. the peptide Ac-RYY(RK)(W)RK-NH₂, where the brackets show allowable variation of amino acid residues. Examples of other suitable peptides are found in US patent applications 2003040472 and US2002004483, and US patent 5869046.

In another embodiment of the present invention, a secretagogue, such as ghrelin or a ghrelin-like compound, is conjugated to a polymer molecule. The polymer molecule may be any suitable polymer molecule, such as a natural or synthetic polymer, typically with a molecular weight in the range of about 1-100 kDa, such as about 3-20, kDa, e.g. 5-10 kDa. The polymer is attached to a reactive group present on the secretagogue, e.g. an amine group or a thiol group.

Examples of suitable polymer molecules include polymer molecules selected from the group consisting of polyalkylene oxide (PAO), including polyalkylene glycol (PAG), such as linear or branched polyethylene glycol (PEG) and polypropylene glycol (PPG), poly-vinyl alcohol (PVA), poly-carboxylate, poly-(vinylpyrrolidone), polyethylene-co-maleic acid anhydride, polystyrene-co-maleic acid anhydride, dextran, including carboxymethyl-dextran. Preferably, the polymer molecule is a PEG molecule, in particular a monofunctional PEG, such as methoxypolyethylene glycol (mPEG). Suitable activated PEG molecules are available from Nektar Therapeutics Inc. (Huntsville Alabama, US) or from Valentis, Inc., Burlingame, CA.
U.S.A.. Alternatively, the polymer molecules can be activated by conventional methods known in the art, e.g., as disclosed in WO 90/13540. Specific examples of activated PEG polymers include the following linear PEGs: NHS-PEG (e.g., SPA-PEG, SSPA-PEG, SBA-PEG, SS-PEG, SSA-PEG, SC-PEG, SG-PEG, and SCM-PEG), and NOR-PEG), BTC-PEG, EPOX-PEG, NCO-PEG, NPC-PEG, CDI-PEG, ALD-PEG, TRES-PEG, VS-PEG, IODO-PEG, and MAL-PEG, and branched PEGs such as PEG2-NHS and those disclosed in U.S. Pat. No. 5,932,462 and U.S. 5,643,575, both of which are incorporated herein by reference.

The PEGylation (i.e. conjugation of the secretagogue polypeptide and the activated polymer molecule) is conducted in accordance with established procedures, e.g., as described in the following references (which also describe suitable methods for activation of polymer molecules): R. F. Taylor, (1991), "Protein immobilisation. Fundamental and applications", Marcel Dekker, N.Y.; S. S. Wong, (1992), "Chemistry of Protein Conjugation and Crosslinking", CRC Press, Boca Raton; G. T. Hermanson et al., (1993), "Immobilized Affinity Ligand Techniques", Academic Press, N.Y.).

It is also contemplated according to the invention to couple the polymer molecules to the secretagogue through a linker. Suitable linkers are well known to the skilled person. A preferred example is cyanuric chloride (Abuchowski et al., (1977), J. Biol. Chem., 252, 3578-3581; U.S. Pat. No. 4,179,337; Shafer et al., (1986), J. Polym. Sci. Polym. Chem. Ed., 24, 375-378.

In yet another embodiment the secretagogue is conjugated to an oligosaccharide molecule, such as dextran, glycan, transferrin, etc. Such conjugation may be achieved in accordance with established technologies, e.g. those available from Neose Technologies, Inc. Horsham, PA.

In yet another embodiment, the secretagogue is conjugated to an Fc region of an IgG molecule, typically in the form of a fusion protein. For instance, a salvage receptor binding epitope of the Fc region of an IgG (i.e. the Fc portion of an immunoglobulin of the isotype IgG) is incorporated into the secretagogue so as to increase its circulatory half-life, but so as not to lose its biological activity. This can take place by any means, such as by mutation of the appropriate region in the
secretagogue to mimic the Fc region or by incorporating the epitope into a peptide tag that is then fused to the secretagogue at either end or in the middle or by DNA or peptide synthesis.

The salvage receptor binding epitope is any suitable such epitope as known to the person skilled in the art, and its nature will depend, e.g., on the type of secretagogue being modified. The epitope is introduced into the secretagogue such that the biological activity of the secretagogue is maintained, i.e., the epitope does not adversely affect the conformation of the secretagogue or affect its binding to ligands that confers its biological activity.

Alternatively to providing the secretagogue in the form of a conjugate, the secretagogue may be modified to include suitable reactive groups, whereby the thus modified secretagogue is capable of forming a conjugate in vivo (after having been administered to an individual) through covalent bonding with available reactive functionalities on blood components. The invention also relates to such modified secretagogues, and methods for their use. Also, the invention relates to conjugates formed in vitro between a modified secretagogue as described above and a blood component. The conjugates formed in accordance with this embodiment are contemplated to have an increased in vivo half life as compared to the corresponding non-modified secretagogue.

In accordance with this embodiment, the secretagogue is modified with a chemically reactive group (reactive entity). The reactive entity may, e.g., be selected from the wide variety of active carboxyl groups, particularly esters, where the hydroxyl moiety is physiologically acceptable. Such groups may be selected from the group consisting of N-hydroxysuccinimide (NHS), N-hydroxy-sulfosuccinimide (sulfo-NHS), maleimide-benzoyl-succinimide (MBS), gamma-maleimido-butyroxy succinimide ester (GMBS) and maleimidopropionic acid (MPA). The principal targets for this group of entities are primary amines on the blood component. Another group of active entities is constituted by a maleimido-containing group such as MPA and gamma-maleimido-butyramide (G MBA) Such groups react with thiol groups present on the blood component.

The blood component with which the modified secretagogue is designed to react
may be any blood component having an available target group, e.g. an amine or a thiol group, and which is suitable as a carrier for binding the modified secretagogue in vivo and thereby extend the circulating half-life thereof. Examples of such blood components are serum albumin and IgG.

As mentioned above the covalent bonding of a modified secretagogue to a blood component may be achieved in vivo by administration of the modified secretagogue directly to the patient. The administration may be done in any suitable form, such as in the form of a bolus or introduced slowly over time by infusion using metered flow or the like. Alternatively, the secretagogue/blood component conjugate may also be prepared ex vivo by combining blood with the modified secretagogue, allowing covalent bonding of the modified secretagogue to reactive functionalities on blood components and then returning or administering the conjugated blood to the individual. Moreover, the above may also be accomplished by first purifying an individual blood component or limited number of components, such as red blood cells, immunoglobulins, serum albumin, or the like, and combining the component or components ex vivo with the chemically reactive secretagogues.

Examples of further suitable entities are described in PCT patent application PCT/DK2004/000519 (Gastrotech Pharma) and Danish patent application no. PA 200401875, both incorporated herein by reference

**Methods for production**

GH-secretagogues, such as ghrelin-like compounds, can be produced using techniques well known in the art. For example, a polypeptide region of a ghrelin-like compound can be chemically or biochemically synthesized and modified. Techniques for chemical synthesis of polypeptides are well known in the art. (See e.g., Vincent in Peptide and Protein Drug Delivery, New York, N. Y., Dekker, 1990.). Examples of techniques for biochemical synthesis involving the introduction of a nucleic acid into a cell and expression of nucleic acids are provided in Ausubel, Current Protocols in Molecular Biology, John Wiley, 1987-1998, and Sambrook et al., in Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989. Other methods for production of GH-secretagogues are disclosed in Example 2 of PCT/DK2004/000519 (Gastrotech Pharma), incorporated herein by reference thereto.
Pharmaceutical compositions

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

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

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.

Suitable pharmaceutical carriers include sterile aqueous solution and various organic solvents and inert solid diluents or fillers. Examples of solid carriers are lactose, terra alba, sucrose, cyclodextrin, talc, gelatine, agar, pectin, acacia, magnesium stearate, stearic acid or lower alkyl ethers of cellulose. Examples of liquid carriers are syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene or water. 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. It is preferred that the formulation has a pH within the range of 3.5-8, such as in the range 4.5-7.5, such as in the range 5.5-7, such as in the range 6.7-5, preferably around 7.3. However, as is understood by one skilled in the art, the pH range may be adjusted according to the individual treated and the administration procedure. For example, some GH secretagogues may be easily stabilised at a lower pH, so in another preferred embodiment of the invention the formulation has a pH within the range 3.5-7, such as 4.6, such as 5.6, such as 5.3-5.7, such as 5.5.

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.

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

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

The pharmaceutically acceptable salt may be an acid addition salts including salts of inorganic acids as well as organic acids. Acid addition salts are formed with free amino groups of the GH secretagogue. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydriodic, metaphosphoric, phosphoric, sulphuric and nitric acids and the like. Representative examples of 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. The metal salt may be an alkali metal or earth alkali metal salt. Examples of metal salts include lithium, sodium, potassium and magnesium salts and the like. Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium and tetramethylammonium salts and the like.

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

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

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

In one particular embodiment the invention relates to the use of a pharmaceutical composition comprising a mixture of at least two different GH-secretagogues (such as e.g. two different ghrelin-like compounds), such as a mixture of a ghrelin-like compound being acylated with a C8 acyl and a ghrelin-like compound being acylated with a C10 acyl. Without being bound by theory it is believed that such a mixture will have a longer half-life in plasma.
In yet another embodiment, the pharmaceutical composition used comprises one or more acylated GH secretagogue(s) (preferably ghrelin-like compound(s)), optionally compounds having different acyl chain lengths preferably selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group, further optionally in combination with a desacylated secretagogue, such as a desacylated Ghrelin-like compound.

In a preferred aspect of the invention the GH-secretagogue (such as a ghrelin-like compound) is administered with a substance capable of increasing the half-life of the GH-secretagogue, for example by incorporating the secretagogue into liposomes, micelles, iscoms, and/or microspheres or other transport molecules. This is of particular interest when an amino acid residue of the ghrelin-like compound is modified with a bulky hydrophobic group and it is desirable to protect the modified amino acid from degradation.

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

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

Conventional liposomes are typically composed of phospholipids (neutral or negatively charged) and/or cholesterol. The liposomes are vesicular structures based on lipid bilayers surrounding aqueous compartments. They can vary in their physiochemical properties such as size, lipid composition, surface charge and number and fluidity of the phospholipids bilayers. The most frequently used lipid for liposome formation are: 1,2-Dilauroyl-sn-Glycero-3-Phosphocholine (DLPC), 1,2-
Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC), 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC), 1,2-Distearyl-sn-Glycero-3-Phosphocholine (DSPC), 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC), 1,2-Dimyristoyl-sn-Glycero-3-Phosphoethanolamine (DMPE), 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine (DPPE), 1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine (DOPE), 1,2-Dimyristoyl-sn-Glycero-3-Phosphate (Monosodium Salt) (DMPA), 1,2-Dipalmitoyl-sn-Glycero-3-Phosphate (Monosodium Salt) (DPPA), 1,2-Dioleoyl-sn-Glycero-3-Phosphate (Monosodium Salt) (DOPA), 1,2-Dimyristoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] (Sodium Salt) (DMPG), 1,2-Dipalmitoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] (Sodium Salt) (DPPG), 1,2-Dioleoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] (Sodium Salt) (DOPG), 1,2-Dimyristoyl-sn-Glycero-3-[Phospho-L-Serine] (Sodium Salt) (DMPS), 1,2-Dipalmitoyl-sn-Glycero-3-[Phospho-L-Serine] (Sodium Salt) (DPPS), 1,2-Dioleoyl-sn-Glycero-3-[Phospho-L-Serine] (Sodium Salt) (DOPS), 1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine-N-(glutaryl) (Sodium Salt) and 1,1',2,2'-Tetramyristoyl Cardiolipin (Ammonium Salt). Formulations composed of DPPC in combination with other lipid or modifiers of liposomes are preferred e.g. in combination with cholesterol and/or phosphatidylcholine.

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

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

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

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".

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

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

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

**Compositions for parenteral administration**

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

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

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

Examples of oily or nonaqueous carriers, diluents, solvents or vehicles for parental use include propylene glycol, polyethylene glycol, animal, synthetic or vegetable oils, and injectable organic esters, and may contain formulatory agents such as preserving, wetting, emulsifying or suspending, stabilizing and/or dispersing agents. Specific examples of oils useful in such compositions include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral compositions include oleic acid, stearic acid, and isostearic acid. Suitable organic esters include fatty acid esters such as ethyl oleate and isopropyl myristate.

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 polyoxyethylene polypropylene 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 compound(s) or pharmaceutically acceptable salt(s) thereof in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization.

**Compositions for oral delivery**

Those GH secretagogue types capable of remaining biologically active in an individual after oral administration (such as short peptides) can be formulated in a wide range of oral administration dosage forms. The pharmaceutical compositions and dosage forms may comprise the compounds of the invention or its pharmaceutically acceptable salt or a crystal form thereof as the active component. The pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, wetting agents, tablet disintegrating agents, or an encapsulating material.
Preferably, the composition will be about 0.5% to 75% by weight of a compound or compounds of the invention, with the remainder consisting of suitable pharmaceutical excipients. For oral administration, such excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like.

In powders, the carrier is a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain 1-70% of the active compound. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term "preparation" is intended to include the composition of the active compound with encapsulating material as carrier providing a capsule in which the active component, with or without carriers, is surrounded by a carrier, which is in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be as solid forms suitable for oral administration.

Drops can be used according to the present invention and may comprise sterile or non-sterile aqueous or oil solutions or suspensions, and may be prepared by dissolving the active ingredient in a suitable aqueous solution, optionally including a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100 °C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container aseptically. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.
Other forms suitable for oral administration include toothpaste, gel dentifrice or chewing gum. Emulsions may be prepared in solutions in aqueous propylene glycol solutions or may contain emulsifying agents such as lecithin, sorbitan monooleate, or acacia. Aqueous solutions can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizing and thickening agents. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well known suspending agents. Solid form preparations include solutions, suspensions, emulsions, syrups and elixirs and may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

Compositions for topical administration

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

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

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

**Compositions for aerosol, nasal or inhalation delivery**

It is contemplated that the GH secretagogues 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 solubilized such as in microdroplets, 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.

**Administration as suppositories**

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

Subcutaneous administration
Any parenteral administration form that will ensure that the ghrelin receptors which normally are the target for peripherally produced ghrelin in the premeal situation will be exposed to sufficient levels of the bioactive form of a GH secretagogue or a salt thereof to ensure robust and appropriate appetite stimulation, without causing 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 GH secretagogue or a salt thereof is administered subcutaneously in an amount sufficient to allow sufficient levels of the bioactive form, e.g. an acylated or anchorgroup containing form, to reach the receptors in time, such as prior to the forthcoming meal. Dose and frequency of administration by the subcutaneous route are as described above.

Bolus administration
From a molecular pharmacological point-of-view it is important to note that it has been found that the ghrelin receptor normally is exposed to short-lived surges in the concentrations of the natural agonist ligand, ghrelin. The GHS-R 1a receptor belongs to the class of receptors, so-called G protein coupled receptors or 7TM receptors, that upon continued exposure to an agonist will be desensitised, 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). Another part of the agonist
mediated desensitization process is receptor internalization (i.e. physical removal of the receptor from the cell surface where it could bind the agonist) as well as receptor down regulation (i.e. decreased production / expression of the receptor). Receptor 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.

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

**Administration**

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

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

The present invention preferably deals with methods for administering a secretagogue, such as ghrelin, in a way which mimics the physiologically pre-meal situation as closely as possible yet providing patients in need of increased food
intake, for example post operative patients with a sufficient extra stimulatory input to their appetite regulating ghrelin receptors, which normally are reached by ghrelin in the pre-meal situation.

Suitable dosing regimens for the various compounds and methods of the present invention are preferably determined taking into account factors well known in the art including type of subject being dosed; age, weight, sex and medical condition of the subject; the route of administration; the renal and hepatic function of the subject; the 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, such as ghrelin or a ghrelin-like compound, is administered subcutaneously.

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

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

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

Pharmaceutical compositions for parenteral administration include sterile aqueous and non-aqueous injectable solutions, dispersions, suspensions or emulsions, as well as sterile powders to be reconstituted in sterile injectable solutions or dispersions prior to use.
Other suitable administration forms include suppositories, sprays, ointments, cremes, gels, inhalants, dermal patches, implants, pills, tablets, lozenges and capsules.

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

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

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

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

**Dosage regimes for treatment of liver failure**

Without being bound by theory, it is believed that ghrelin circulating in the body is partially cleared by the liver. Accordingly, a dosing regimen for treatment of liver failure patients is preferably based on ghrelin pharmacokinetics and pharmacodynamics in patients with liver failure, as known by one skilled in the art. For example, in one preferred embodiment of the invention ghrelin is administered as a subcutaneous injection at a dose of 0.1-1000 μg/kg, such as at a dose of 1-1000 μg/kg, such as at a dose of 1-100 μg/kg, such as at a dose of 1-50 μg/kg, such as at a dose of about 10 μg/kg.

**Formulation**

In a preferred aspect the present invention contemplates pharmaceutical compositions useful for practicing the therapeutic methods described herein. Pharmaceutical compositions of the present invention contain a physiologically tolerable carrier together with at least one species of a secretagogue, such as ghrelin or a ghrelin-like compound as described herein, dissolved or dispersed therein as an active ingredient.

In one aspect the invention relates to a pharmaceutical composition comprising at least one secretagogue, such as ghrelin or a ghrelin-like compound as defined above in formula I. In a preferred embodiment the pharmaceutical composition comprises at least two different ghrelin-like compounds as defined above in formula I in order to increase the effect of the treatment. The difference may for example be compounds having different acylations, for example wild-type ghrelin and L-692-429 (Merck).

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.
In a preferred embodiment of the invention the formulation comprises the secretagogue or a salt thereof as a lyophilisate and the formulation further comprises a solvent, said lyophilisate and said solvent being in separate compartments until administration.

In another embodiment the formulation is a solution of the secretagogue or a salt thereof.

**Combination treatments**

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

In a preferred embodiment, the GH-secretagogue is administered to an individual suffering from, or at risk of suffering from, renal failure, in combination with one or more drugs used for treating or alleviating complications of renal failure, such as one or more of: EPO, dietary potassium/fluid and protein restriction/resin, correction of acidose (sodium bicarbonate or Calcium carbonate), 1-alfa-cholecalciferole and 1,25-(OH)-D3, prokinetics, antihypertensive treatment (e.g. ACE inhibitors), antidiabetic therapy, growth hormone and/or IGF-1.

In one preferred embodiment, the secretagogue is administered to the individual suffering from liver failure in combination with one or more drugs used for treating or alleviating liver failure, such as e.g. one or more of: colestyramine, ondansetron,
rifampicin, variceal sclerotherapy, lactulose, metronidazole, ascitis paracentesis, sodium and fluid restriction, spironolactone, liver surgery therapies, liver transplant and associated medications and treatment regimes.

Combinations wherein all active ingredients are appetite-regulating agents

The secretagogue(s) according to the invention can also be administered in combination with other appetite-regulating agents, including more than one type of growth hormone secretagogue, such as another ghrelin-like compound, such as a ghrelin-like compound comprising a structure defined by formula I, described herein. Other secretagogues suitable for combination administration with another secretagogue compound are any of the secretagogue compounds described herein. In one preferred embodiment of the present invention, wild type ghrelin (most preferably human wild type ghrelin) is administered in combination with a different, ghrelin-like compound – this combination is envisaged to enhance and/or prolong the effect of the secretagogues on the ghrelin receptor. In another preferred embodiment of the present invention, a ghrelin-like compound that is not wild type ghrelin is administered in combination with a different ghrelin-like compound that is not wild-type ghrelin – again, this combination is envisaged to enhance and/or prolong the effect of the secretagogues on the ghrelin receptor. In a similar way, several different secretagogues may be administered to an individual to increase efficacy on the ghrelin receptor – such as greater than 2 different secretagogue types, such as 3, such as 4, such as 5, such as 6, such as 7, such as greater than 8 different secretagogue types. The secretagogue according to the invention, such as ghrelin or a ghrelin-like compound(s) can also be administered in combination with a pharmacologically effective amount of a growth hormone, including hGH.

In one preferred embodiment of the present invention the secretagogue, such as ghrelin or a ghrelin-like compound, may be administered in combination with IGF-1, IGFBP-3, or ALS, preferably with IGF-1. The rationale behind this combination treatment is to increase the level of IGF-1, IGFBP-3, and/or ALS found to be low in cachectic individuals.

In a further embodiment of the invention, the secretagogues, such as ghrelin or a ghrelin-like compound, may be administered in combination with compounds known to stimulate appetite, such as melanocortin receptor antagonists, neuropeptide Y
receptor agonists including agonists selective for individual subtypes of the
neuropeptide Y receptors, leptin or leptin receptor agonists, cannabinoids including
marijuana and marijuana derivatives, antipsychotics, especially atypical
antipsychotics such as sertindole, Sulpirid, Clozapine, Risperidone, Quetiapin,
Amisulpride, Ziprasidon, and Olanzapine.

The GH secretagogue may also be administered in combination with an ingredient
or therapy useful in a supportive care regimen, such as one or more of the following:
- G-CSF (and analogues thereof)
- EPO (and analogues thereof)
- Cannabinoid(s)
- Progestagen(s)
- Androgen(s) (such as SARM and androgen receptor modulators).
- Analgetics such as opioids

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

Medical packaging

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

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

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

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

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

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

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

Example 1

Competition binding assays
Transfected COS-7 cells were transferred to culture plates one day after transfection at a density of $1 \times 10^5$ cells per well aiming at 5 - 8 % binding of the radioactive ligand. Two days after transfection competition binding experiments were performed for 3 hours at 4°C using 25 pM of $^{125}$I-ghrelin (Amersham, Little Chalfont, UK).

Binding assays were performed in 0.5 ml of a 50 mM Hepes buffer, pH 7.4, supplemented with 1 mM CaCl$_2$, 5 mM MgCl$_2$, and 0.1 % (w/v) bovine serum albumin, 40 microgram/ml bacitracin. Non-specific binding was determined as the binding in the presence of 1 micromole of unlabeled ghrelin. Cells were washed twice in 0.5 ml of ice-cold buffer and 0.5-1 ml of lysis buffer (8 M Urea, 2 % NP40 in 3 M acetic acid) was added and the bound radioactivity was counted.

Determinations were made in duplicate. Initial experiments showed that steady state binding was reached with the radioactive ligand under these conditions.

Example 2

Preclinical model

In a rat model of chronic renal failure the beneficial effect of ghrelin administration will be observed.

Chronic renal failure (CRF) will be induced in 20 rats by one-step 5/6 nephrectomy. All surgical interventions will be performed under anesthesia, induced by intraperitoneal administration of chloral hydrate (400 mg/kg). The experimental CRF is induced by removal of the upper and the lower pole of the left kidney, followed by complete removal of the right kidney 7 d later. In the 20 control rats, sham procedures involving kidney decapsulation will be performed performed.

Simultaneously with the initial partial nephrectomy/sham surgery, all rats will be bilaterally orchidectomized. A tablet (diameter, 0.5 cm), containing either 15 mg of testosterone (Innovative Research of America, Toledo, OH) or placebo, is inserted subcutaneously through a tunnel extending to approximately 2 cm lateral of the nephrectomy incision site. Gradual resorption of the tablets guaranteed stable
plasma testosterone concentrations in the low normal range of adult male rats for approximately 3 wk. All rats are given a standard diet.

Both control and CRF rats will be divided into two group with 10 rats in each group receiving either saline or ghrelin 200μg/kg s.c. for 14 days. The treatment will start four days after the surgical procedure.

Measurements:

- Bodyweight (each day) and body-composition.
- Food intake as measured by weight of the remaining food each day
- Plasma hormone level: IGF-I, leptin, Insulin,
- Gastric emptying measured by oral administration of 51Cr solution and following detection of 51Cr in feaces.
- Cytokines: TNF-α, IL-6, CRP

Example 3

PK-study in patients with renal failure

Objectives: Establish the safety and pharmacokinetics of ghrelin in patients with chronic renal failure.

Design: Open label, parallel group study in patients with renal impairment (n=6) and in healthy male volunteers (n=6).

Endpoints: Pharmacokinetic parameters, safety

Trial product: Single dose, subcutaneously administered ghrelin at a dose of e.g. 5-10 μg/kg.

Example 4

Clinical Proof-of-concept study

II. A randomised placebo-controlled cross-over trial demonstrating long-term ghrelin therapy in patients with chronic renal failure.
Objectives: To demonstrate the effect of long-term ghrelin administration on appetite, food intake, nutritional status and quality of life in patients with chronic renal failure.

Design: Randomised, double-blinded placebo-controlled, parallel-group trial (n=200).

Endpoints: Appetite as assessed by visual analogue scales, eating related symptoms, food intake, body weight, lean body mass and quality of life.

Trial product: Ghrelin e.g. 5-10 μg/kg administered as a subcutaneous injection 1-2 times daily for 3-6 months.

Example 5
Pharmacokinetic study in patients with liver failure.

Objectives: Establish the safety and pharmacokinetics of ghrelin in patients with liver failure.

Design: Open label, parallel group study in patients with liver failure (n=6) and in healthy male volunteers (n=6).

Endpoints: Pharmacokinetic parameters, safety

Trial product: Single dose, subcutaneously administered ghrelin at a dose of e.g. 10 μg/kg.

Example 6
A randomised placebo-controlled cross-over trial evaluating long-term ghrelin therapy in patients with liver failure.

Objectives: To evaluate the effect of long-term ghrelin administration on appetite, food intake, nutritional status and quality of life in patients with liver failure.

Design: Randomised, double-blinded placebo-controlled, parallel-group trial (n=200).

Endpoints: Appetite as assessed by visual analogue scales, eating related symptoms, food intake, body weight, lean body mass and quality of life.

Trial product: Ghrelin e.g. 10 μg/kg administered as a subcutaneous injection 1-2 times daily for 3-6 months.
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Claims

1. Use of a secretagogue compound or pharmaceutically acceptable salt thereof for the preparation of a medicament for the stimulation of appetite, food intake and/or weight gain in an individual suffering from liver failure.

2. The use according to claim 1, wherein said liver failure is chronic.

3. The use according to any of the preceding claims, wherein said liver failure is due to alcoholic liver failure or hepatitis.

4. The use according to any of the preceding claims, wherein said individual is suffering from liver failure caused by hepatitis C.

5. The use according to any of the preceding claims, wherein said individual has experienced an involuntary weight-loss prior to commencement of treatment, preferably having a lean body mass of less than 80% of normal.

6. The use according to any of the preceding claims, wherein said secretagogue is administered in a formulation having a pH within the range 3.5-7, such as pH 4-6 or pH 5-6.

7. The use according to any of the preceding claims, wherein said secretagogue is administered in a formulation for parenteral, intravenous, intramuscular or subcutaneous administration.

8. The use according to any of the preceding claims, wherein the secretagogue is administered using an administration form selected from the group consisting of: buccal delivery, sublingual delivery, transdermal delivery, needle-free injection, dermal patches, implants, a dropper, pipette, ampoules, pre-filled syringes, transdermal administration, a cartridge for an injection pen, small volume infusion, or in multi-dose containers with an added preservative.

9. The use according to any of the preceding claims, wherein the secretagogue is administered using an administration form selected from the group consisting of:
buccal delivery, sublingual delivery, transdermal delivery, needle-free injection, dermal patches, implants, a dropper, pipette, ampoules, pre-filled syringes, transdermal administration, a cartridge for an injection pen, small volume infusion, or in multi-dose containers with an added preservative.

10. The use according to any of the preceding claims, wherein said secretagogue is administered in a formulation comprising the secretagogue or a salt thereof as a lyophilisate and the formulation further comprises a solvent, said lyophilisate and said solvent being in separate compartments until administration.

11. The use according to any of the preceding claims, wherein said secretagogue is administered as a solution of the secretagogue or a salt thereof, such as a saline solution.

12. The use according to any of the preceding claims, wherein said secretagogue is administered using intra-arterial administration.

13. The use according to any of the preceding claims, wherein said secretagogue is administered as a premeal bolus using either parenteral or subcutaneous administration.

14. The use according to any of the preceding claims, wherein said secretagogue is administered as a bolus during a meal or after commencing a meal, preferably less than 180 minutes after commencing a meal.

15. The use according to any of the preceding claims, wherein said secretagogue is administered as a bolus injection or a fast running infusion prior to a meal or during a meal.

16. The use according to any of the preceding claims, wherein said secretagogue is administered from one to three times daily, each administration being within 45 minutes of a meal.

17. The use according to any of the preceding claims, wherein the secretagogue is administered in a concentration equivalent to from 0.1 μg to 1 mg ghrelin per kg bodyweight, such as from 0.5 μg to 0.5 mg ghrelin per kg bodyweight, such as from
1.0 µg to 0.1 mg ghrelin per kg bodyweight, such as from 1.0 µg to 50 µg ghrelin per kg bodyweight,

18. The use according to any of the preceding claims, wherein the secretagogue is administered as a conjugate.

19. The use according to any of the preceding claims, wherein the secretagogue is administered conjugated to one or more of the following:
- a peptide having effect on nociceptin receptor ORL1
- a natural or synthetic polymer attached to a reactive group present on the secretagogue,
- an oligosaccharide molecule, such as dextran, glycan, transferrin, etc.
- N-hydroxysuccinimide (NHS)
- N-hydroxy-sulfosuccinimide (sulfo-NHS)
- maleimide-benzoyl-succinimide (MBS)
- gamma-maleimido-butryroxy succinimide ester (GMBS)
- maleimidopropionic acid (MPA)

20. The use according to any of the preceding claims, wherein the secretagogue is modified to include suitable reactive groups, whereby the thus modified secretagogue is capable of forming a conjugate in vivo (after having been administered to an individual) through covalent bonding with available reactive functionalities on blood components.

21. The use according to any of the preceding claims, wherein the secretagogue is either PEGylated or alternatively conjugated to an Fc region of an IgG molecule

22. The use according to any of the preceding claims, wherein the secretagogue is incorporated into liposomes, micelles, iscoms, and/or microspheres or other transport molecules

23. The use according to any of the preceding claims, wherein the secretagogue is administered in a composition comprising one or more of the following: methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, benzalkonium chlorides.
24. The use according to any of the preceding claims, wherein the pharmaceutically acceptable salt is one of the following: a hydriodic salt, trichloroacetic salt, cinnamic salt, glycolic salt, malonic salt, mandelic salt, picric salt, pyruvic salt, salicylic salt, ethanesulfonic salt, pamoic salt, bis(methylene salicylic salt, ethanedisulfonic salt, gluonic salt, ethylenediaminetetraacetic (EDTA) salt, p-aminobenzoic salt, lithium salt, methylammonium salt, dimethylammonium salt, salt derived from metaphosphoric acid, salt derived from lactic acid, salt derived from glycolic acid or gluconic acid, trimethylammonium salt, ethylammonium salt, hydroxyethylammonium salt, diethylammonium salt, ferric hydroxide salt, isopropylamine salt, 2-ethylamino ethanol salt, procaine salt, butylammonium salt and tetramethylammonium salt.

25. The use according to any of the preceding claims, wherein the secretagogue is administered in combination with one or more of the following compounds: a growth hormone, such as hGH; IGF-1, IGFBP-3, ALS, a melanocortin receptor antagonist, a neuropeptide Y receptor agonist, leptin or leptin receptor agonist, a cannabinoid, an antipsychotic, Sulpirid, Clozapine, Risperdone, Quetiapin, Amisulpride, Ziprasidon, or Olanzapine.

26. The use according to any of the preceding claims, wherein the secretagogue is a ghrelin-like compound and is administered in combination with at least one different ghrelin-like compound.

27. The use according to any of the preceding claims, wherein the secretagogue is ghrelin or a pharmaceutically acceptable salt thereof.

28. The use according to any of the preceding claims, wherein the secretagogue is a ghrelin-like compound or a pharmaceutically acceptable salt thereof

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

\[ Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2, \]  

wherein

\[ Z^1 \]  
is an optionally present protecting group

35
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 $X^2$ 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.

29. The use according to claim 28, wherein $m$ is an integer in the range of from 1-9, such as from 1-8, such as from 1-7, such as from 1-6, such as from 1-5, such as from 1-4, such as from 1-3, such as from 1-2, such as 2.

30. The use according to any of claims 28 to 29, wherein $X^2$ is selected from the group of modified Ser, modified Cys and modified Lys, such as wherein $X^2$ is modified Ser.

31. The use according to any of claims 28 to 30, wherein the ghrelin-like compound is selected from a compound of

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

formula III $Z^1$ – Gly – Ser – (X$^2$) – (X$^3$)$_{n-1}$ – $Z^2$, and

formula IV $Z^1$ – Gly – (X$^2$) – (X$^3$)$_{n-1}$ – $Z^2$. 
32. The use according to claim 31, wherein the ghrelin-like compound is having formula III.

33. The use according to any of claims 28 to 32, 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

34. The use according to any of claims 28 to 33, wherein \(n\) is an integer in the range of from 1-25, such as 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.

35. The use according to any of claims 28 to 34, wherein \((X^3)_n\) is selected from one or more of the sequences shown below:

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

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

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

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

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

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

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

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

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

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

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

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

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

Phe Leu Ser Pro Glu His Gln Arg Val Gln

35 Phe Leu Ser Pro Glu His Gln Arg
Phe Leu Ser Pro Glu His Gln
Phe Leu Ser Pro Glu His
5
Phe Leu Ser Pro Glu
Phe Leu Ser Pro
10
Phe Leu Ser
Phe Leu

Phe

36. The use according to any of claims 28 to 35, wherein the acyl group is selected from a C1-C35 acyl group, such as a C1 – C20 acyl group, such as a C1 – C15 acyl group, such as a C6 – C15 acyl group, such as a C6 – C12 acyl group, such as a C8 – C12 acyl group.

37. The use according to any of claims 28 to 36, wherein the acyl group is selected from the group of C7 acyl group, C8 acyl group, C9 acyl group, C10 acyl group, C11 acyl group, and C12 acyl group.

38. The use according to any of claims 28 to 37, wherein the acyl group is selected from the group of C8 acyl group, and C10 acyl group.

39. The use according to any of claims 28 to 38, wherein the acyl group is selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.

40. The use according to any of claims 28 to 39, wherein the medicament is administered in a concentration equivalent to from 10 ng to 10 mg ghrelin per kg bodyweight.
41. The use according to claim 40, wherein the medicament is administered in a concentration equivalent to from 0.1 μg to 1 mg ghrelin per kg bodyweight, such as from 0.5 μg to 0.5 mg ghrelin per kg bodyweight, such as from 1.0 μg to 0.1 mg ghrelin per kg bodyweight, such as from 1.0 μg to 50 μg ghrelin per kg bodyweight, such as from 1.0 μg to 10 μg ghrelin per kg bodyweight.

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

43. The use according to claim 42, wherein the medicament is administered as a bolus prior to or during a meal, said bolus comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 2.0 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. The use according to any of the preceding claims, wherein the medicament is administered prior to or during a meal, preferably less than 180 minutes prior to a meal, such as less than 90 minutes prior to a meal, for example less than 45 minutes prior to a meal, such as less than 30 minutes prior to a meal, for example less than 25 minutes prior to a meal, such as less than 20 minutes prior to a meal, for example less than 15 minutes prior to a meal, such as about 10 minutes prior to a meal, for example about 5 minutes prior to a meal, such as immediately prior to a meal, or during a meal.

45. The use according to any of the preceding claims, wherein the medicament is administered after commencing a meal, preferably less than 90 minutes after commencing a meal, for example less than 45 minutes after commencing a meal, such as less than 30 minutes after commencing a meal, for example less than 25 minutes after commencing a meal, such as less than 20 minutes after commencing a meal, for example less than 15 minutes after commencing a meal, such as less than 10 minutes after commencing a meal, for example less than 5 minutes after commencing a meal.
46. The 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. The use according to any of the preceding claims, wherein the patient has a lean body mass of less than 80% of normal, such as less than 60% of normal and/or a body mass index below 17 kg/m².

48. The use according to any of the preceding claims, wherein the medicament is given until the lean body mass is more than 60% of normal, preferably more than 80% of normal, more preferably more than 90% of normal.

49. Use of a secretagogue compound for the preparation of a medicament for the stimulation of appetite, food intake and/or weight gain in an individual suffering from renal failure.

50. The use according to claim 49, wherein the secretagogue is as described in any of the claims 27 to 39.

51. The use according to any of claims 49 to 50, wherein the medicament is in a formulation as described in any of claims 6-26.

52. The use according to any of claims 49 to 51, wherein the medicament is administered as described in any of claims 40 to 48.

53. The use according to any of claims 49-52, wherein said renal failure is chronic renal failure.

54. The use according to any of claims 49-53, wherein said renal failure has a chemical cause

55. The use according to any of claims 49-53, wherein said renal failure is caused by an infection.
56. The use according to any of claims 49-53, wherein said renal failure has an immunologic cause.

57. The use according to any of claims 49-53, wherein said renal failure has an idiopathic cause.

58. The use according to any of claims 49-53, wherein said renal failure is due to congenital renal disease.

59. The use according to any of claims 49-58, wherein said individual is euemic.

60. The use according to any of claims 49-59, wherein said individual has end-stage renal failure.

61. The use according to any of claims 49-60, wherein said individual has hypoalbuminemia and is preferably unresponsive to dialysis.

62. The use according to any of claims 49-61, wherein said individual is suffering from, or at risk of suffering from cystinosis and/or oxalosis.

63. The use according to any of claims 50-62, wherein said individual is suffering from, or at risk of suffering from, chronic glomerulonephritis.

64. The use according to any of claims 50-63, wherein the individual's glomerular filtration rate is within the range of 60-89 ml/min.

65. The use according to any of claims 50-63, wherein the individual's glomerular filtration rate is within the range of 30-59 ml/min.

66. The use according to any of claims 50-63, wherein the individual's glomerular filtration rate is within the range of 15-29 ml/min.
67. The use according to any of claims 50-63, wherein the individual's glomerular filtration rate is less than 15 ml/min.