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(54) Title: TREATMENT OF EATING DISORDERS AND INDUCTION OF LIPOLYSIS

(57) Abstract: The present invention relates to PYY or functional equivalents thereof for use in pharmaceutical compositions. The pharmaceutical compositions are in particular useful in the treatment of eating disorders, such as disorders characterised by craving and binges. The invention further discloses the use of said composition for regulation of lipolysis, e.g. stimulation of lipolysis for the treatment of obesity. The invention further relates to methods of treatment using said compositions. Further included is the combination of PYY or functional equivalents thereof with a secondary active ingredient such as an anti-emetic drug.
Treatment of eating disorders and induction of lipolysis

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

Field of invention

The present invention relates to novel therapeutic uses of PYY or functional equivalents thereof. More specifically, PYY or a functional equivalent thereof may be used for prevention or reduction of craving for food in an individual suffering from such craving, e.g. in individuals suffering from an eating disorder.

In a specific aspect, the invention relates to treatment of eating disorders, such as binge eating disorder and night eating syndrome. The invention relates to a composition for the preparation of a pharmaceutical composition for the above mentioned therapeutic uses.

The present application relates to the use of PYY or functional equivalents thereof for stimulation of fat mobilisation by lipolysis.

Background of invention

Food craving is an intense and uncontrollable desire for food, e.g. food in general or specific food types or items, such as food having a high content of carbohydrate and/or fat, e.g. snacks. Craving is usually independent of a feeling of hunger. Craving can lead to abnormally large consumption of food and resulting psychological problems and/or weight problems. Food craving may lead to health-threatening conditions such as obesity. Craving may occur independently of any other eating disorder or may be related to an eating disorder, e.g. any of the eating disorders disclosed herein.

Food cravings as used herein may be defined according to The Questionnaire for Weight and Eating Patterns-R (QEWP-R) (Spitzer, RL; Devlin, M; Walsh, BT et al. Binge eating disorder: a multisite field trial of the diagnostic criteria. Int. J Eat. Disord. 1992;11:191-203; Nangle, DW; Johnson, WG; Carr-Nangle, RE & Engler, LB. Binge eating disorder and the proposed DSM-IV criteria: psychometric analysis of
the Questionnaire of Eating and Weight Patterns. Int. J. Eat. Disord. 1994;16:147-57), by either 1) Being upset about overeating, and being upset about not being able to control what and how much that was eaten, and Not engaging in compensatory behaviour such as purging, vomiting, fasting, excessive exercise, excessive use of laxatives or suffering from bulimia nervosa (including the non-purging and purging subtypes) or anorexia nervosa; or 2) Being upset about overeating, and Being upset about not being able to control what and how much that was eaten, and During the past six months often having eaten within any two hour period what most people would regard as an unusually large amount of food, and 3) not engaging in compensatory behaviour such as purging, vomiting, fasting, excessive exercise, excessive use of laxatives or suffering from bulimia nervosa (including the non-purging and purging subtypes) or anorexia nervosa.

Alternatively, Food Cravings as used herein may be defined using the Bulimia scale of the Eating Disorder Inventory-2 (EDI-2) questionnaire (Garner, DM. Eating Disorder Inventory-2: professional manual, Odessa (FL): Psychological Assessment Resources, 1993) 1) by having a score of at least 2, such as at least 3, e.g. at least 4, such as at least 5 on the Bulimic subscale of EDI-2 and 2) by having answered “never” or “rarely/seldom” to the question pertaining to compensatory vomiting in order to lose weight in the Bulimia scale of the EDI-2. The Bulimia scale of the EDI-2 consists of seven questions, and responses are made on a 6-point Likert-type scale ranging from “never” to “always”.

Alternatively, Food Cravings as used herein may be defined according to the Food Cravings Questionnaires Trait (FCQ-T) (Cepeda-Benito, A; Gleaves, DH; Williams, TL & Erath, SA. The development and validation of the state and trait food-cravings questionnaires. Behaviour Therapy. 2000;31:151-173) 1) by having an average score of at least 3, such as at least 3.5, such as at least 4, such as at least 4.5 or such as at least 5 in each of the nine subscales of the FCQ-T, thus corresponding to a sum of the averages of the nine subscales of at least 27, such as at least 31.5, such as at least 36, such as at least 40.5 or such as at least 45, and 2) not engaging in compensatory behaviour such as purging, vomiting, fasting, excessive exercise, excessive use of laxatives or suffering from bulimia nervosa (including the non-purging and purging subtypes) or anorexia nervosa.
Furthermore, Food Cravings may be defined according to the Binge Eating Scale (BES) (Gormally, J; Black, S; Daston, S & Rardin, D. The assessment of binge eating severity among obese persons. Addict. Behav. 1982;7:47-55; and Gladis, MM; Wadden, TA; Vogt, R; Foster, G; Kuehnel, RH & Barlett, SJ. Behavioural treatment of obese binge eaters: do they need different care? J Psychosom. Res. 1998;44:375-84) 1) by having a score of at least 16, such as at least 18, such as at least 20, such as at least 22, such as at least 24, such as at least 26 or such as at least 27, and 2) not engaging in compensatory behaviour such as purging, vomiting, fasting, excessive exercise, excessive use of laxatives or suffering from bulimia nervosa (including the non-purging and purging subtypes) or anorexia nervosa.

Preferably, the food craving diagnosis of the individual to be treated in accordance with the invention is defined using the FCQ-T scale with the appropriate cut-off values, as defined above. In another embodiment of the invention, the food cravings diagnosis is defined using one of the other methods outlined above. In a further preferred embodiment of the invention, the food cravings diagnosis is defined using all of the methods above in combination, such that the subject fulfils any one of the criteria outlined above. In another embodiment of the invention, the food cravings diagnosis is defined using all of the methods outlined above in combination, such that the subject fulfils all of the criteria outlined above.

A great number of people suffer from eating disorders, such as binge eating disorder and/or night eating syndrome and thereby have an increased risk of acquiring additional health problems as well as a lack of quality of life. These types of syndromes have been getting more attention lately and are now being characterized as clinical diseases. A major problem is the diagnosis of these diseases, as the syndromes are merely characterised by a behavioural pattern of the patient. The characteristics of these syndromes are summarised below.

**Binge Eating Disorder**

Binge Eating Disorder (BED) is a type of eating disorder not otherwise specified and is characterized by recurrent binge eating without the regular use of compensatory measures to counter the binge eating. People with binge eating disorder frequently eat large amounts of food and feel a loss of control over their eating behaviour. This disorder is different from binge-purge syndrome (bulimia nervosa) because people
with binge eating disorder usually do not purge afterward by vomiting or using laxatives. Most people with serious binge eating problems frequently experience episodes of eating what others would consider an abnormally large amount of food and frequently feel unable to control what or how much to eat. A person suffering from BED often eats without being hungry, eats more rapidly than normal and eats until uncomfortably full. The patient may feel ashamed or disgusted by their behaviour. Eating alone or in secret out of embarrassment of the quantity of food being eaten are other characteristics of binge eating disorder. The patient may experience feelings of disgust, depression, or guilt after overeating.

Diagnosing of Eating disorders as Binge-eating disorders may be diagnosed according to: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) published by the American Psychiatric Association, as cited here below.

**DSM IV Binge-Eating Disorder 1994**

A - Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following:

1. eating in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat in a similar period of time under similar circumstances

2. a sense of lack of control over eating during the episode (e.g. a feeling that one cannot stop eating or control what or how much one is eating)

B - The binge-eating episodes are associated with three (or more) of the following:

1. eating much more rapidly than normal

2. eating until feeling uncomfortably full

3. eating large amounts of food when not feeling physically hungry

4. eating alone because of being embarrassed by how much one is eating

5. feeling disgusted with oneself, depressed, or very guilty after overeating

C - Marked distress regarding binge eating is present

D - The binge eating occurs, on average, at least 2 days a week for 6 months

Note: The method of determining frequency threshold is counting the number of days on which binges occur or in counting the number of episodes of binge eating.

E - The binge eating is not associated with regular use of inappropriate compensatory behaviors (e.g., purging, fasting, excessive exercise) and does not occur exclusively during the course of anorexia nervosa or bulimia nervosa.
Night eating syndrome

Night-eating syndrome (NES) has not yet been formally defined as an eating disorder. Underlying causes are being identified, and treatment plans are still being developed. It seems likely that a combination of biological, genetic, and emotional factors contribute to the problem.

A person suffering from NES shows little or no appetite for breakfast and delays first meal for several hours after waking up. This is due to lack of hunger or based on being upset about how much was eaten the night before. Often the patient eats more food after dinner than during the meal. This may mean that more than half of the daily food intake occurs after dinner but before breakfast. Most people who suffer from the syndrome are prone to stress, especially at night the person may be moody, tense, anxious, nervous, agitated, etc. and has trouble falling asleep or staying asleep and thus have poor sleep, often waking up three to four times a night. Each time they wake up they walk to the kitchen to eat a "snack" of high carbohydrate (sugar and starch) food such as biscuits, cakes or crisps. The person often feels tense, anxious, upset, or guilty while eating. This pattern should persist for at least two months before being characterised as a night eating syndrome. NES is thought to be stress related and is often accompanied by depression. The behaviour of an NES patient is different from the behaviour of an individual suffering from a binge eating disorder. During binge eating, relatively large amounts of food are consumed over a relatively short space of time. Night-eating syndrome involves continuous eating throughout evening hours. The heavy preference for carbohydrates, which trigger the brain to produce so-called "feel-good" neuro-chemicals, suggests that night eating may be an unconscious attempt to self-medicate mood problems. NES may run in families. It appears to respond to treatment with the selective serotonin reuptake inhibitor (SSRI) sertraline (a prescription medication). NES is remarkable for characteristic disturbances in the circadian rhythm of food intake, while the circadian sleep rhythm remains normal.

Night-eating syndrome shows distinctive changes in hormones related to sleep, hunger and stress. The night time rise in the hormone that accompanies sleep, melatonin, is greatly decreased in night eaters, probably contributing to their sleep disturbances. Similarly, night-eaters fail to show a nighttime rise in the hormone leptin,
which suppresses hunger, and the stress hormone cortisol is elevated throughout a 24-hour period.

Food craving may, but need not be one of the eating behavioural symptoms giving rise to NES.

Regulation of energy uptake.
People who suffer from eating disorders will very often encounter health problems due to an unbalanced diet. This may be due to deregulation of the system regulating eating behaviour in the individual.

In normal healthy individuals energy uptake is regulated by hormones that regulate food intake, by modifying the sense of appetite, and regulate absorption of nutrients and solutes from the gut.

Hormones that regulates food intake can be separated into long and short term regulating hormones. Long term regulating hormones as insulin and leptin act slowly to promote the stability of body fat stores long, whereas the short term regulating hormones, as ghrelin and cholecystokinin, act rapidly to influence the individual meal by sensing of satiety and “fullness”. Insulin and leptin are released into the blood in proportion to the amount of body fat. When body fat stores are reduced, declining levels of these hormones are sensed by the brain and are transduced into increases in appetite and metabolic efficiency that persist until the lost weight is recovered. Ghrelin and cholecystokinin are factors that trigger the onset and termination of eating and thereby ghrelin and cholecystokinin function in a meal-to-meal control system that itself is sensitive to changes in insulin and leptin levels. In this way, the size and frequency of individual meals can be adjusted so as to minimize changes in body fat content.

The uptake of nutrients from the gut is regulated by various factors including gut hormones. The gut hormones VIP, CCK, and motilin relate to the motility of the upper gastrointestinal tract whereas Peptide YY (PYY) and Neuropeptide Y (NPY) affect the absorption in the intestine.
PYY

The gut hormone peptide YY (PYY), and the neuropeptide, neuropeptide Y (NPY), are structurally related to pancreatic polypeptide (PP) (figure 1). PYY and NPY exert their action through NPY receptors (Y1R, Y2R, Y4R and Y5R). The PP, NPY and PYY peptides consist of 36 amino acids with an amidated C-terminal. Two forms of PYY, PYY1-36 and PYY3-36, the latter being a truncated form of the former, have been found in circulation. PYY3-36 is produced by the cleavage of PYY1-36 by the enzyme dipeptidyl peptidase IV (DPP-IV). PYY1-36 binds to and activates at least three NPY receptor subtypes (Y1, Y2 and Y5) whereas PYY3-36 is more selective for the Y2 receptor (Y2R). Only the C-terminal part of the PYY3-36 peptide is required for the binding to the Y2 receptor (Berglund, M.M. et al., 2003). Throughout this document, the notation PYY covers both PYY1-36 and PYY3-36.

PYY was initially isolated from porcine intestine (Tatemoto, K. and Mutt, C., 1980) and named Peptide YY due to the tyrosine residues present in the N- and C-terminal of the molecule.

PYY is expressed in endocrine cells lining the gastrointestinal tract and particularly in the distal portion. PYY is secreted in response to food ingestion. Within 15 minutes the plasma level of PYY rise and the level of PYY will reach a plateau after approximately 90 min. The maximum level of PYY reached is proportional to the calories ingested, suggesting that PYY may function as a sensor of food ingestion. In addition PYY is also expressed by neurones, such as in peripheral neurons, particularly enteric neurons. Furthermore, PYY is found in a restricted set of central neurons. The expression pattern of PYY in both endocrine cells and neurons suggest that PYY may be involved in regulation of multiple functions in the individual (Ekblad, E. and Sundler, F., 2002).

A suggested role of PYY may be to regulate the secretion and absorption of fluid and electrolytes in the gastrointestinal tract and intestine and PYY have therefore been suggested as treatment of diarrhoea (US 6,588, 708) by prolonging the residence time. Furthermore, PYY3-36 has been suggested to be involved in the system regulating feeding behaviour. It has been found that peripheral administration of PYY3-36 inhibited food intake in rodents. Moreover, direct intra-pituitary administration of PYY3-36 inhibited food intake. A linkage between the PYY
effect on feeding behaviour and Y2R has been suggested by the demonstration that NPY receptor Y2 null mice are resistant to the anorectic effects of peripherally administered PYY3-36 (Batterham, R.L. and Bloom, S.R., 2003).

The hypothalamic arcuate nucleus, a key brain area regulating appetite, has access to nutrients and hormones within the peripheral circulation. NPY neurons within the arcuate nucleus express the Y2R. The arcuate nucleus contains two distinct subgroups of neurons that control food intake. On group of neurons produces NPY, which acts in the brain to stimulate feeding (Stanley, B. G. et al, 1986), whereas an adjacent subgroup of neurons produces melanocortin peptides, which act in the same brain areas as NPY, but inhibit eating (Fan, W. et al, 1997). Typically, when one of these subsets is activated, the other one is inhibited.

Lipid Metabolism in Animals

Fatty acids are stored in triglycerides (triacylglycerols) in fat droplets in the cytoplasm of adipose cells. In the intestines, lipases secreted by the pancreas hydrolyze dietary triglycerides. The monoglycerides and fatty acids produced are taken up by the cells lining the intestine and then assembled into chylomicrons and transported into the lymph fluid and from there into the bloodstream.

Lipolysis is defined as the catabolism of a triglyceride into three fatty acids and one glycerol molecule and occurs mainly in adipose tissue. The free fatty acids are transported in the blood by albumin. To utilize energy bound in free fatty acids, the molecules are degraded to acetyl CoA in the mitochondria of eukaryotic cells by the beta-oxidation pathway. Fatty acid biosynthesis occurs in the cytoplasm of eukaryotic cells. Although it occurs by sequential addition of acetyl groups and involves similar chemical reactions, fatty acid biosynthesis is not beta-oxidation in reverse.

Lipid and carbohydrate metabolism occur to different extents in different organs. The liver regulates the flux of metabolites to brain, muscle and adipose tissue, and ultimately controls the concentration of blood glucose. Glycolysis and fatty acid metabolism are coordinated in adipose tissue, so that glucose imported from the liver is converted to glycerol, and fatty acids are esterified in triglycerides. Muscle oxidizes glucose, fatty acids and ketone bodies, and returns lactate to liver for conversion to glucose by gluconeogenesis. The brain uses glucose as a fuel except in prolonged
fasting or starvation, when it will use ketone bodies as a fuel. Under some conditions, fatty acid degradation occurs more rapidly than glycolysis.

Insulin stimulates biosynthetic processes and inhibits catabolism in liver, muscle, and adipose tissue. The effects of insulin encourage the storage of glucose and lipids in the form of triglycerides and insulin further inhibits lipolysis and ketogenesis. Glucagon is secreted when blood glucose levels are too low. It has the opposite effects of insulin, on metabolism, including the breakdown of lipids and glycogen.

Lipids in the Blood:

Lipids ingested as food are digested in the small intestine where bile salts are used to emulsify them and pancreatic lipase hydrolyzes lipids into fatty acids, glycerol, soaps, or mono- and diglycerides.

Since lipids are not soluble in blood, they are transported as lipoproteins after reaction with water-soluble proteins in the blood. Fatty acids are generally transported in this form as well. Excess lipids in the blood are eventually converted into adipose tissue. If lipid levels in the blood become too low, the body synthesizes lipids from other foods, such as carbohydrates, or removes lipids from storage.

Fat is used as energy source when insulin levels are low, except in brain tissue. Thus fat is preferably mobilised and use in situations of low blood sugar.

Hydrolysis of triglyceride in adipocytes is regulated by catecholamines that bind to β-adrenergic receptors (β-AR) to activate hormone-sensitive lipase (Fain and Garcia-Sainz, 1983). Adipocytes express three βAR subtypes, but the role for each subtype is not clearly defined. The β-3 adrenoceptor has a profile quite distinct from that of β-1 and β-2 adrenergic receptors and an important role of the β-3 receptor is in the regulation of lipid metabolism. Following β-3 antagonists have been shown to induce changes in energy metabolism via lipolysis and thermogenesis.

References


Summary of invention

The situation described above reflects the healthy subject, whereas this system of appetite regulation is abolished in patients suffering from craving and/or an eating disorder. The patients may feel an urge to eat, thus the start of a "meal" is not necessarily controlled by hunger or appetite, and/or the patients do not stop eating because of a normal sensation of satiety. The syndromes may be caused by complex disorders that both may be related to biological defects and/or psychological problems.

The consumption of an inappropriate meal by a patient can be characterised as an eating disorder event. In order to minimize the health problems associated with eating disorders it is a goal of this invention to reduce the number and/or extent of
food cravings, to reduce or inhibit the number of binge eating events and/or number of "snack" meals of a NES patient during the night.

Peptide YY (PYY) has surprisingly been found to be an effective compound for the treatment of eating disorders.

An aspect of the invention relates to a composition comprising PYY or a functional equivalent thereof, for the preparation of a pharmaceutical composition for prevention or reducing craving for food in an individual.

Further the invention relates to a composition comprising PYY or a functional equivalent thereof, for the preparation of a pharmaceutical composition for the treatment of an eating disorder by

a) decreasing the frequency of eating disorder events (EDEs) and/or
b) preventing an eating disorder event and/or
c) reducing food and/or caloric intake during the event.

An aspect of the invention relates to a method of treatment comprising administration of the pharmaceutical composition according to the invention.

It is further described herein that PYY stimulates mobilization of fatty acids.

An aspect of the invention relates to a composition comprising PYY or a functional equivalent thereof, for the preparation of a pharmaceutical composition for mobilization of fatty acids and stimulation of lipolysis and further for the increase of blood concentration of free fatty acids.

The medicament is preferably for administration before bedtime whereby administration is followed by a fasting period.

Description of figures

Figure 1

Structure of the family of the PP-fold peptides
NPY, PYY, and PP share a common hairpin-like three-dimensional structure called the PP-fold. All three peptides are 36 amino acids long with an amidated carboxy-terminus. The general structure of the PP-fold peptides has been established using x-ray crystallography of avian PP and confirmed in several studies using nuclear magnetic resonance. Amino acid residues 1–8 form a type II proline helix followed by a loop. Residues 15–32 form an \( \alpha \)-helix, and the four most carboxy-terminal residues are in a flexible loop conformation. The amino acid sequence of mammalian NPY is highly conserved, PYY display 8 variable amino acids, whereas the amino acid sequence of PP is the least conserved PP-fold peptide. The general three-dimensional structure seems to be conserved in all PP-fold peptides (Berglund MM et al, 2003).

Figure 2

**PYY plasma concentration in response to subcutaneous PYY1-36 administration.**

PYY1-36 was administered to subjects as described in example 3. The plasma level of PYY was measured during a 4 hour time period following injection. The plasma level of PYY increases with in 15 minutes after administration.

A plasma level of 80-100 pmol/l is obtained using a dosage of 200 pmol/kg FFM.

Figure 3

**PYY plasma concentration in response to subcutaneous PYY3-36 administration.**

PYY3-36 was administered to subjects as described in example 3. The plasma level of PYY was measured during a 4 hour time period following injection. The plasma level of PYY increases with in 15 minutes after administration.

A plasma level of 100-120 pmol/l is obtained using a dosage of 100 pmol/kg FFM.

Figure 4

**PYY3-36 increases the concentration of free fatty acids (FFA) in plasma**

Subjects were infused with PYY3-36 for 90 minutes as described in example 5. The plasma level of FFA is measured during a 6 hour period, where the subjects receive a meal after 4 hours. The level of FFA increases from the start of treatment until meal intake.
Sequence listing

SEQ ID 1: Human PYY

Definitions:

AA: See "Amino acid".

Amino acid: Entity comprising an amino terminal part (NH2) and a carboxy terminal part (COOH) separated by a central part comprising a carbon atom, or a chain of carbon atoms, comprising at least one side chain or functional group. NH2 refers to the amino group present at the amino terminal end of an amino acid or peptide, and COOH refers to the carboxy group present at the carboxy terminal end of an amino acid or peptide. The generic term amino acid comprises both natural and non-natural amino acids. Natural amino acids of standard nomenclature as listed in J. Biol. Chem., 243:3552-59 (1969) and adopted in 37 C.F.R., section 1.822(b)(2) belong to the group of amino acids listed in Table 1 herein below. Non-natural amino acids are those not listed in Table 1. Examples of non-natural amino acids are those listed e.g. in 37 C.F.R. section 1.822(b) (4), all of which are incorporated herein by reference. Amino acid residues described herein can be in the "D" or "L" isomeric form.

<table>
<thead>
<tr>
<th>Symbols</th>
<th>1-Letter</th>
<th>3-Letter</th>
<th>Amino acid</th>
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<tr>
<td>Y</td>
<td>Tyr</td>
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<td>G</td>
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</table>
V  Val  valine
P  Pro  proline
K  Lys  lysine
H  His  histidine
Q  Glu  glutamic acid
E  Trp  tryptophan
R  Arg  arginine
D  Asp  aspartic acid
N  Asn  asparagine
C  Cys  cysteine

Table 1. Natural amino acids and their respective codes.

15  **Amino acid residue**: the term "amino acid residue" is meant to encompass amino acids, either standard amino acids, non-standard amino acids or pseudo-amino acids, which have been reacted with at least one other species, such as 2, for example 3, such as more than 3 other species. In particular amino acid residues may comprise an acyl bond in place of a free carboxyl group and/or an amine-bond and/or amide bond in place of a free amine group. Furthermore, reacted amino acid residues may comprise an ester or thioester bond in place of an amide bond.

**Antibody**: Are immunoglobulin molecules and active portions of immunoglobulin molecules. Antibodies are for example intact immunoglobulin molecules or fragments thereof retaining the immunologic activity.

20  **Antigen**: The molecule recognised by an antibody. Usually a peptide, polypeptide or a multimeric polypeptide. Antigens are preferably capable of eliciting an immune response.

**Appetite**: Appetite in an individual is assessed by measuring the amount of food ingested and by assessing the individual's desire to eat. Appetite (i.e. 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, no desire at all, to 5, extreme desire. Other scales and ratings are also used, but, in general, some type of a visual analogue scale (VAS) is used to assess an individual's desire to eat.
**BED**: Binge eating disorder.

**BMI**: Body mass index measures an individual's height/weight ratio. It is determined by dividing the weight in kilograms by the square of the height in meters. A BMI between 18.5 and 24.9 is considered normal.

**Body fat mass**: Body fat mass can be measured e.g. by the fat fold technique: In this technique, a pincer-type calliper 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.

**Concentration equivalent**: A concentration equivalent is an equivalent dosage being defined as the dosage of a compound having the same response (as evaluated e.g. from a dosage-response curve) in vitro and/or in vivo as a known compound.

**Eating disorder event**: See “EDE”.

**EDE**: An eating disorder event refers to an inappropriate meal consumed by an individual suffering from an eating disorder, such as NES or BED.

**Frequency**: The number of occurrences of a certain event within a certain period of time (e.g. the number of occurrences per day or per week).

**Half-life**: See T1/2.

**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 any of the various 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.

**Ligand**: A molecule, for example a peptide, capable of specific binding to one or more cognate receptors. An antigen is, for example, a ligand to its cognate antibodies.
“Loss of body weight”: Defined herein as a reduction of an individual’s overall weight, which include both relative and absolute reductions of weight, as well as BMI reductions.

“Loss of body fat”: Defined herein as either a reduction of an individual’s overall fat mass or a reduction in the percentage of an individual’s body fat.

Medical disorder: By the term “medical disorder” is meant any disease or syndrome having a detrimental effect on an individual’s physical and/or mental health.

MTD: Maximum tolerated dose. The maximum tolerated dose of a substance in a given test period should not induce a) overt toxicity, such as appreciable death of cells or organ dysfunction; b) a material reduction in life span, except by cancer induction; c) 10% or greater retardation of body weight gain (in growing animals). In a clinical setting, the MTD can be defined as the highest dose that dose not give rise to intolerable adverse effects, neither of a subjective nor of an objective (measurable, e.g. detectable by physical examination, blood sampling or other tests) nature. The MTD can be determined both on an individual basis and in a population of subjects receiving the substance in question.

NES: Night eating syndrome.

Parenteral: For the purpose of this document “parenteral” is defined as being outside the alimentary canal. Thus, the term “parenteral administration” of a compound encompasses e.g. subcutaneous, intramuscular, intravenous, intranasal, buccal, intradermal and transdermal administration, as well as inhalation of said compound. Furthermore, “parenteral” is also meant to encompass administration via rectal suppositories.

Peptide: Plurality of covalently linked amino acid residues defining a sequence and linked by amide bonds. The term is used analogously with oligopeptide and polypeptide. The amino acids may be both natural amino acids and non-natural amino acids, including any combination thereof. The natural and/or non-natural amino acids may be linked by peptide bonds or by non-peptide bonds. The term peptide also embraces post-translational modifications introduced by chemical or enzyme-catalyzed reactions, as are known in the art.

PYY: Peptide YY. Herein PYY represents both PYY1-36 and PYY3-36, the full length and a truncated version of PYY, respectively.

Receptor: A receptor is a molecule, such as a protein, glycoprotein and the like, that can specifically (non-randomly) bind to another molecule.
Recombinant DNA (rDNA) molecule: A DNA molecule produced by operatively linking two DNA segments. Thus, a recombinant DNA molecule is a hybrid DNA molecule comprising at least two nucleotide sequences not normally found together in nature.

$T_{1/2}$: The half-life is the time for the concentration of a compound to decrease 50%.

Detailed description of the invention

The present invention relates to the finding that administration of PYY or functional equivalents thereof is particularly useful for the treatment of food cravings, such as food cravings in association with an eating disorder. The PYY or functional equivalents useful according to the invention is described in a separate section below.

Food cravings

In the present context, food craving is intended to mean an undesirable eating behaviour that may include eating unusually large amounts of food every now and then, or very frequent eating of small amounts of food or snacks continuously or during discrete periods of time. Food cravings are typically not occurring in response to hunger. Furthermore, as used herein food cravings are typically not associated with compensatory behaviour such as purging, forced vomiting, fasting, excessive exercise or the excessive use of laxatives. In particular, as used herein subjects suffering from food cravings are distinctive from patients suffering from bulimia nervosa, including the purging and non-purging kinds of bulimia nervosa, and anorexia nervosa.

In one embodiment of the invention the food craving is associated with an eating disorder event, e.g. selected from the group of: binge eating and night eating. Eating disorder events will be discussed in further detail below.

In another embodiment food craving is associated with smoke cessation. Food craving may be one way that former smokers compensate for reduced or stop smoking activities and may contribute to increasing the body weight of people having stopped or reduced smoking. Accordingly, the composition of the invention may find an important use in reducing and/or removing the craving for food in an individual which has or desires to reduce or stop smoking.
In yet another embodiment food craving is associated with being on or having finalized a diet with the purpose of reducing or maintaining body weight.

It is a well recognized phenomenon that people being on a diet tend to focus a lot of their attention on food – so much that in certain instances the focus becomes a craving for food. The composition according to the present invention is contemplated to be useful for reducing and/or removing the craving for food in such individuals.

In one embodiment, the composition according to the present invention is contemplated to reduce one of the numerical scores outlined in the background section defining craving (the Bulimia scale of the EDI-2 questionnaire, the FCQ-T scale or the BES) by at least 5%, such as at least 10%, such as at least 15%, such as at least 20%, such as at least 25%, such as at least 30%, such as at least 40%, such as at least 50%, such as at least 60%, such as at least 70%, such as at least 80% or such as at least 90%.

In another embodiment, the composition according to the present invention is contemplated to reduce all of the numerical scores outlined above defining craving (the Bulimia scale of the EDI-2 questionnaire, the FCQ-T scale or the BES) by at least 5%, such as at least 10%, such as at least 15%, such as at least 20%, such as at least 25%, such as at least 30%, such as at least 40%, such as at least 50%, such as at least 60%, such as at least 70%, such as at least 80% or such as at least 90%.

In one embodiment, the food craving as described herein is craving for food with a high content of carbohydrate and/or fat, e.g. with a fat content of at least 30%, such as at least 40%, such as at least 50%, such as at least 60%, such as at least 70%, such as at least 80%, and such as at least 90% and/or a carbohydrate content of at least 30%, such as at least 40%, such as at least 50%, such as at least 60%, such as at least 70%, such as at least 80%, and such as at least 90%.

The psychological problems arising from food cravings may include being upset about overeating, feeling disgusted or embarrassed with oneself, or being depressed. The weight problems include becoming overweight as defined by having a
BMI in the range $25 \leq \text{BMI} < 30$, or becoming obese as defined by having a BMI $\geq 30$.

**Eating disorders**

Binge Eating Disorders are characterised by recurrent consumption of inappropriate meals. Patients suffering from Night Eating Syndrome eat during the night, and consume most of their daily intake of calories during the night.

These types of eating disorders have a substantial impact on the lives of the patients. The individual suffering from an eating disorder is likely to have health problems due to malnutrition. The diseases may also have a severe impact on the social life of the patients.

**Eating disorder event**

The eating disorders listed above are characterised by the recurrent consumption of inappropriate meals. Such a meal or behaviour is referred to as an eating disorder event (EDE). Binge eating relates to eating of large quantities at relatively long intervals, whereas the meals of a NES patient are relatively small but frequent. The severity of the syndromes may be characterised by the frequency of meals or the display of behaviour. Therefore, a treatment should decrease the number of meals, the frequency of the display of the inappropriate behaviour, e.g. the number of EDEs, and/or the size of individual meals (in particular in case of BED).

The treatment may aim at preventing the occurrence of an eating disorder event, or to decrease the frequency or extent (e.g. in terms of food intake) of an eating disorder event over a longer period.

To characterise the illness, the patient must obtain knowledge about the number and intervals of meals and the amounts of food consumed. The frequency of an eating disorder event (EDE) may e.g. be once a week, once a day, twice a day, three times a day or 5 times a day. The person suffering from NES may have one, two, three or more night eating syndrome events per night. In severe cases the NES patient may have e.g. 5 EDEs per night, or 7 EDEs per night, or 10 EDEs per night, or 15 EDEs per night, or 20 EDEs per night or more than 20 EDEs per night.
Therefore, a treatment should decrease the number of meals or the frequency of the display of the inappropriate behaviour, e.g. the number of EDEs. Alternative treatment may decrease the number of calories ingested during an EDE.

Evaluation of the eating disorder events may be performed as described in example 3 herein. The subjects are divided in three groups A, B and C, wherein the A group is the control and the B and C group receives different treatments.

In an embodiment according to the invention the active treatment group (B or C) has a reduction of frequency of binge-eating episodes of at least 5% more than the placebo group (group A) with statistically significant difference (p<0.05).

In an embodiment the frequency of EDE is decreased by at least 5 %, such as at least 10 %, 15 %, 20 %, 30%, 40 %, 50 %, 60 %, 70, 80 or 90 %. In preferred embodiment the frequency of EDE is decreased by at least 25, such as 45% or 75 %.

The treatment may aim at preventing the occurrence of an eating disorder event, or to decrease the frequency of an eating disorder event over a longer period.

The disorder may also be characterised by the food and/or caloric intake during an eating disorder event. In this case, the treatment may aim at reducing the amount of food or calories consumes during an eating disorder event or during a period of eating disorder event(s). In a preferred embodiment a reduction of at least 10 % of calorie intake during an EDE is observed for the active treatment group (B or C) compared to the placebo group (group A) with statistically significant difference (p<0.05). Preferably the calorie intake is reduced with at least 15 %, such as 20 % or such as 30 %. More preferably a reduction of at least 25 %, such as 40 or such as 50 % is observed.

Regulation of lipid metabolism

An aspect of the present invention relates to a medicament for stimulating lipolysis. Without being bound by the theory it is believed that PYY mobilises free fatty acids from storages by stimulating lipid catabolism. In an embodiment the medicament is for increasing the blood concentration of free fatty acids.
The effect of PYY administration may be improved by controlling the food intake after PYY administration. In order to avoid dramatic changes in the blood sugar levels – insulin levels, the amount of food consumed after PYY administration should be limited to such as less than 1, 0.5 or 0.25 MJ in the first meal.

The glycemic index indirectly measures how fast a food is likely to raise your blood sugar. The glycemic index is based on glucose, which is one of the fastest carbohydrates available. Glucose is given an arbitrary value of 100 and other carbohydrates are given a number relative to glucose. Faster carbohydrates (higher numbers) are great for raising low blood sugars and for covering brief periods of intense exercise. Slower carbohydrates (lower numbers) are helpful for preventing overnight drops in the blood sugar and for long periods of exercise. The impact food will have on the blood sugar depends on many other factors such as ripeness, cooking time, fiber and fat content, time of day, blood insulin levels, and recent activity. A diet low in fast carbohydrates is preferred to a diet rich in fast carbohydrates.

Therefore the diet should comprise a high content of slow carbohydrates in order to minimise insulin fluctuations.

It is further relevant to time administration of PYY in relation to food intake, in order to obtain the highest efficiency. Preferably PYY is administered at least 30 minutes post-meal, and such as at least 3 hours pre-meal. More preferably PYY is administered before bedtime, where by, presuming that the subject does not eat during the night, PYY is administered well before the next meal. Details relating to preferred administration schemes are found in the section relation to administration.

By applying these administrations schemes PYY may be used for the treatment of any indication in which reduced caloric intake, reduced appetite and/or reduced food intake is desirable. Such indications include, e.g. the metabolic syndrome (insulin-resistance syndrome (syndrome X)), diabetes mellitus (including type1, non-insulin dependent diabetes mellitus (NIDDM or type 2), gestational diabetes mellitus, maturity onset diabetes of the young (MODY) and late onset autoimmune diabetes of adulthood (LADA)), overweight, obesity, other eating disorders (e.g.bulimia
nervosa), glucose intolerance, dyslipidemia, hypertension, atherosclerosis and other cardiovascular disorders.

5 **PYY**

Throughout this document, peptide YY (PYY) is used as a general term covering PYY1-36 and PYY3-36. PYY1-36 is a 36 AA polypeptide with C- as well as N-terminal tyrosine amino acid residues. The polypeptide is produced by cleavage of a pre-polypeptide and is furthermore cleaved by depeptidyl peptidase IV yielding PYY3-36, as described above. Surprisingly, PYY is capable of suppressing the occurrence of an eating disorder event and to reduce the frequency of eating disorder events. PYY may decrease the frequency of eating disorder events and/or prevent an eating disorder event and/or reduce food and/or caloric intake during the event.

15 **Functional equivalents**

Human PYY1-36 is identified by SEQ ID NO: 1. PYY3-36 is a truncated form of PYY where the two most N-terminal residues are deleted. Functional equivalents of PYY include PYY molecules originating from different species, such as mouse, rat, monkey, swine, bovine or other mammalian species. A functional equivalent may also be a homologue to PYY.

The invention relates to a composition comprising PYY or a functional equivalent thereof, for the preparation of a pharmaceutical composition for the treatment of an eating disorder by

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a) decreasing the frequency of eating disorder events and/or
b) preventing an eating disorder event and/or
c) reducing food and/or caloric intake during the event.

30 In a preferred aspect, the invention relates to a composition comprising PYY or a functional equivalent thereof, for the preparation of a pharmaceutical composition for the treatment of an eating disorder by

a) decreasing the frequency of eating disorder events and/or
b) preventing an eating disorder event.
In a preferred embodiment the composition comprises human PYY. In a more preferred embodiment the composition comprises human PYY1-36 as defined by SEQ ID NO 1. In a further preferred embodiment the composition comprises human PYY3-36 as defined in SEQ ID NO. 1.

Homologues

A homologue shall be construed as a molecule that shares some identity to the molecule, here PYY represented by both PYY1-36 and PYY3-36. The homology may be expressed as the percentage of amino acid residues in the candidate sequence that are identical with the residue of a corresponding sequence to which it is compared, after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent identity for the entire sequence, and not considering any conservative substitutions as part of the sequence identity. Neither N- or C-terminal extensions nor insertions shall be construed as reducing identity or homology. Methods and computer programs for the alignment are well known in the art. Sequence identity may be measured using sequence analysis software (e.g., Sequence Analysis Software Package, Genetics Computer Group, University of Wisconsin Biotechnology Centre, 1710 University Ave., Madison, Wis. 53705). This software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

A homologue of one or more of 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.

As described above a functional equivalent of any of the predetermined sequences herein may be defined as:

i) homologues comprising an amino acid sequence capable of being recognized by an antibody, said antibody also recognizing PYY (PYY1-36 and/or PYY3-36), and/or

ii) homologues comprising an amino acid sequence capable of binding selectively to an NPY receptor, and/or
iii) homologues having a substantially similar or higher binding affinity to NPY receptors than PYY (PYY1-36 or PYY3-36), and/or

iv) homologues with at least 60% identity to human PYY identified by SEQ ID NO 1, and/or

v) homologues consisting of fragments of human PYY identified by SEQ ID NO 1, wherein the fragments comprise a stretch of at least 6 continuous amino acids of SEQ ID NO 1.

Human PYY1-36 has the sequence shown in SEQ ID NO: 1. Human PYY3-36 is 34 amino acids long and has the sequence shown in SEQ ID NO: 1 except for the deletion of the two N-terminal amino acids.

Examples of homologues comprise 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 pola-
rity, 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 Gin 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 substitutions, including charge, size, and the like. Exemplary amino acid substitutions which take one 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.

An embodiment of the invention relates to a composition comprising human PYY or a functional equivalent thereof for the production of a pharmaceutical composition for the treatment of eating disorders.

In a preferred embodiment the homologue has an amino acid sequence at least 60 \% identical to SEQ ID NO 1.

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

In a more preferred embodiment the percentages mentioned above relate to the identity of the sequence of a homologue as compared to SEQ ID NO 1.
In a preferred embodiment the functional equivalent comprise the amino acids corresponding to the 6 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO.1 (Tyr Pro Ile Lys Pro Glu). The functional equivalent may comprise 8 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro), or such as 10 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu) or such as 12 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala), or such as 14 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro), or such as 16 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Gly Glu) or such as 18 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr), or such as 22 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala), or such as 24 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu), or such as 26 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His), or such as 28 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu), or such as 30 N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr), or such as 34N-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln).

In a further preferred embodiment the functional equivalent comprise the amino acids corresponding to the 6 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO.1 (Ile Lys Pro Glu Ala Pro), or such as 8 N-terminal amino acids of PYY3-36
as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu) or such as 10 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala), or such as 12 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro), or such as 14 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu), or such as 16 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn), or such as 18 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr), or such as 20 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Ala), or such as 22 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala), or such as 24 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His), or such as 26 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu), or such as 28 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Tyr Leu), or such as 30 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Val Thr), or such as 32 N-terminal amino acids of PYY3-36 as defined in SEQ ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Val Thr Arg Gln).

In a preferred embodiment the functional equivalent comprise the amino acids corresponding to the 6 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Val Thr Arg Gln Arg Tyr), or such as the 8 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Asn Leu Val Thr Arg Gln Arg Tyr), or such as the 10 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr), or such as the 12 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr), or such as the 14 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr), or such as the 16 C-terminal
amino acids of PYY1-36 as defined in SEQ ID NO 1 (Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr Tyr Ala), or such as the 18 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Arg Tyr Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Gln Arg Tyr Tyr Ala), or such as the 20 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Leu Asn Arg Tyr Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Gln Arg Tyr Tyr Ala), or such as the 22 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Glu Glu Leu Asn Arg Tyr Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Gln Arg Tyr Tyr Ala), or such as the 24 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Ser Pro Glu Glu Leu Asn Arg Tyr Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr Tyr Ala), or such as the 26 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr Tyr Ala), or such as the 28 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr Tyr Ala), or such as the 30 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr Tyr Ala), or such as the 32 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr Tyr Ala), or such as the 34 C-terminal amino acids of PYY1-36 as defined in SEQ ID NO 1 (Tyr Ala Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr).

In another preferred embodiment the functional equivalent may comprises internal amino acids of PYY1-36 such as amino acid 16-21 of PYY 1-36 as defined in sequence ID NO 1 (Glu Leu Asn Arg Tyr Tyr), or such as amino acid 15-22 of PYY 1-36 as defined in sequence ID NO 1 (Glu Glu Leu Asn Arg Tyr Tyr Ala), or such as amino acid 14-23 of PYY 1-36 as defined in sequence ID NO 1 (Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser), or such as amino acid 13-24 of PYY 1-36 as defined in sequence ID NO 1 (Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu), or such as amino acid 12-25 of PYY 1-36 as defined in sequence ID NO 1 (Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg), or such as amino acid 11-26 of PYY 1-36 as defined in sequence ID NO 1 (Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His), or such as amino acid 10-27 of PYY 1-36 as defined in sequence ID
NO 1 (Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr), or such as amino acid 9-28 of PYY 1-36 as defined in sequence ID NO 1 (Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu), or such as amino acid 8-29 of PYY 1-36 as defined in sequence ID NO 1 (Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn), or such as amino acid 7-30 of PYY 1-36 as defined in sequence ID NO 1 (Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu), or such as amino acid 6-31 of PYY 1-36 as defined in sequence ID NO 1 (Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val), or such as amino acid 5-32 of PYY 1-36 as defined in sequence ID NO 1 (Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr), or such as amino acid 4-33 of PYY 1-36 as defined in sequence ID NO 1 (Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg), or such as amino acid 3-34 of PYY 1-36 as defined in sequence ID NO 1 (Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln), or such as amino acid 2-35 of PYY 1-36 as defined in sequence ID NO 1 (Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gin Arg Tyr) or such as amino acid 2-36 of PYY 1-36 as defined in sequence ID NO 1 (Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gin Arg Tyr), or such as amino acid 4-36 of PYY 1-36 as defined in sequence ID NO 1 (Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gin Arg Tyr), or such as amino acid 4-36 of PYY 1-36 as defined in sequence ID NO 1 (Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gin Arg Tyr), or such as amino acid 4-36 of PYY 1-36 as defined in sequence ID NO 1 (Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gin Arg Tyr), or such as amino acid 4-36 of PYY 1-36 as defined in sequence ID NO 1 (Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gin Arg Tyr).

In an embodiment the functional equivalent comprise any of the above sequences with conservative amino acid substitutions, such as one substitution, or such as two substitutions, or such as two substitutions, or such as more than two substitutions, or such as more than four substitutions.

Further included are functional equivalent known from the literature, such as the PYY agonists described in WO 03/057235 and references therein.
PYY 2-36 and PYY 4-36 may in addition to the eating disorders disclosed herein be used for the treatment of any indication in which reduced caloric intake, reduced appetite and/or reduced food intake is desirable. Such indications include, e.g. the metabolic syndrome (insulin-resistance syndrome (syndrome X)), diabetes mellitus (including type1, non-insulin dependent diabetes mellitus (NIDDM or type 2), gestational diabetes mellitus, maturity onset diabetes of the young (MODY) and late onset autoimmune diabetes of adulthood (LADA), overweight, obesity, other eating disorders (e.g. bulimia nervosa), glucose intolerance, dyslipidemia, hypertension, atherosclerosis and other cardiovascular disorders.

Covalent modifications

The functional equivalent may comprise any type of modifications. Nearly 200 structurally distinct covalent modifications have been identified thus far, ranging in size and complexity from conversion of amides to carboxylic acids, to the attachment of multiple complex oligosaccharides. Such modifications include phosphorylation, acetylation, ubiquination, lipidation (acetylation, prenylation, farnesylation, geranylation, palmitoylation, myristoylation), methylation, carboxylation, sulfonation and O- or N-glycosylations.

A subset of modifications is dependent on vitamin C as a cofactor. This include proline and lysine hydroxylations and carboxy terminal amidation.

In an embodiment PYY or the functional equivalent comprise a C-terminal amidation. In a preferred embodiment the C-terminal tyrosine residue of PYY or a functional equivalent is amidated.

Protecting group

The functional equivalent may according to the invention comprise 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 in-
clude - 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, benzyloxy carbonyl 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
PYY or the functional equivalent of PYY may be conjugated to another entity, in order for example, to prolong its half-life. Conjugation can improve the delivery of targeted doses, prevent breakdown, and increase bioavailability in circulation. The conjugate may be any molecule that confers the desired property to PYY. For instance, PYY or a functional equivalent thereof may be conjugated to a polymer molecule, such as polyethylene glycol (PEG).


Most methods use amine-reactive reagents or thiol-reactive reagent. In the preparation of conjugates advantages may be achieved through the use of certain linkers. For example, linkers that contain a disulfide bond that is sterically "hindered" are often preferred, due to their greater stability in vivo, thus preventing release of the PYY moiety prior to binding at the site of action. It is generally desired to have a conjugate that will remain intact under conditions found everywhere in the body except the intended site of action, at which point it is desirable that the conjugate have good "release" characteristics.
Different conjugates have been described, for example, use of the A chain of ricin is described in US Pat. No. 4,340,535 incorporated herein by reference. Examples of peptide conjugates based on Ac-RYY(RK)(W1)RK-NH₂ (where the brackets show allowable variation of amino acid residues) may be found in US patent application 2003040472. Further examples include Fc fusions, wherein the PYY or functional equivalent thereof is fused to the Fc portion of an antibody molecule.

In an embodiment of the invention PYY or the functional equivalent is conjugated to another entity.

The molecules may be conjugated as described above, or by peptide bonds, before or after synthesis and purification. The fusion may be obtained by any suitable methods, for example, but not exclusively, by recombinant DNA technology. For instance, the nucleotide sequence encoding PYY or a functional equivalent thereof may be fused to a nucleotide sequence encoding the peptide constituting the other entity. The fusion polypeptide may be expressed and purified as a single polypeptide molecule, using any suitable method, as described herein. The fusion polypeptide may include a linker, such as a peptide of at least 2 AA; such as at least 5 AA, such as at least 8 AA, such as at least 15 AA, such as at least 20 AA, typically located between the PYY part of the fusion protein and the other entity.

In one embodiment PYY or a functional equivalent thereof is conjugated with another entity using a linker of at least 2AA.

Methods for production of PYY

PYY or a functional equivalent thereof can be produced using techniques well known in the art. For example, a polypeptide region of a PYY 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

An example of how PYY according to the invention is produced is described in brief below. More specifically PYY of the present invention may be produced by the following process:

(a) constructing, by conventional techniques, an expression vector containing an operon with a DNA sequence encoding PYY or a functional equivalent thereof, thereby producing the vector of the invention;

(b) transfecting the expression vectors into a host cell by conventional techniques to produce the transfected host cell of the invention; and

(c) culturing the transfected cell by conventional techniques to produce the PYY of the invention.

The host cell may be cotransfected with a second vector for optimization of the production process. The two vectors may contain different selectable markers. The coding sequences of PYY may comprise cDNA or genomic DNA or both.

A suitable host cell for the expression of PYY or a functional equivalent thereof may be either a bacterial cell such as Escherichia coli, or a eukaryotic cell, such as S. cerevisiae or P. pastoris. In particular a mammalian cell line may be used, such as Hela, CHO or any other suitable host cell known by the person skilled in the art.

The general methods by which the vectors of the invention may be constructed, transfection methods required to produce the host cell of the invention and culture methods required to produce the polypeptide of the invention from such host cells are all conventional techniques. Likewise, once produced, the polypeptide of the invention may be purified according to standard procedures as described below.

**Purification of PYY**

After production, PYY or a functional equivalent thereof is preferably purified. The method of purification used is dependent upon several factors, including the purity...
required, the source of PYY, the intended use and the species in which PYY was produced.

Any suitable conventional methods of purifying polypeptides including precipitation and column chromatography are well known to one of skill in the purification arts, including cross-flow filtration, ammonium sulphate precipitation, affinity column chromatography, gel electrophoresis and the like may be used.

**PYY composition**

According to the invention, a PYY composition comprises PYY or a functional equivalent thereof which is preferably produced by chemical or biochemical synthesis or recombinant methods, and is preferably free of any contaminants present in blood such as infectious agents.

In a preferred embodiment the PYY composition have a concentration of at least 2 nM, 4 nM, 6 nM, 8 nM, 10 nM, 15 nM, 16 nM, 20 nM, 25 nM, 50 nM, such as at least 0.2 µM, such as at least 0.5 µM, such as at least 1 µM, such as at least 2 µM, such as at least 5 µM, such as at least 10 µM, such as at least 20 µM, such as at least 50 µM, such as at least 0.2 mM, such as at least 0.5 mM, such as at least 1 mM, such as at least 2 mM, such as at least 5 mM of PYY or a functional equivalent thereof.

The PYY composition may be stored as a dry composition, for example lyophilized (freeze-dried) or spray dried to improve the stability of PYY. Such compositions are reconstituted with liquid solutions prior to use. Generally, the protein concentration of the reconstituted formulation is about 2-40 times greater than the protein concentration in the mixture before lyophilization; thus this allows the production of a PYY composition of a high concentration. When reconstituted with a diluent comprising a preservative (such as bacteriostatic water for injection, BWFI), the reconstituted formulation may be used as a multi-dose formulation. Such a formulation is useful, for example, where the patient requires frequent subcutaneous administrations.

In another embodiment the PYY composition may be a liquid composition of high stability. The PYY composition may be meant for mixing with a suitable diluent prior to use.
The PYY composition may further comprise pharmaceutically acceptable salts, as well as comprise pharmaceutically acceptable carriers and diluents.

**Pharmaceutical compositions**

Pharmaceutical compositions of the present invention may be prepared by conventional techniques, e.g. as described in Remington: The Science and Practice of Pharmacy 1995, edited by E. W. Martin, Mack Publishing Company, 19th edition, Easton, Pa. The compositions may appear in conventional forms, for example solutions or suspensions.

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

According to the present invention, the pharmaceutical composition comprises PYY or a functional equivalent thereof.

The pharmaceutical composition according to the present invention further preferably comprises pharmaceutically acceptable salts, a pharmaceutically acceptable carrier and/or a diluent. The pharmaceutical composition may further comprise vehicles, excipients and/or transport molecules.

The pharmaceutical composition may be produced prior to use by mixing a PYY composition with an appropriate diluent.

The compositions of the present invention may preferably be administered to an individual in any way so as to achieve a beneficial effect, preferably to decrease the number of eating disorder events.

An aspect of the invention relates to a method of treatment comprising administration of the pharmaceutical composition according to the invention.
The pharmaceutical composition according to the invention is preferably formulated for parenteral administration, such as via a subcutaneous, intradermal, intramuscular or intravenous route. In a further preferred embodiment of the invention, the pharmaceutical composition is administered parenterally. More preferably, the composition is administered via the subcutaneous route. Other drug-administration methods, which are effective to deliver the drug to a target site or to introduce the drug into the bloodstream, are also contemplated.

The composition of the invention may be administered in combination with a second pharmaceutical composition. The compositions may be administered simultaneously, either as separate compositions or combined in a unit dosage form, or administered sequentially as two separate pharmaceutical compositions.

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

**Second active ingredient**

The patient suffering from an eating disorder event may benefit from additional treatments. This may e.g. involve anti-depressants, such as selective serotonin reuptake inhibitors (SSRIs), serotonin noradrenalin reuptake inhibitors (SNRIs), norepinephrine serotonin reuptake inhibitors (NSRIs), selective noradrenalin reuptake inhibitors, tetracyclic antidepressants, non-selective monoamine reuptake inhibitors including tricyclic antidepressants (TCAs), selective reversible monoamine reuptake inhibitors and antidepressants with other mechanisms of action, e.g. mirtazapin. Examples of SSRIs are citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine and sertraline. An example of an SNRI is venlafaxine. An example of an NSRI is milnacipran.

In an embodiment of the invention, the composition comprises a second active ingredient in addition to PYY or a functional equivalent thereof.

The second active ingredient may be selected from the group of: selective serotonin reuptake inhibitors (SSRIs), serotonin noradrenalin reuptake inhibitors (SNRIs), norepinephrine serotonin reuptake inhibitors (NSRIs), selective noradrenalin reuptake
inhibitors, tetracyclic antidepressants, non-selective monoamine reuptake inhibitors including tricyclic antidepressants (TCAs), selective reversible monoamine reuptake inhibitors and antidepressants with other mechanisms of action, e.g. mirtazapin. Examples of SSRIs are citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine and sertraline.

In another embodiment, an anti-emetic may be used as a second active ingredient. This is of particular interest in cases where the treatment with PYY or a functional equivalent thereof gives rise to nausea and/or emesis.

Anti-emetic drugs
In the present context, an “anti-emetic” drug is one which counteracts (i.e. reduces or removes) nausea or emesis (vomiting). The experience of nausea and emesis may have many causes and the relief or reduction of the symptoms may be obtained by various mechanisms. The major groups of drugs useful for the treatment of nausea and emesis are; Neuroleptics/anti-psychotics, Antihistamines, Anticholinergic agents, Steroids (corticosteroids), 5HT3-receptor antagonists (serotonin receptor antagonist), NK1-receptor antagonists (Neurokinin 1 substance P receptor antagonists), Antidopaminergic agents/dopamine receptor antagonists, Benzodiazepines, Cannabinoids. Here below is a non-exhaustive list of members of the different groups of compounds.

1. Neuroleptics/anti-psychotics
   a. Dixyrazine
   b. Haloperidol
   c. Promazine (Compazine®)

2. Antihistamines
   a. Piperazine derivatives
      i. cyclizine
      ii. medizine
      iii. cinnarizine
   b. Promethazine
   c. Dimenhydrinate
   d. Diphenhydramine
   e. Hydroxyzine
   f. Buclizine
   g. Meclizine hydrochloride (Bonine, Antivert)

3. Anticholinergic agents (Inhibitors of the acetylcholine receptors.)
   a. Scopolamine
   b. Glycopyrron
c. Hyoscine
   i. Artane (Trihexy-5 trihexyphenidyl hydrochloride)
   ii. Cogentin (benztropine mesylate)
   iii. Akineton (biperiden hydrochloride)
   iv. Disipal (Norflex orphenadrine citrate)
   v. Kemadrin (procyclidine hydrochloride)

4. Steroids (corticosteroids)
   a. Betamethasone
   b. Dexamethasone
   c. Methylprednisolone
   d. Prednisone®
   e. Trimethobenzamide (Tigan)

5. 5HT3-receptor antagonists (serotonin receptor antagonist)
   a. Granisetron
   b. Dolasetron
   c. Ondansetron (hydrochloride)
   d. Tropisetron
   e. Ramosetron
   f. Palonosetron
   g. Alosetron
   h. Bemsetron
   i. Zatisetron
   j. Batanopiride
   k. MDL-73147EF;
   l. Metoclopramide
   m. N-3389 (endo-3,9-dimethyl-3,9-diazabicyclo[3,3,1]non-7-yl-1 H-
      indazole-3-carboxamide dihydrochloride),
   n. Y-25130 hydrochloride
   o. MDL 72222
   p. Tropanyl-3,5-dimethylbenzoate
   q. 3-(4-Allylpirazin-1-yl)-2-quinoxalinecarbonitrile maleate
   r. Zacopride hydrochloride
   s. Mirtazapine (Antidepressant)

6. NK1-receptor antagonists (Neurokinin 1 substance P receptor antagonists)
   a. Aprepitant
   b. MPC-4505
   c. GW597599
   d. MPC-4605
   e. GR205171 (a selective tachykinin NK1 receptor antagonist)
   f. L-759274
   g. SR 140333
   h. CP-96,345
   i. BIIF 1149, NKP 608C, NKP 608A, CGP 60829, SR 140333 (Nolpitant-
      tium besilate/chloride), LY 303870 (Lanepitant), MDL-105172A, MDL-
      103896, MEN-11149, MEN-11467, DFN 333A, YM-49244, YM-
      44778, ZM-274773, MEN-10930, S-19752, Neuronom, YM-35375,
   j. Benserazide and carbidopa
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k. TAK-637 [(aR,9R)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-
tetrahydro-9-methyl-5-(4-methylphenyl)-7H-[1,4]diazocino[2,1-g]
[1,7]naphthyridine-6,13-dione]

l. PD 154075 ([2-benzofuran]-CH2OCO-(R)-alpha-MeTrp-(S)-
NHCH(CH3) Ph)

m. FK888, chemical modification of the parent compound, (D-Pro4, D-
Trp7, 9, 10, Phe11) SP4-11.

7. Antidopaminergic agents/dopamine receptor antagonists

a. Domperidone
b. Prochlorperazine
c. Metoclopramide
d. Chlorpromazine (Thorazine)
e. Droperidol (Inapsine)
f. Promethazine (Phenergan)

8. Benzodiazepines (Valium® and others)

9. Non-psychoactive cannabinoids,

a. Cannabidiol (CBD)
b. Cannabidiol dimethylheptyl (CBD-DMH)
c. Tetra-hydro-cannabinol (THC)
d. Cannabinoid agonists such as WIN 55-212 (a CB1 and CB2 receptor
agonist)
e. Dronabinol (Marinol®)

10. Further cannabinoids

a. Nabilone (Cesamet)

11. c-9280 (Merck)

A 5HT3-receptor antagonist is particularly preferred. The anti-emetic is used in an
effective amount which is sufficient to either remove or reduce the nausea and/or
emesis to an acceptable level.

This combination may be useful for the treatment of a disease selected from the
group of; metabolic disorders, the metabolic syndrome (insulin-resistance syndrome
(syndrome X)), diabetes mellitus (including type1, non-insulin dependent diabetes
mellitus (NIDDM or type 2), gestational diabetes mellitus, maturity onset diabetes of
the young (MODY) and late onset autoimmune diabetes of adulthood (LADA), over-
weight, obesity, an eating disorder, insulin-resistance syndrome (syndrome X), glu-
cose intolerance, dyslipidemia, hypertension, atherosclerosis and other cardiovascu-
lar disorders.
A particular embodiment relates to a composition comprising PYY or a functional equivalent thereof and a second active ingredient, where in the second active ingredient is an anti-emetic drug for the manufacture of a medicament for

b) treatment of overweight and/or
c) treatment of obesity and/or
d) treatment of syndrome X and/or
e) treatment of disorders of appetite regulation and/or
f) treatment of eating disorders and/or
any combination of the above

In a preferred embodiment the pharmaceutical composition may be a kit-in-part.

The pharmaceutical composition may also be a kit-in-part further including anti-depressants, such as selective serotonin reuptake inhibitors (SSRIs), serotonin noradrenalin reuptake inhibitors (SNRIs), norepinephrine serotonin reuptake inhibitors (NSRIs), selective noradrenalin reuptake inhibitors, tetracyclic antidepressants, non-selective monoamine reuptake inhibitors including tricyclic antidepressants (TCAs), selective reversible monoamine reuptake inhibitors and antidepressants with other mechanisms of action, e.g. mirtazapin. Examples of SSRIs are citalopram; escitalopram, fluoxetine, fluvoxamine, paroxetine and sertraline. An example of an SNRI is venlafaxine. The kit-in-part may be used for simultaneous, sequential or separate administration. An example of an NSRI is milnacipran.

In a further embodiment the kit of parts include an anti-emetic drug.

**Pharmaceutically acceptable salts**

Pharmaceutically acceptable salts of the present compounds, where they can be prepared, are also intended to be covered by this invention. These salts will be ones which are acceptable in their application to a pharmaceutical use. By that it is meant that the salt will retain the biological activity of the parent compound and the salt will not have untoward or deleterious effects in its application and use in treating diseases.
Pharmaceutically acceptable salts are prepared in a standard manner. If the parent compound is a base it is treated with an excess of an organic or inorganic acid in a suitable solvent. If the parent compound is an acid, it is treated with an inorganic or organic base in a suitable solvent.

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

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

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

Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, ammonium salts and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydriodic, phosphoric, 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 p-toluensulfonic acids and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the
pharmaceutical acceptable salts listed in J. Pharm. Sci. 1977,66,2, which is incorporated herein by reference. Examples of metal salts include lithium, sodium, potassium and magnesium salts and the like.

According to the invention organic acid salts of organic acids such as for example acetic acid is preferred.

Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium and tetramethylammonium salts and the like.

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

Also included within the scope of compounds or pharmaceutical acceptable acid addition salts thereof in the context of the present invention are any hydrates (hydrated forms) thereof.

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.

**Pharmaceutically acceptable carriers and diluents**

The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.
Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, organic esters such as ethyl oleate, and water-oil emulsions.

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

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

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

Stabilizers
The active compound of the invention may be unstable, thus the composition preferably contain stabilizers, preservatives or conservatives to increase the stability of the compounds.

A pH-buffering agent may be used to stabilize the active compound of the composition. The buffering agent may be acetate, carbonate, bicarbonate, phosphate, citrate, tris or hepes. In a preferred embodiment the buffering agent is acetate.

According to the invention the composition preferably has a pH between 2.0 and 9.0, or such as between 2.5 and 8.0, or such as 3.0 and 7.0, or such as between 3.5 and 6.0, or such as between 3.5 and 5.0 or such as between 4.0 and 5.0, or such as between 4.0 and 5.0, or such as between 4.0 and 4.5. Preferably the pH of the com-
positions is less than 6, preferably less than 5.5, preferably less than 5, preferably less than 4.8, preferably less than 4.6, preferably less than 4.4, preferably less than 4.2.

Tween 20, Tween 60, Tween 80, Span 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glycerol mono-stearate, mannitol, polysorbates and sodium lauryl sulphate are possible stabilizers.

In a preferred embodiment mannitol may be used as stabilizer.

For the preparation of a lyophilised composition ad lyoprotectant may be used to stabilize the active ingredient (Townsend and DeLuca, "Use of lyoprotectants in the freeze-drying of a model protein, ribonuclease A" Journal of Parenteral Science & Technology 42 (6): 190-199 (Nov.-Dec. 1988)).

The lyoprotectant may preferably be a sugar such as sucrose or trehalose such as sucrose, dextran, or hydroxypropyl-/142-cyclodextrin.

Transport molecules

Transport molecules act by having incorporated into or anchored to it the compound according to the invention. Any suitable transport molecules known to the skilled person may be used. Examples of transport molecules may be liposomes, micelles, and/or microspheres.

A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Pat. Nos. 4,235,871, 4,501,728 and 4,837,028, all of which are incorporated herein by reference.

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

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

The size of a micelle or its aggregation number is governed largely by geometric factors. The radius of the hydrocarbon core cannot exceed the length of the extended hydrocarbon chain of the surfactant molecule. Therefore, increasing the chain length or ascending homologous series increases the aggregation number of spherical micelles. If the surfactant concentration is increased beyond a few percent and if electrolytes are added (in the case of ionic surfactants) or the temperature is raised (in the case of nonionic surfactants), the micelles increase in size. Under these conditions, the micelles are too large to remain spherical and become ellipsoidal, cylindrical or finally lamellar in shape.

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

The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol. Examples of oily or nonaqueous carriers, diluents, solvents or vehicles include propylene glycol, polyethylene glycol, vegetable oils (e.g., olive oil), and injectable organic esters (e.g., ethyl oleate), and may contain formulatory agents such as preserving, wetting, emulsifying or suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution for constitution before use with a suitable vehicle, e.g., sterile, pyrogen-free water. Aqueous solutions should be suitably buffered if necessary, and the liquid diluents 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.

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

The pharmaceutical composition comprises PYY or a functional equivalent thereof or a pharmaceutically acceptable salt thereof, (and for example protease inhibitors). Such compositions can be prepared in water or saline, and optionally mixed with a nontoxic surfactant. Compositions for intravenous or intra-arterial administration may include sterile aqueous solutions that may also contain buffers, liposomes, diluents and other suitable additives.

Oils useful in parenteral compositions include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils useful in such compositions include peanut,
soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral compositions include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

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

The parenteral compositions typically will contain from about 0.00002 to about 2% 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 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 PYY or a functional equivalent thereof or pharmaceutically acceptable salt thereof in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filter sterilization.

An individual in need
According to the invention an individual in need is any subject, preferably a human being that benefit from PYY administration as described herein, subjects suffering from metabolic disorders, obesity, syndrome x, eating disorders (BED and NES) are contemplated. Further included are individuals in need of stimulation of lipolysis or an increased blood level of FFA’s.

Administration
The pharmaceutical composition may be prepared so it is suitable for one or more particular administration methods.

The pharmaceutical composition comprising PYY or a functional equivalent thereof may be administered to an individual in need thereof.

The composition comprising PYY or a functional equivalent thereof can, according to the invention, be administered parenterally, e.g. by injection. Thus, PYY or a functional equivalent thereof may, according to the invention, be administered parenterally, such as intravenously, intra-arterially, intraperitoneally, intramuscularly, subcutaneously, intranasally or transdermally. The composition may further be administered by inhalation, topical application to the eye, intranasal application, intravaginal absorption, application to the skin, and rectal suppositories.

The pharmaceutical compositions containing PYY or a functional equivalent thereof may be administered intravenously, intra muscularily or subcutaneously for example by injection of a unit dose. The term "unit dose" when used in reference to a pharmaceutical composition of the present invention refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e. carrier, or vehicle. Alternatively, the phar-
maceutical composition may be prepared in a multiple dose form and, by use of the multi-dose delivery device, single doses can be administered when needed. As an alternative to standard injections the pharmaceutical composition may be administered by infusions, such infusions are preferably short.

It should be noted that the normal PYY response, which occurs during the course of a meal, is a short-lived surge in plasma concentrations of PYY, and that, due to the relatively short half-life of the peptide, a s.c injection of PYY will ensure that a similar short-lived concentration peak of PYY can be obtained. The administration route must ensure that the non-degraded, bioactive form of the peptide will be the dominating form in the circulation, which will reach the ligands, for example the NPY receptors, and stimulate these.

The effect of PYY or functional equivalents according to the invention is believed to be mediated via actions of PYY outside the central nervous system, with the exception of the NPY neurons located in the hypothalamus. Thereby PYY or functional equivalents do not affect NPY neurons located elsewhere in the central nervous system.

Accordingly, PYY or functional equivalents capable of binding to the NPY Y2 receptor may circulate in the bloodstream to the receptors in the hypothalamus; however, these molecules should preferably not be capable of crossing the blood-brain barrier, and thereby not be able to enter into other parts of the central nervous system.

For the treatment of food craving and/or an eating disorder event as defined herein. PYY may be administered to mimic the effect of a meal by administration prior to a meal, for example three times a day. The present invention relates to a treatment of food craving and/or eating disorders which may be characterized by recurrent inappropriate intake of excessive meals. The treatment may aim at preventing an eating disorder event by immediately eliminating or reducing the eating urge.

**Craving and/or Eating Disorder Event dependent administration**

In an embodiment the PYY composition is administered when the patient senses that a food craving and/or eating disorder event is to occur. The administration may also be based on a scheme, which reflects the timing of food cravings and/or eating
disorder events. The food cravings and/or eating disorder event may be associated with time or specific trigger actions.

Trigger actions may for example be mood changes, dieting (the patients make themselves hungry, and then binge eat in response to the hunger) smoking cessation or reduction in the amount of tobacco smoked, and threatening situations.

The dose administered should be an "effective amount" or an amount necessary to achieve an "effective level" in the individual patient. Furthermore, since the "effective level" is used as the preferred endpoint for dosing, the actual dose and schedule can vary, depending on individual differences in pharmacokinetics, drug distribution, and metabolism. The "effective level" can be defined, for example, as the blood or tissue level desired in the patient that corresponds to a concentration of one or more compounds to be used in accordance with the invention. The effective level may be defined as the dose required to achieve a plasma concentration of at least, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140 or 150 pmol/l or alternatively a plasma concentration of 20-50, 30-100, 40-80, 50-70 or approximately 60 pmol/l. Preferably an increase in plasma PYY level is observed within 90 minutes after administration, such as within 75; 60; 45 or 30 minutes after administration. Preferably with in 45 minutes of administration.

**Daily administration**

The treatment may aim at reducing the number of eating events over a period of time, thus in a further embodiment the treatment is for decreasing the frequency of food cravings and/or eating disorder events. In case, for example, the patient has a PYY deficiency, the level of PYY may be normalized by daily administration of the pharmaceutical composition. Administration of said pharmaceutical composition may be once a day, or twice a day, or three times a day, or five times a day, or more than five times a day. In order to obtain PYY levels that resemble the PYY levels of a healthy individual, the pharmaceutical composition may be administered prior to a meal, such as within 5 minutes of a meal, such as within 20 minutes of a meal, such as within 60 minutes of a meal. According to the invention, the method of administration of the pharmaceutical composition may include daily administration of the pharmaceutical composition even if the PYY level of the patient does not indicate that the patient is suffering from a PYY deficiency.
In some situations it may be beneficial to combine the two administration forms. Thus, the pharmaceutical composition may be administered daily in predetermined amounts and, in addition, when the patient feels that food cravings and/or an eating disorder event is about to occur. In an embodiment of the invention, the pharmaceutical composition is administered for the treatment of NES or BED.

In an embodiment of the invention, administration of the pharmaceutical composition decreases the frequency of eating disorder events. The number of events may be decreased by at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or 100 per cent.

In an embodiment of the invention the pharmaceutical composition is administered within 10-180, such as within 20-120 or such as within 30-90 minutes before bedtime.

In a further embodiment of the invention, the pharmaceutical composition is administered at bedtime. The pharmaceutical composition may be administered before bedtime, such as within 5 minutes before bedtime, such as within 10 minutes before bedtime, such as within 15 minutes before bedtime, such as within 30 minutes before bedtime, such as within 45 minutes before bedtime, such as within 60 minutes before bedtime. In a further embodiment, the pharmaceutical composition is administered during the night. The term daily dose should be construed as the total dose administered in 24 hours. The pharmaceutical composition may be administered at regular intervals during the night, such as every second hour, or such as every three hours or every four hours. In another embodiment, the pharmaceutical composition may be administered when the patient wakes up, or when the patient feels an urge to eat, thus the pharmaceutical composition may be administered when there is a need.

In one embodiment, the composition according to the present invention is contemplated to reduce one of the numerical scores outlined above defining craving (the Bulimia scale of the EDI-2 questionnaire, the FCQ-T scale or the BES) by at least 5%, such as at least 10%, such as at least 15%, such as at least 20%, such as
at least 25%, such as at least 30%, such as at least 40%, such as at least 50%, such as at least 60%, such as at least 70%, such as at least 80% or such as at least 90%.

In another embodiment, the composition according to the present invention is contemplated to reduce all of the numerical scores outlined above defining craving (the Bulimia scale of the EDI-2 questionnaire, the FCQ-T scale or the BES) by at least 5%, such as at least 10%, such as at least 15%, such as at least 20%, such as at least 25%, such as at least 30%, such as at least 40%, such as at least 50%, such as at least 60%, such as at least 70%, such as at least 80% or such as at least 90%.

In a further embodiment, the average food or caloric intake during an eating disorder event is reduced by at least 5, such as at least 10, such as at least 15, such as at least 20, such as at least 25, such as at least 30, such as at least 40, such as at least 50, such as at least 60, such as at least 70, such as at least 80, such as at least 90 or such as at least 95 per cent.

In an embodiment pharmaceutical composition may be administered for the stimulation of fat mobilisation or lipolysis. In a second embodiment the pharmaceutical composition is for the increasing the blood concentration of free fatty acids (FFAs) in the blood. It is contemplated that the efficiency of administration increases if the pharmaceutical composition is administered separate from meals, preferably the pharmaceutical composition is administered to a fasting individual. In an embodiment the composition is administered at least 30 minutes after food intake, such as at least 45 minutes, such as at least 60 minutes, such as at least 75, such as at least 90 minutes after food intake. It is further preferred that the individual continuous fasting for at least 2 hours, such a 2.5, such as 3, such as 4, such as 5, such as 6, such as 7, such as 8 hours following administration of the pharmaceutical composition. In a more preferred embodiment the individual is fasting at least 4 hours, such as 5 hours or such as 6 hours after administration of the pharmaceutical composition. In order to follow such an regime of administration it is preferred that the pharmaceutical composition is administered before bedtime, such as within 5 minutes before bedtime, such as within 10 minutes before bedtime, such as within 15 minutes before bedtime, such as within 30 minutes before bedtime, such as within
45 minutes before bedtime, such as within 60 minutes before bedtime. In an individual with regular eating and sleeping habits a night sleep of 6-8 hours is a suitable time frame before food intake.

It is preferred that the medicament is administered to a fasting individual, and that the individual continues to fasten for at least 1 hour, such as 2 hours, such as at least 2.5 hours, such as 3 hours or preferably 4 hours after administration of the medicament in order to obtain an effective increase in the blood concentration of free fatty acids (FFAs). More preferably the subject continues fastening for at least 4 hours, such as 5 hour or at least 6 hours. The preferred timing of administration may be before bedtime, such as within 3 hours before bedtime, such as within 2.5 hours, such as within 2.0 hours, such as within 1.5 hours, such as within 1.0 hours, such as within 0.5 hours or such as with in 0.5 hour before bedtime.

With out being bound by the theory the consummation of food, by increasing blood sugar and following insulin concentration stimulates storage of lipids, and thereby abolish the lipid mobilizing effect of PYY. Most effective administration of PYY may be at least one hour after a meal and at least 4 hours, such as 6 hours or such as 8 hours before the next meal. It is further contemplated that administration to diabetic are preferably at least one hour after insulin administration, such as 2 hours or such as 3 hours after insulin administration. Likewise it is preferred that PYY is administered at least 1hour, such as 1.5 hours, such as 2 hours, such as at least 2,5 hours, such as 3 hours or preferably 4 hours before administering insulin.

An example of an increase in blood FFA concentration upon PYY is described in example 5 and figure 4. In an embodiment the concentration of FFA increases at least 1.5 fold, such as at least 2 folds, such as 3 fold, such as 4 fold, or such as 5 folds or such as 8 folds in the period from administration of the PYY composition until the next meal is consumed.

Thus using this administration scheme a treatment suitable for mobilizing the fatty acids from adipose tissue, allowing more effective utilization of the energy stored as fat. This may be particular useful for the treatment of indication in which mobilization of fat as an energy resource is preferred, such as for reducing caloric intake, reducing appetite and/or reducing food intake is desirable. Such indications include,
e.g. the metabolic syndrome (insulin-resistance syndrome (syndrome X)), diabetes mellitus (including type 1, non-insulin dependent diabetes mellitus (NIDDM or type 2), gestational diabetes mellitus, maturity onset diabetes of the young (MODY) and late onset autoimmune diabetes of adulthood (LADA)), overweight, obesity, other eating disorders (e.g. bulimia nervosa), glucose intolerance, dyslipidemia, hypertension, atherosclerosis and other cardiovascular disorders.

In a particular embodiment the PYY composition is for:

a) treatment of overweight and/or

b) treatment of obesity and/or

c) treatment of syndrome X and/or

d) treatment of disorders of appetite regulation and/or

e) treatment of eating disorders and/or

any combination of the above

The pharmaceutical preparations described herein may also be arranged in unit dosage forms. In such a form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as powders in compartments. In this embodiment the powders may be mixed with a solvent prior to or during use.

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

The pharmaceutical compositions may be administered by intravenous infusion. The duration of an infusion may be less than 120 minutes, such as less than 100 minutes, such as less than 80 minutes, such as less than 60 minutes, such as less than 40 minutes, such as less than 20 minutes, such as less than 10 minutes or such as less than 5 minutes.

The dosage requirements will vary with the particular drug composition employed, the route of administration and the particular subject being treated. Ideally, a patient to be treated by the present method will receive a pharmaceutically effective amount of the compound not exceeding the maximum tolerated dose (MTD), which is generally no higher than that required before drug resistance develops.

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

Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug. For the present invention the dosage will vary depending on the compound employed and the mode of administration.

In certain situations the dosages may be calculated base on the fat free mass (FFM) of the subject. Thus the dosage may be in concentration equivalent to PYY1-36 or PYY3-36.

For the present invention the dosage will vary depending on the compound employed and the mode of administration. Dosage levels may vary between about 4 ng/kg body weight to 20 μg/kg body weight daily, preferably between about 10 ng/kg body weight to 1 μg/kg body weight, more preferably between 50 to 750 ng/kg body weight. Alternative dosages in relation to FFM may vary between about 5 ng/kg FFM
to 25 μg/kg FFM daily, preferably between about 12.5 ng/kg FFM to 1.25 μg/kg FFM, more preferably between 62.5 to 875 ng/kg FFM. To obtain dosages in relation to FFM the dosage/kg bodyweight should be multiplied by the factor 1.25.

The dosage may be administered when needed, such as up to ten times daily, such as one to five times daily, such as two or three times daily, or preferably such as once a day, thus the daily dosage maybe up to 2-5 times the dosages mentioned above. Alternatively, the dosage may be administered less frequently than once daily as described herein above.

A preferred dosage of a composition employed according to the invention is in a concentration equivalent to PYY or a functional equivalent thereof of from 4 ng to about 20 μg per kg bodyweight, or such as from 10 ng to 1 μg per kg bodyweight, more preferably from 50 to 750 ng per kg bodyweight. The dosage of PYY is preferably 20-200 ng/kg, 20-160 ng/kg, 40-160 ng/kg, 40-120 ng/kg, 40-80 ng/kg, 60-120 ng/kg or such as approximately 60 ng/kg or 80 ng/kg.

The preferred dosages may be in a concentration equivalent to PYY or a functional equivalent thereof of from 1 pmol/kg to 5 nmol/kg, such as from 5 pmol/kg to 1 nmol/kg, or such as from 20 pmol/kg to 500 pmol/kg alternatively such as from 40 to 160 pmol/kg or such as from 75 to 120 pmol/kg. PYY1-36 is preferably administered in dosages of, such as from 50-400 pmol/kg, such as 80-320 pmol/kg, such as 150-250 pmol/kg, such as about 200 pmol/kg. PYY3-36 is preferably administered in dosages of such as from 30-350 pmol/kg, such as 50-280 pmol/kg, such as 80-200 pmol/kg, such as about 120 pmol/kg. Dosages in relation to FFM may be calculated by multiplying the indicated dosages with 1.25.

In a second embodiment the dosage may be 5-50 pmol/kg, 5-40 pmol/kg, 5 to 30 pmol/kg, 10-40 pmol/kg, 10-30 pmol/kg such as 5 to 25 pmol/kg, such as 5 to 20 pmol/kg, and most preferably 10-20 pmol/kg, 15-30 pmol/kg or approximately 15 pmol/kg or 20 pmol/kg.

The dosages are preferably administered once a day, or such as two times a day, or such as three times a day, or such as four times a day, or such as five times a day, or such as more than five times a day.
In one preferred embodiment of the present invention, the compositions are administered in dosages of PYY or a functional equivalent from about 400 ng to about 2 mg, more preferably from about 10 μg to about 200 μg, or from about 5 μg to about 250 μg, more preferably from about 20 μg to about 200 μg, more preferably from about 20 μg to about 100 μg. Most preferably the dosage may be 1-20 μg, 2-16 μg, 4-16 μg, 4-12 μg, 4-8 μg, 6-12 μg, 6-10 μg or approximately 8 μg.

In a preferred embodiment the composition is administered in dosages of PYY or a functional equivalent from 100 pmol to 500 nmole, or such from 500 pmol to 100 nmol, or such as from 1 nmol to 50 nmol, or such as from 2 to 25 nmol, or such as from 4 to 20 nmol. Alternatively the preferred dosage may be 0,25-5 nmol, 0,5-4 nmol, 1-4 nmol, 1-3 nmol, 1-2 nmol, 1,5-3 nmol or more preferably 1,5-2,5 nmol or most preferably approximately 2 nmol. En a further preferred embodiment a PYY1-36 dosages includes such as from 5-40 nmol, such as 8-32 nmol, such as 15-25 nmol, such as about 20 nmol, whereas a dosage of PYY3-36 includes such as from 3-35 nmol, such as 5-28 nmol, such as 8-200 nmol, such as about 12 nmol.

In another embodiment, the PYY or functional equivalent is administered subcutaneously in a dosage of 5-30 pmol/kg, such as 5-25 pmol/kg, such as 5-20 pmol/kg or such as 10-20 pmol/kg bodyweight, in order to achieve an effective level in the individual treated. The presently preferred dosage is 10-20 pmol/kg bodyweight of PYY1-36 or PYY 3-36. In second preferred embodiment the dosages of PYY1-36 is 150-250 pmol/kg and/or the dosages of PYY3-36 is 80-150 pmol/kg.

The dosages of PYY or the functional equivalent is preferably administered once a day, or such as two times a day, or such as three times a day, or such as four times a day, or such as five times a day.

The pharmaceutical preparations described herein may also be arranged in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as powders in compartments. In this embodiment the powders may be mixed with a solvent prior to or during use.
In a preferred embodiment the PYY composition is administered in unit dosage form, from about 400 ng to about 2 mg of PYY or a functional equivalent thereof, more preferably from about 10 μg to about 200 μg, or from about 5 μg to about 250 μg, more preferably from about 20 μg to about 200 μg, more preferably from about 20 μg to about 100 μg. The unit dosage form may comprise from 100 pmol to 500 nmole, or such from 500 pmol to 100 nmol, or such as from 1 nmol to 50 nmol, or such as from 2 to 25, such as from 4 nmol to 20 nmol of PYY or a functional equivalent thereof. The compositions are preferably administered once a day, or such as two times a day, or such as three times a day, or such as four times a day, or such as five times a day. In a further preferred embodiment a unit dosage of PYY1-36 includes such as from 5-40 nmol, such as 8-32 nmol, such as 15-25 nmol, such as about 20 nmol, whereas a unit dosage of PYY3-36 includes such as from 3-35 nmol, such as 5-28 nmol, such as 8-200 nmol, such as about 12 nmol.

In certain embodiment the pharmaceutical composition may be administered by infusions. The dosages of PYY or a functional equivalent for infusion may be from 0.01 pmole/kg minute to 500 pmole/kg minute such as from 0.05 pmole/kg minute to 100 pmole/kg minute, or such as from 0.1 pmole/kg minute to 50 pmole/kg minute, or such as from 1 pmole/kg minute to 25 pmole/kg minute.

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

In general, daily should be construed as meaning within a 24-hour period. It is thereby included that the treatment may be administered during the night. Furthermore, since the "effective level" is used as the preferred endpoint for dosing, the actual dose and schedule can vary, depending on individual differences in pharmacokinetics, drug distribution, and metabolism. The "effective level" can be defined,
for example, as the blood or tissue level desired in the patient that corresponds to a concentration of one or more compounds according to the invention.

The effective level may refer to an amount of the active ingredient of the composition according to the invention that is able to obtain certain blood or tissue levels of a desired compound in the patient. The effective level may be the amount of the active ingredient of the composition according to the invention that is able to obtain a reduced frequency of eating disorder events or prevent an eating disorder event.

The frequent administration of the composition according to the invention is not suitable for administration by medical personnel, neither would hospitalization be appropriate, thus there is a need for the composition to be self-administered.

In one embodiment of the invention, the compositions of the present invention are self-administered. The pharmaceutical composition may be administered by use of an injection device, for example by the use of a system similar to insulin pens.

In one embodiment of the invention the composition is administered by use of a single or a multi-dose injection device.
Examples

The following examples illustrate the invention without limiting it thereto.

Example 1

5 Binding assay and functional assay

_Transfections and tissue culture:_ COS-7 cells can be grown Dulbecco’s Modified Eagle’s Medium 1885 supplemented with 10% fetal calf serum, 2mM glutamine and 0.01 mg/ml gentamicin. The expression plasmids containing the cDNAs encoding the wild type or the mutated receptors can be transiently expressed after transfection according to the calcium phosphate precipitation method and assay can be performed 48 hours after transfection.

_Binding assay:_ One day after transfection the cells will be transferred and seeded in multi-well plates for assay. The number of cells to be plated per well will be chosen so as to obtain 5 to 10% binding of the radioligand added. Two days after transfection the cells will be assayed in competition binding assays using \(^{125}\text{I}-\text{PYY}(3-36)\) as a tracer. Radioligand will be bound in a buffer composed of 0.5 ml of 50 mM Hepes buffer, pH 7.4, supplemented with 1 mM CaCl\(_2\), 5 mM MgCl\(_2\), and 0.1% BSA, and displaced in a dose dependent manner by unlabelled ligands. The assay will be performed in duplicate for 3 hours at 4°C, and stopped by washing twice in the buffer. Cell associated, receptor bound radioligand will be determined by the addition of lysis buffer (48% urea, 2% NP-40 in 3M acetic acid). The concentration of radioligand in the assay corresponds to a final concentration of approximately 20 pM.

_Functional assay._

COS-7 can be cultured as described above and contransfections can be performed. The activation of Phospholipase C by chimeric G-proteins (Conklin B) formed between both Gq and Gai the Y2 receptor can be measured through the inositol phosphate (IP) turnover in the cell. The IP turnover may be recorded by use of following assay:

One day after transfection COS-7 cells are incubated for 24 hours with 5 :Ci of \(^{3}\text{H}\)-myo-inositol (Amersham, PT6-271) in 1 ml medium supplemented with 10% fetal calf serum, 2 mM glutamine and 0.01 mg/ml gentamicin per well. Cells are washed twice in buffer, 20 mM HEPES, pH 7.4, supplemented with 140 mM NaCl, 5 mM KCl, 1 mM MgSO\(_4\), 1 mM CaCl\(_2\), 10 mM glucose, 0.05 % (w/v) bovine serum; and are incubated in 0.5 ml buffer supplemented with 10 mM LiCl at 37°C for 30 min.
The indicated curves are furthermore incubated with adenosine deaminase ADA (200U/mg, Boeringer Mannheim, Germany) for 30 min in a concentration of 1U/ml. After stimulation with various concentrations of peptide for 45 min at 37°C, cells will be extracted with 10% ice-cold perchloric acid followed by incubation on ice for 30 min. The resulting supernatants are neutralized with KOH in HEPES buffer, and the generated [³H]-inositol phosphate is purified on Bio-Rad AG 1-X8 anion-exchange resin. Determinations will be made in duplicates.

Example 2

Synthetic production of PYY and functional equivalents thereof

The polypeptide of the present invention may be produced by a conventional peptide synthesis method. Amino acid derivatives and synthesis reagents, can be obtained from commercial sources. Peptide chain extension is performed by mainly using Applied Biosystem 433A synthesizer produced by Perkin Elmer, and a protected peptide derivative-resin is constructed by the Boc or Fmoc method. The protected peptide resin obtained by the Boc method is deprotected with anhydrous hydrogen fluoride (HF) in the presence of p-cresol thereby releasing the peptide, which is then purified. The protected peptide resin obtained by the Fmoc method is deprotected with trifluoroacetic acid (TFA) or dilute with TFA containing various scavengers, and the released peptide is purified. Purification is performed in reversed phase HPLC on a C4 or C18 column. The purity of the purified product is confirmed by reverse phase HPLC, and its structure is confirmed by amino acid composition analysis and mass spectrometry.

Example 3

Measurements of PYY plasma levels.

The experiment is performed by subcutaneous injections of placebo and 4 escalating doses of PYY1-36 or PYY3-36 as set out in table 1. The dosages of PYY is calculated base on the fat free mass (FFM) of the subject.
<table>
<thead>
<tr>
<th>Stof</th>
<th>Dosis, pmol/kg FFM</th>
<th>Number of subjects (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYY1-36</td>
<td>12,5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>25</td>
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<td></td>
<td>150</td>
<td>10</td>
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<tr>
<td></td>
<td>200</td>
<td>10</td>
</tr>
<tr>
<td>PYY3-36</td>
<td>12,5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>25</td>
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<tr>
<td></td>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 1. Dosages of PYY and number of subjects (n)

The results are presented as mean±SE, paired t-test (SAS) and repeated measures (SAS).

The PYY injections is performed at time 0 minutes and the plasma concentrations of PYY upon PYY1-36 and PYY3-36 administration is measured at t=0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210 and 240 minutes.

PYY assay

The plasma concentration of PYY is measured using radioimmunoassay of PYY. The assays are performed using PYY antiserum (code no. 8412-5) (Euro-Diagnostica, Malmoe, Sweden). The antiserum recognizes both human PYY 1-36 and PYY 3-36. Synthetic human PYY 1-36 (Peninsula, Merseyside, UK) and porcine $^{125}\text{I}$-PYY (code no. IM259) is purchased from Amersham Biosciences, Buckinghamshire UK) for use as standards. Detection limit of the assay is below 2 pmol/l and 50 % inhibition is obtained with 40 pmol/l PYY. Recovery of PYY added to plasma in concentrations between 5 and 50 pmol/l deviates less than 15 % from expected values. Intra-assay coefficient of variation is below 5 %. The antiserum shows no cross reaction with human NPY or human PP in concentrations up to 500 pmol/l.
The results are shown in figure 2 and 3.

5 Example 4

Clinical protocol Binge Eating Disorder (BED)

45 subjects that meet the proposed diagnostic criteria/research criteria for BED according to the Diagnostic and Statistical Manual IV (DSM-IV) [see below] are included in the study. The discrete period of time in A1 is a 2-hour period, and the method of determining frequency under item D is counting the number of days on which binges occur.

The study is performed in a double-blinded, placebo-controlled fashion. Subjects are divided into three groups (n=15 in each), groups A, B and C. The subjects are given diaries where they note the type and amount of food ingested and at what time. This initial phase of the study is 4 weeks ("Run-in Phase"), after which the subjects start treatment with one of three regimens, as defined below. The subjects keep diaries where they note the type and amount of food ingested and at what time throughout the treatment phase. The treatment duration is 4 weeks ("Treatment Phase").

Dosing: The subjects of group A receive subcutaneous placebo injections (NaCl) three times daily (distributed evenly over the hours awake). The subjects of group B receive 60 pmol/kg body weight of PYY 3-36 s.c. three times daily and the subjects of group C receive 100 pmol/kg body weight of PYY 3-36 s.c. three times daily.

The subjects’ diaries are reviewed by the investigators and the number of binge-eating episodes are determined and further the amount of food/calories ingested per binges/event (EDE) is recorded.

The reduction in the frequency of eating disorder events (binge-eating episodes/events), as defined by calculating the number of binge-eating episodes during the 4-week Treatment Phase divided by the number of binge-eating episodes during the Run-in Phase for each subject, and then statistically comparing the result from group B with group A, and the result from group C with group A.
The change in the amount of food ingested during the events is calculated by calculating the number of calories ingested during the binge-eating episodes during the 4-week Treatment Phase divided by the number of calories ingested during the binge-eating episodes during the Run-In Phase for each subject, and then statistically comparing the result from group B with group A, and the result from group C with group A.

Example 5

Induction of lipolysis by PYY infusion.

Lean subjects (BMI: 18-23 kg/m²) and obese subjects (BMI: 27-40 kg/m²) were infused with PYY3-36 to study its effect on the plasma levels of free fatty acids. 4 individuals received PYY3-36 and 24 subjects received placebo treatment.

PYY3-36 composition: 6 nmol/ml in 0.9% NaCl.

PYY3-36 administration: 0.6 pmol/kg body weight/min.

Placebo administration: 0.9% NaCl.

The subjects were fasting from 10 PM the night before the study. The subjects were infused for a period of 90 minutes starting at 9 AM the following day. Following the infusion, the subjects rested for 2.5 hours prior to the consumption of an ad libitum lunch consisting of an average Danish diet with 34 energy percent (E%) fat, 50 E% carbohydrate and 16 E% protein.

Plasma FFA determination: Blood samples are analysed using a Wako 994-75409 NEFA-C test kit (ACS-ACOD method; Wako Chemicals GmbH, Neuss, Germany) with intraassay and interassay CVs < 4.5 % and < 4.2 %, respectively.

Plasma samples were drawn from the patients every 30 minutes during the study, and the amount of free fatty acids were determined as describe here above. The results showed an increase in plasma levels of free fatty acids of approximately 3-fold over placebo in the 2.5 hour period following the completion of the i.v. infusion
(figure 4). The increase was observed in both lean and obese subjects. This result demonstrated that infusion of PYY3-36 in fasted individuals induces lipolysis and mobilisation of free fatty acids into the blood. The level of free fatty acids in the blood decreases following calorie intake.
Claims

1. A composition comprising PYY or a functional equivalent thereof for the manufacture of a medicament for stimulation of lipolysis.

2. The composition according to claim 1 for increasing the blood concentration of free fatty acids (FFAs).

3. A composition comprising PYY or a functional equivalent thereof, for the preparation of a pharmaceutical composition for preventing or reducing craving for food in an individual.

4. The composition according to claim 3, wherein the food craving is associated with an eating disorder event.

5. The composition according to claim 4, wherein the eating disorder event is associated with binge eating disorder or night eating syndrome.

6. The composition according to claim 5; wherein the food craving is associated with smoking cessation and/or dieting.

7. The composition according to any of claims 3-6, wherein the food craving is craving for food with a high content of carbohydrate and/or fat.

8. A composition comprising PYY or a functional equivalent thereof, for the preparation of a pharmaceutical composition for the treatment of an eating disorder by
   a) decreasing the frequency of eating disorder events and/or
   b) preventing an eating disorder event and/or
   c) reducing food and/or caloric intake during the eating disorder event.

9. The composition according to claim 1-8, comprising human PYY.

10. The composition according to claim 9, comprising human PYY1-36.
11. The composition according to claim 10, comprising human PYY3-36.

12. A composition comprising PYY or a functional equivalent thereof and a second active ingredient, where in the second active ingredient is an anti-emetic drug for the manufacture of a medicament for
   g) treatment of overweight and/or
   h) treatment of obesity and/or
   i) treatment of syndrome X and/or
   j) treatment of disorders of appetite regulation and/or
   k) treatment of eating disorders and/or
   any combination of the above

13. The composition according to any of the preceding claims, wherein the pH of the composition is between 4.0 and 9.0

14. The composition according to any of the preceding claims, wherein the composition comprises a second active ingredient.

15. The composition according to any of the preceding claims, wherein the medicament is for parenteral administration.

16. The composition according to any of the preceding claims, wherein the medicament is for subcutaneous administration.

17. The composition according to any of the preceding claims, wherein the medicament is for intranasal administration.

18. The composition according to any of the preceding claims, wherein the medicament is for administration at least 30 minutes post-meal.

19. The composition according to any of the preceding claims, wherein the medicament is for administration at two hours pre-meal.

20. The composition according to any of the preceding claims, wherein the medicament is for administration before bedtime.
21. The composition according to claim 20, wherein the pharmaceutical composition is for administration within 10-180, 20-120, 30-90 minutes before bedtime.

22. The composition according to 20 or 21, wherein the pharmaceutical composition is for:
   a) treatment of overweight and/or
   b) treatment of obesity and/or
   c) treatment of syndrome X and/or
   d) treatment of disorders of appetite regulation and/or
   e) treatment of eating disorders and/or
   any combination of the above

23. The composition according to any of the preceding claims, wherein the pharmaceutical composition is for the treatment of NES or BED.

24. The composition according to any of the preceding claims, wherein the pharmaceutical composition is for administration at regular intervals, such as once every day, such as twice daily, such as three times every day.

25. The composition according to any of the preceding claims, wherein the pharmaceutical composition is for administration when the patient senses that an eating disorder event is to occur.

26. The composition according to any of the preceding claims, wherein the pharmaceutical composition is for administration during the night.

27. The composition according to any of the preceding claims, wherein administration of the medicament reduces the frequency of eating disorder event by at least 5%.

28. The composition according to any of the preceding claims, wherein the pharmaceutical composition maybe self-administered.
29. The composition according to any of the preceding claims, wherein the pharmaceutical composition is administered by use of a single or a multi-dose injection device.

30. A method of treatment comprising administration of the pharmaceutical composition according to claim 1-29.

31. The method of treatment according to any of the claim 30, wherein PYY or a functional equivalent thereof is administered in dosages of 100 pmole to 500 nmole.
Figure 2

Plasma concentrations of PYY after subcutaneous PYY1-36 administration

PYY1-36

- Placebo (n=12)
- 50 pmol/kg FFM (n=12)
- 100 pmol/kg FFM (n=12)
- 150 pmol/kg FFM (n=10)
- 200 pmol/kg FFM (n=9)

P_{dose\times time} < 0.0001
P_{dose} < 0.0001
P_{time} < 0.0001
P_{baseline} = 0.0007
Figure 3

Plasma concentrations of PYY after subcutaneous PYY3-36 administration

- Placebo (n=12)
- 25 pmol/kg FFM (n=12)
- 50 pmol/kg FFM (n=12)
- 75 pmol/kg FFM (n=10)
- 100 pmol/kg FFM (n=12)
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