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# **Appetite Regulatory Peptides and Insulin Resistance**

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<http://dx.doi.org/10.5772/50977>

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## **1. Introduction**

The discovery of leptin in 1994 provoked the interest in the adipose tissue which was no longer considered as an inert tissue storing energy in the form of triglycerides but as the greatest endocrine organ in human body [1, 2]. As a growing number of people suffer from obesity and metabolic syndrome, understanding the mechanisms by which various hormones and neurotransmitters have influence on energy balance, weight control and insulin resistance has been a subject of intensive research.

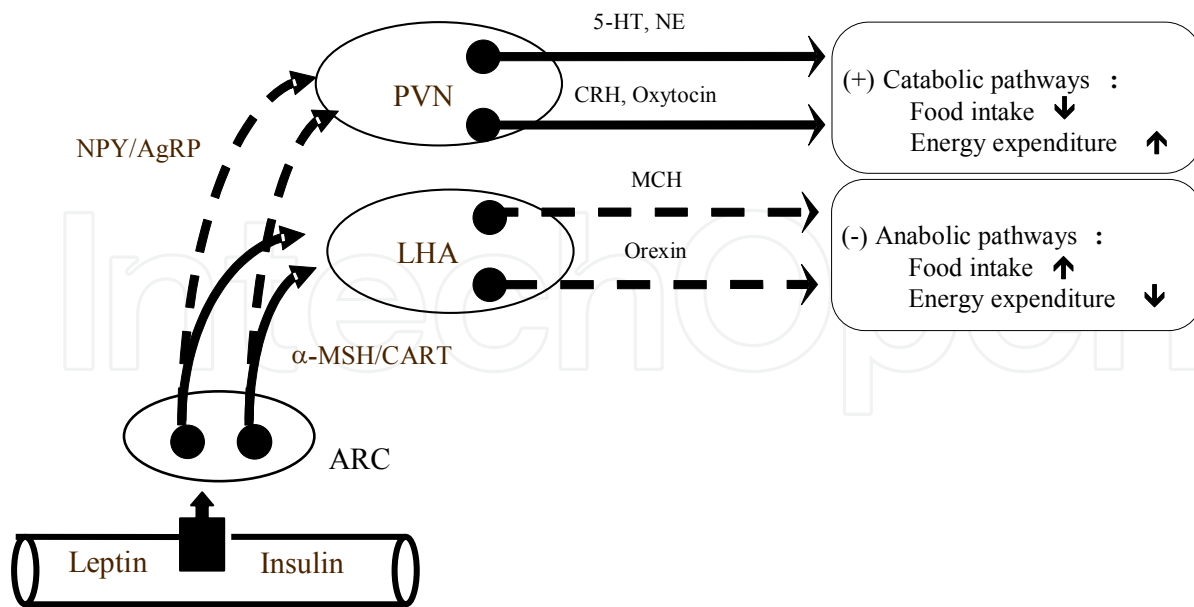
The regulation of appetite and feeding is a homeostatic mechanism. A powerful and complex physiological system exists to balance energy intake and expenditure, in order that sufficient energy is available and body weight remains stable [2, 3]. This system is composed of both afferent signals and efferent effectors. A large number of factors originating throughout the body send afferent signals to a smaller number of functional centers in the central nervous system (CNS), that then mediate interactions with efferent pathways to regulate energy expenditure and energy intake [4]. Thus, central circuits in the brain rely on peripheral signals indicating satiety levels and energy stores, as well as higher cortical factors such as emotional and reward pathways [5].

There are numerous peptides involved in the regulation of energy homeostasis, some of which are produced centrally and others peripherally in the gastrointestinal tract (GI), with some produced at both locations. These peptides are known as members of the 'gut-brain axis' [6]. Since the discovery of secretin, which was confirmed to stimulate pancreatic exocrine secretion, more than 40 other GI tract hormones have been discovered. Anticipation of a meal and the presence of food in the stomach and the small intestine stimulate secretion of many of these hormones from the gut through mechanical and chemical stimuli. These signals are involved in the initiation of food intake as well as termination of meals [7]. However, many of the same hormones are also expressed in the

CNS, acting to translate metabolic information between the GI tract and the brain [8]. In normal subjects, body weight is tightly regulated despite day-to-day variations in food intake and energy expenditure. Obesity is due to a state in which energy intake exceeds energy expenditure over a prolonged period of time. In humans it is also of note that psychological and emotional factors can drive food intake in excess of actual need [7].

In summary, signals relaying information such as the nutritional and energy status of the body converge within the CNS. Thus, CNS mediates energy balance in the body, the hypothalamus playing a main role in this process. The arcuate nucleus (ARC) is a key hypothalamic nucleus in the regulation of appetite and is involved in integrating peripheral satiety and adiposity signals via orexigenic and anorexigenic neuropeptide transmission to other hypothalamic and extrahypothalamic brain regions [9]. Proximity of ARC to the median eminence and the fact that it is not fully insulated from the circulation by the blood brain barrier makes it strategically positioned to integrate the great number of peripheral signals controlling food intake [5]. There are two major neuronal populations in the ARC implicated in the regulation of feeding. One population co-expresses Neuropeptide Y (NPY) and agouti-related protein (AgRP) and increases food intake. The second population of neurons co-expresses cocaine- and amphetamine-related transcript (CART) and pro-opiomelanocortin (POMC), the precursor to the melanocortin receptor agonist,  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH), and inhibits food intake. Neuronal projections from these two populations then communicate with other hypothalamic areas involved in appetite regulation such as the paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial nucleus (DMN) and lateral hypothalamic area (LHA) [10].

Receptors for leptin and insulin are expressed on both of these types of neurons, suggesting that they are responsive to circulating levels of these hormonal signals, acting as effectors for altering food intake in response to variations in energy balance as indicated by body adiposity [11]. Leptin is secreted by adipocytes and circulates at concentrations proportional to fat mass. Restriction of food intake for a relatively longer period, results in a suppression of leptin levels, which can be reversed by refeeding or administration of insulin. Insulin is a major metabolic hormone. Like leptin, levels of plasma insulin vary directly with changes in adiposity being influenced to a great extent by peripheral insulin sensitivity. The latter is related to total body fat stores and fat distribution, with visceral fat being the key determinant [12]. Plasma insulin increases at times of positive energy balance and decreases at times of negative energy balance. However, unlike leptin, insulin secretion increases rapidly after a meal, whereas leptin levels are relatively insensitive to meal ingestion. Both leptin and insulin cross the blood-brain barrier, stimulate anorexigenic  $\alpha$ -MSH/CART neurons and inhibit orexigenic NPY/AgRP neurons, thus activating the catabolic pathways and inhibiting the anabolic pathways (Figure 1). When energy stores are low, production of leptin from adipose tissue, and thus circulating leptin concentrations fall, leading to increased production of hypothalamic neurotransmitters that strongly increase food intake, such as NPY, galanin, and AgRP and decreased levels of  $\alpha$ -MSH, CART, and neurotensin that reduce food intake and increase energy expenditure [8, 13].



CRH, corticotrophin-releasing hormone; MCH, melanin-concentrating hormone; 5-HT, 5-hydroxytryptamine; NE, norepinephrine; ( - - → ) inhibition; ( -> ) stimulation.

**Figure 1.** Schematic action of leptin and insulin on the hypothalamus.

In addition to leptin and insulin, receptors for ghrelin are also located on arcuate AgRP/NPY neurons, which are activated by central ghrelin administration. Furthermore, peripheral ghrelin administration activates neurons in the ARC and AgRP and NPY have been demonstrated to be requisite mediators of the hyperphagia induced by systemic ghrelin [11]. So, meal-generated satiety signals from the GI tract do interact with longer-term adiposity signals, such as insulin and leptin in energy balance [8].

NPY is one of the most abundant peptides of the hypothalamus and one of the most potent orexigenic factors. The majority of neurons expressing NPY in the hypothalamus are found within the ARC and most co-express AgRP [14]. Synthesis and release of NPY are both regulated by leptin binding to its hypothalamic receptor. NPY links afferents reflecting the nutritional status of the organism from endocrine, gastrointestinal, and central and peripheral nervous systems to effectors of energy intake and expenditure [15]. NPY stimulates appetite inducing hyperphagia, increase of fat depots, decrease of thermogenesis, and suppression of sympathetic activity. NPY is known to be involved in other physiological functions, such as cardiovascular regulation, affective disorder, memory retention, neuroendocrine control. When leptin levels increase after food intake, the latter binds to its receptors in the hypothalamus which leads to discontinuation of NPY secretion [5, 16]. The decrease of NPY concentration in obesity probably plays a role of a counter-regulatory factor intended to prevent further weight gain. Thus, NPY becomes one of the main regulators of food intake, body weight and energy expenditure.

*Ghrelin* is a fast-acting hormone, seemingly playing a role in meal initiation. Secreted predominantly from the stomach, ghrelin is the natural ligand for the growth hormone secretagogue-receptor (GHS-R) in the pituitary gland, thus fulfilling the criteria of a brain-

gut peptide [5, 6]. Although the majority of ghrelin is produced peripherally, there are ghrelin immunoreactive neurons within the hypothalamus that have terminals on hypothalamic NPY/AgRP, POMC and corticotropin releasing hormone (CRH) neurons [17], as well as orexin fibres in the LHA [18]. It was found that ghrelin acts to promote appetite in two ways—directly, by depolarizing the orexigenic NPY/AgRP neurons, and indirectly, by increasing the tonic inhibition exerted by the NPY/AgRP neurons over the anorexigenic POMC/CART neurons. Both of these ultimately enhance appetite [4, 17, 18]. The ability of ghrelin to increase food intake and body weight is mediated through the stimulation of NPY production in the hypothalamic ARC, where it antagonizes the inhibitory effect on NPY secretion displayed by leptin and insulin [19].

The purpose of this chapter is to provide background information on the relationship between the main appetite regulatory peptides NPY and ghrelin, and insulin resistance. The role of NPY and ghrelin in food intake and body weight control in humans, and their mechanism of action are discussed, focusing on association with glucose metabolism and insulin resistance.

## 2. Neuropeptide Y (NPY)

NPY is one of the most abundant peptides of the hypothalamus and one of the most potent orexigenic (appetite-increasing) factors [20]. It is a 36-amino acid peptide that was first isolated from porcine brain in 1982 [21] (Figure 2). It is a member of the PP-fold family of peptides which consists of NPY, peptide YY (PYY), pancreatic polypeptide (PP) and peptide Y (PY) [22].



**Figure 2.** Structure of NPY.

NPY is synthesized by cell bodies in the ARC and transported axonally to the PVN where the highest concentrations are found [23]. NPY-expressing neurons are prominent also in the DMN and the VMN [24]. All these regions of the brain influence feeding behavior and energy balance.

NPY is also found in circulating blood, where it comes mainly from the adrenal medulla and sympathetic nerves, but this peripheral hormone does not cross the blood-brain barrier [25,

26]. NPY is present in the pancreas, in both the islet cells and in sympathetic nerve terminals [27, 28].

#### *Regulation of NPY synthesis and release*

The levels of hypothalamic NPY mRNA and NPY release increase with fasting and decrease after refeeding [29-31]. When fed with a high-carbohydrate diet; diabetic rats exhibit increased gene expression of the NPY in the hypothalamic ARC, and high-fat diet suppressed NPY expression [32]. Thus, NPY synthesis as well as its receptorial expression are sensitive to changes in the metabolic status and food availability. While starvation and food deprivation increase NPY release [30], in obese subjects the activity of NPY neurones is down-regulated in the attempt to restrain overeating of palatable food [33]. These facts support the existence of a long loop control system in energy metabolism between the brain and adipose tissue. Several peripheral factors are involved in the modulation of this system. Among them insulin and leptin display an inhibitory effect; glucocorticoids and ghrelin act as stimulatory afferent signals [34].

It has been reported that *leptin* is a major inhibitory regulator of the activation by ghrelin of the orexigenic network of NPY [35]. Circulating leptin crosses the blood brain barrier and binds to the long form of the leptin receptor, Ob-Rb, in the hypothalamus [36]. The Ob-Rb is expressed widely within the hypothalamus but particularly in the ARC, VMN, DMN and LHA. Several experimental studies demonstrate that systemic administration of leptin inhibits NPY gene overexpression through a specific action in the ARC. Cells within the ARC express both NPY and leptin receptors, and leptin directly activates anorectic POMC neurons and inhibits orexigenic AgRP/NPY neurons [37]. All available data can be summarized as follows: when leptin levels increase after food intake, the latter binds to its receptors in the hypothalamus which leads to discontinuation of NPY secretion. When production of leptin from adipose tissue is reduced, the circulating leptin concentrations fall, and this lead to enhanced production of NPY that strongly increase food intake.

Receptors for *ghrelin* are also located on arcuate AgRP/NPY neurons, which are activated by central ghrelin administration. Ghrelin acts by depolarizing the orexigenic NPY/AgRP neurons, and by increasing the tonic inhibition exerted by the NPY/AgRP neurons over the anorexigenic POMC/CART neurons [11]. c-Fos expression increases within NPY-synthesizing neurons in the ARC after peripheral administration of ghrelin [38], and ghrelin fails to increase food intake following ablation of the ARC [39]. Studies of knockout mice demonstrate that both NPY and AgRP signalling mediate the effect of ghrelin, although neither neuropeptide is obligatory [40].

*Insulin* is a major metabolic hormone produced by the pancreas and the first adiposity signal to be described. Little or no insulin is produced in the brain itself [41, 42]. Insulin levels are dependent on peripheral insulin sensitivity that is related to total body fat stores and fat distribution, with visceral fat being a key determinant of insulin sensitivity [12]. Once insulin enters the brain, it acts as an anorexigenic signal, decreasing food intake and subsequently body weight. It was found that both the NPY and melanocortin systems are important downstream targets for the effects of insulin on food intake and body weight. Insulin

penetrates the blood–brain barrier via a saturable, receptor-mediated process, at levels which are proportional to the circulating insulin [43]. Insulin receptors are widely distributed in the brain, with highest concentrations found in the olfactory bulbs and the hypothalamus [44]. Within the hypothalamus, there is particularly high expression of insulin receptors in the ARC; they are also present in the DMH, PVN, and suprachiasmatic and periventricular regions [45]. Hypothalamic NPY is a potential mediator of the regulatory effects of insulin. The increase of NPY levels in the PVN and prepro-NPY mRNA in the ARC during fasting are inhibited by intracerebroventricular (icv) administration of insulin. Fasting, therefore, increases NPY biosynthesis along an ARC-PVN pathway in the hypothalamus via a mechanism dependent on low insulin levels [46]. NPY expression is increased in insulin-deficient, streptozocin-induced diabetic rats and this effect is reversed with insulin therapy [47, 48]. Insulin receptors have been found also on POMC neurons in the ARC [49]. Administration of insulin into the third ventricle of fasted rats increases POMC mRNA expression and the reduction of food intake caused by icv injection of insulin is blocked by a POMC antagonist [49]. Furthermore, POMC mRNA is reduced by 80% in rats with untreated diabetes, and this can be attenuated by peripheral insulin treatment which partially reduces the hyperglycaemia [50].

*Glucocorticoid hormones* play a critical role in energy balance and also appear to mediate at least some of their actions through the central NPY axis. They may regulate NPY-induced insulin release and NPY signaling within the VMH of the hypothalamus. Glucocorticoid-receptor immunoreactivity is found within the rat CNS, including the ARC, VMH, and PVN [51]. Many of these receptors are expressed at the nucleus of NPY-containing, endocrine-related neurons and coexist in regions containing high NPY receptor density [52]. In rats, excessive corticosterone promotes body fat gain and hyperinsulinemia [53] and also increases NPY synthesis and Y1-receptor mRNA expression, at least within the ARC [54, 55]. Conversely, removal of glucocorticoids by adrenalectomy reduces hyperphagia and body weight of obese (*fa/fa*) rats [56], abolishes obesity induced by VMH lesions [57], and prevents obesity induced by chronic central NPY infusion in normal rats [58]. However, it has been reported that adrenalectomy does not alter NPY-1 (Y1)-receptor mRNA expression in the ARC [54]. Chronic icv infusion of NPY induces hyperphagia, hyperinsulinemia, and insulin resistance in rats, and these effects are blocked by previous adrenalectomy [59]. Wisialowski et al. [60] have demonstrated that adrenalectomy also abolishes the insulin release caused by an acute icv injection of NPY and this is associated with significant reduction in Y1- and Y5-receptor mRNA expression specifically within the VMH. These experiments imply that glucocorticoids are necessary for icv NPY to stimulate insulin release and suggest that the latter manifest this regulatory role through alterations in Y1- and Y5-receptor expression in the VMH [60]. Taken together, all these observations indicate that glucocorticoids have a regulatory role in long-term central NPY signaling.

As concerns plasma NPY, its concentrations rise in response to muscular exercise [61] and in disorders such as pheochromocytoma and renal failure [62].

#### *Mechanisms of action of NPY – NPY receptors*

PP-fold family of peptides bind to seven transmembrane-domain G-protein-coupled receptors [63]. Heterogeneity among NPY (and PYY) receptors was first proposed on the

basis of studies on sympathetic neuroeffector junctions, where NPY (and PYY) can exert three types of action: 1) a direct (e.g., vasoconstrictor) response; 2) a postjunctional potentiating effect on norepinephrine (NE)-evoked vasoconstriction; and 3) a prejunctional suppression of stimulated NE release. The two latter phenomena are probably reciprocal, since NE affect NPY mechanisms similarly [64]. Six different NPY receptors have been identified ([65], of which five have been cloned and characterized. Y1–Y5 receptors have been demonstrated in rat brain, but Y6, identified in mice, is absent in rats and inactive in primates [66]. The Y1, Y2, Y4 and Y5 receptors, cloned in the hypothalamus, have all been postulated to mediate the orexigenic effects of NPY. Biological redundancies are likely to exist between Y1 and Y5 receptor signaling [67].

NPY initiates appetite drive directly through its receptors, particularly the Y1-5 (NPY5-R), and by the simultaneous inhibition of anorexigenic melanocortin signalling in the ARC [68]. NPY5-R is thought to be the main receptor involved in NPY-induced food intake since a reduction in food intake after an icv injection of antisense oligonucleotides directed against NPY5-R is demonstrated in rats [69].

Although the large number of Y receptors has made it difficult to delineate their individual contributions, recent studies analyzing NPY and Y receptor-overexpressing, knockout, and conditional-knockout mouse models have started to unravel some of the complexity. To elucidate the role of NPY1-R in food intake, energy expenditure, and other possible functions, Kushi et al. [70] have generated NPY1-R-deficient mice (Y1-R<sup>-/-</sup>) by gene targeting. Contrary to their hypothesis that the lack of NPY signaling via Y1-R would result in impaired feeding and weight loss, Y1-R<sup>-/-</sup> mice showed a moderate obesity and mild hyperinsulinemia without hyperphagia. The authors suggest either that the Y1-R in the hypothalamus is not a key molecule in the leptin/NPY pathway, which controls feeding behavior, or that its deficiency is compensated by other receptors, such as NPY5-R. Probably the mild obesity found in Y1-R<sup>-/-</sup> mice was caused by the impaired control of insulin secretion and/or low energy expenditure [70]. This model could be useful for studying the mechanism of mild obesity and abnormal insulin metabolism in noninsulin-dependent diabetes mellitus.

In order to investigate the role of different Y receptors in the NPY-induced obesity syndrome, Lin et al. [71] used recombinant adeno-associated viral vector to overexpress NPY in mice deficient of selective single or multiple Y receptors (including Y1, Y2, and Y4). Results from this study demonstrated that long-term hypothalamic overexpression of NPY lead to marked hyperphagia, hypogonadism, body weight gain, enhanced adipose tissue accumulation, hyperinsulinemia, and other hormonal changes characteristic of an obesity syndrome. NPY-induced hyperphagia, hypogonadism, and obesity syndrome persisted in all genotypes studied (Y1<sup>-/-</sup>, Y2<sup>-/-</sup>, Y2Y4<sup>-/-</sup>, and Y1Y2Y4<sup>-/-</sup> mice). However, triple deletion of Y1, Y2, and Y4 receptors prevented NPY-induced hyperinsulinemia. These findings suggest that Y1, Y2, and Y4 receptors under this condition are not crucially involved in NPY's hyperphagic, hypogonadal, and obesogenic effects, but they are responsible for the central regulation of circulating insulin levels by NPY [71].

A lot of investigators' data point that NPY5-R mediates the feeding response to exogenous and endogenous NPY. It may be involved in energy balance and is, therefore, a susceptibility candidate gene for obesity and related disorders such as the metabolic syndrome and type 2 diabetes mellitus. It is hypothesized that the feeding effect of NPY may indeed be mediated by a combination of receptors rather than a single one. Also, increasing evidence points to the existence of other as yet unidentified Y receptors, which may mediate NPY's orexigenic actions, and it remains possible that, under certain physiological conditions, NPY may bind and activate receptors for which it normally has no or only low affinities.

Analogues of NPY with high selectivity for the Y1 and Y5 receptor subtypes strongly stimulate food intake in rodents, and icv administration of specific Y5 receptor agonists increases food intake and body weight in mice [72]. A clinical study examining a therapeutic intervention based on the NPY system has been performed by Erundu et al. [73]. The authors tested the hypothesis that blockade of the NPY5-R will lead to weight loss in humans using MK-0557, a potent, highly selective, orally active NPY5-R antagonist. MK-0557 has no significant binding to the human NPY1-R, NPY2-R, NPY4-R, or mouse NPY6-R at concentrations of 10  $\mu$ M. These data indicate a >7500-fold selectivity for the NPY5-R relative to the other NPY receptor subtypes. MK-0557 was administered to 547 obese subjects, who showed statistically significant weight loss at 12 weeks compared to subjects treated with placebo [73]. These observations clearly indicate that antagonizing the NPY5-R induces weight loss in humans. After that a long-term trial over 52 weeks was performed in 1661 subjects (832 completed). There was a mean weight loss of 3.4 kg in those who completed the trial, which was significantly greater than the weight loss seen in the placebo-treated group. Significantly more subjects lost  $\geq 5\%$  and  $\geq 10\%$  of initial body weight with the NPY5-R antagonist than did so on placebo. The authors conclude, however, that the magnitude of the weight loss observed was not clinically significant, and this conclusion is supported by the observation that there were no significant improvements in secondary endpoints such as glucose and lipid levels and blood pressure measurements [73]. While several potential new obesity therapies that act through the CNS pathways or peripheral adiposity signals are in early-phase clinical trials, the above study serves to point out that manipulation of the homeostatic mechanisms involving hypothalamic/brainstem pathways for a clinically significant outcome in obese patients remains a major challenge [74].

#### *Link between NPY, obesity and insulin resistance*

NPY is a powerful stimulant of food intake. Numerous studies in rodent models have demonstrated that administration of NPY into the PVN stimulates feeding [75, 76] and that repeated injections of NPY result in persistent feeding and the development of obesity by promoting fat accumulation [77, 78]. Central administration of NPY was found to reduce energy expenditure, resulting in reduced brown fat thermogenesis [79], suppression of sympathetic nerve activity [80] and inhibition of the thyroid axis [81]. There are some data that NPY activates hypothalamic-pituitary-adrenal axis (HPA) [82], that is implicated in the regulation of metabolism and energy balance. An acute injection of NPY into the PVN produces increases in circulating ACTH and corticosterone in both conscious and

anesthetized rats [83]. ARC NPY neurons project to the ipsilateral PVN [84], and repeated icv injection of NPY into the PVN in normal rats causes hyperphagia, an increase in basal plasma insulin level and morning cortisol level, independent of increased food intake, increased metabolic activity of white adipose tissue and muscle insulin resistance, and results in obesity [85, 86]. Several of these metabolic effects are still present when increased food intake is prevented by food restriction [85]. It was shown that Y5 receptor subtype is involved in the activation of HPA axis mediated by NPY [82].

Interestingly, injection of NPY directly into the VMH significantly increases food intake [75], and NPY-induced feeding is enhanced in VMH-lesioned rats [87]. Lesions of the VMH in rodents also cause multiple changes in metabolic status, including hyperphagia, hyperglycemia, and hyperinsulinaemia [88]. Enhanced NPY expression in the VMH is associated with obesity [89]. Furthermore, NPY has been shown to directly inhibit over one fifth of spontaneously active rat VMH neurons, and this inhibition is potentiated by overfeeding [90]. Therefore, the mechanism by which acute icv NPY stimulates insulin release in the absence of feeding may be by inhibiting the spontaneous activity of the VMH through Y1 and Y5 receptors. A reduction of these receptors with adrenalectomy would then reduce the ability of NPY to inhibit VMH neurons. These data suggest the VMH may also be a site of action for NPY in the development of obesity; however, the mechanisms by which NPY is involved in each aspect of central energy regulation remain to be defined.

Some investigators found that acute icv NPY administration had no effect on plasma glucose levels, indicating that NPY-induced insulin release is not simply a secondary response to changes in peripheral glucose [60, 91]. The decreased basal insulin levels and lack of insulin release in response to NPY injection in adrenalectomized rats with downregulation of Y1-and Y5-receptor mRNA in the VMH, demonstrated in the study of Wisialowski et al. [60], highlights the role for Y1-and Y5-receptors in the etiology of NPY-induced hyperinsulinemia, insulin resistance, and obesity.

Van den Hoek et al. [92] found that icv administration of NPY in the third ventricle in rats acutely hampers the capacity of insulin to suppress endogenous glucose production via activation of sympathetic nerves innervating the liver. The authors discussed a possible explanation for the role of NPY in sympathetic overdrive and hepatic insulin resistance that are typical for obese subjects with the metabolic syndrome [92]. In a study of Singhal et al. [93] the ability of resistin to increase hepatic insulin resistance and modulate the levels of various mediators in the liver was abolished in mice lacking NPY as well as in mice pretreated with icv NPY Y1 receptor antagonist. The authors established a crucial link between NPY and resistin's ability to regulate hepatic insulin resistance possibly via induction of SOCS3 (suppressor of cytokine signaling-3), tumor necrosis factor (TNF)- $\alpha$  and interleukin 6 (IL6). Additionally, NPY is critical to mediating the decrease in STAT3 (signal transducer-activated transcript-3) phosphorylation by central resistin [93].

It was found that the obesity syndrome, induced by injection of NPY into the CNS of rats, closely resembles the phenotype of either leptin deficient *ob/ob* mice, or leptin resistant *db/db* mice [58, 85]. In these animals, genetic alterations of the satiety effect of leptin within the

hypothalamus result in an overexpression of NPY leading to a complex syndrome including hyperphagia, increased fat storage and obesity. The experimental studies in *ob/ob* mice demonstrate that systemic administration of leptin inhibits NPY gene overexpression through a specific action in the ARC and exerts a hypoglycemic action that is partly independent of its weight-reducing effects. It must be pointed that both effects occur before reversal of the obesity syndrome. Defective leptin signaling due to either leptin deficiency (in *ob/ob* mice) or leptin resistance (in *db/db* mice) therefore leads directly to hyperglycemia and the overexpression of hypothalamic NPY, that is implicated in the pathogenesis of the obesity syndrome [94]. Moreover, the obesity syndrome produced by icv administration of NPY is characterised by increased expression of the *ob* gene in adipose tissue [58]. On the other hand leptin, the *ob* gene product, has been shown to inhibit NPY synthesis and release from hypothalamic nuclei in *ob/ob* mice. Correction of the obese state induced by genetic leptin deficiency reduces elevated levels of both blood glucose and hypothalamic NPY mRNA [95].

Although NPY seems to be an important orexigenic signal, NPY-null mice have normal body weight and adiposity [96, 97]. This absence of an obese phenotype may be due to the presence of compensatory mechanisms or alternative orexigenic pathways, such as those which signal via AgRP [98]. It is possible that there is evolutionary redundancy in orexigenic signalling in order to avert starvation. This redundancy may also contribute to the difficulty elucidating the receptor subtype that mediates NPY-induced feeding [99].

In searching the role of NPY in human obesity and metabolic disorders, polymorphisms in the NPY5-R gene have been studied by other authors in several populations. Thus, NPY5-R gene was sequenced by Jenkinson et al. [100], and several single nucleotide polymorphisms (SNPs) were genotyped in the Pima Indians with three novel SNPs being identified, which were described as polymorphism 1, 2, and 3 (P1, P2, and P3). All three SNPs are in non-coding regions. There were genotype differences in lean and obese Pima Indians for P2 and for a 3 SNP haplotype [100]. A silent single nucleotide polymorphism within the NPY5-R coding sequence showed no evidence of association with BMI in children and adolescents [101]. In contrast, a novel polymorphism in the intervening segment between exons of the genes encoding NPY1-R and NPY5-R was associated with reduced serum triglyceride (TG) levels and HDL-cholesterol in a severely obese cohort [102] that should be considered as a protective lipid profile. Roche et al. [103] investigated the potential implication of NPY, NPY-Y1 and -Y5 subtype receptors [rNPY-Y1/-Y5] in the development of human obesity. Two complementary genetic approaches were used: 1) linkage analyses between obesity and polymorphic markers located nearby NPY and rNPY-Y1/-Y5 genes in 93 French Caucasian morbidly obese families; 2) single strand conformation polymorphism (SSCP) scanning of the coding region of the NPY and rNPYY1 genes performed in 50 unrelated obese patients ascertained. No evidence of linkage between morbid obesity or obesity-related quantitative traits and NPY and rNPY-Y1/Y5 regions was found in this population. Moreover, SSCP scanning revealed no mutation in the coding region of NPY and rNPY-Y1 genes among obese subjects. The authors suggest that NPY and NPY-Y1/ Y5 receptors are unlikely to be implicated in the development of human morbid obesity, at least in the French Caucasian population [103].

In addition to the above data, genetic association of NPY receptor Y5 (NPY5R) SNPs with metabolic syndrome was studied in 439 Mexican American individuals by Coletta et al. [104]. Minor alleles for five of nine genetic variants (rs11100493, rs12501691, P1, rs11100494, rs12512687) of the NPY5-R SNPs were found to be significantly associated with both increased plasma TG levels and decreased high-density lipoprotein (HDL) concentrations [104]. In addition, the minor allele for SNP P2 was significantly associated with a decreased homeostasis model assessment of  $\beta$ -cell function (HOMA-% $\beta$ ). Linkage disequilibrium between SNPs pairs indicated one haplotype block of five SNPs (rs11100493), and low HDL-cholesterol are highly associated with insulin resistance states, such as type 2 diabetes mellitus, obesity, and the metabolic syndrome. So, these results provide evidence for association of SNPs in the NPY5R gene with atherogenic dyslipidemia in insulin resistance. In the course of identification of genes implicated in the development of human obesity, further genome-wide searches could be successful for identifying multiple predisposing loci.

It has become apparent, that upon vigorous electrical stimulation or intense stressors motor neurons on the sympathetic nerve system (SNS) may secrete NPY as well as NE [105]. Acting through NPY receptors on vascular and adipose tissue, secreted NPY may play an important role in the pathophysiology of obesity and metabolic syndrome. Thus, Kuo et al. [105] demonstrated that stress exaggerated diet-induced obesity through a peripheral mechanism in the abdominal white adipose tissue that is mediated by NPY. The authors found that stressors such as exposure to cold or aggression lead to NPY release from SNS, which in turn upregulates NPY and its Y2 receptors (NPY2-R) in a glucocorticoid-dependent manner in the abdominal fat. This positive feedback response by NPY lead to abdominal fat enhancement. Release of NPY and activation of NPY2-R stimulated fat angiogenesis, macrophage infiltration, and the proliferation and differentiation of new adipocytes, resulting in abdominal obesity and a metabolic syndrome-like condition. NPY, like stress, stimulated fat growth, whereas pharmacological inhibition or fat-targeted knockdown of NPY2R is anti-angiogenic and anti-adipogenic. Thus, manipulations of NPY2-R activity within fat tissue offer new ways to remodel fat and treat obesity and metabolic syndrome [105].

NPY may be an important intra-islet paracrine hormone [38]. When produced by pancreatic islets, its expression is dependent on the prevailing endocrine environment. Islet NPY appears to constrain insulin release under a variety of conditions. Whether peripheral NPY has a hormone-like action and directly influences glucose metabolism and/or insulin secretion *in vivo* is under investigation. In this context NPY, at high concentrations, may contribute to the modulation of insulin secretion *in vitro*. NPY nerve fibers occur in the mouse pancreas and that most of these NPY nerve fibers are nonadrenergic. Furthermore, in the mouse, NPY enhances basal plasma insulin levels at high dose levels under *in vivo* conditions. At lower dose levels it inhibits glucose-induced, but not cholinergically induced insulin secretion [106]. It has also been reported that NPY may reduce plasma glucose concentrations during exercise by inhibiting glycogen breakdown in the splanchnic compartment [107, 108]. Moreover, the potential relation between circulating NPY and the pathophysiological consequences of obesity need further investigation.

Vettor et al. [109] found that peripheral NPY infusion in normal rats increased the overall rate of glucose disposal by increasing insulin responsiveness in skeletal muscle. Plasma leptin was significantly increased by hyperinsulinaemia, but was not affected by NPY infusion. Both the early and late phase of the insulin response to hyperglycaemia were significantly reduced by NPY. Based on their data for an increased glycolytic flux combined with a blunted increase in lactate, the authors suggested that NPY may raise insulin mediated glucose disposal by increasing its utilisation through the oxidative pathways. Intravenous NPY did not influence glucose metabolism in adipose tissue and leptin release [109].

The above data indicate that NPY has different effects on insulin secretion when administered acutely via intracerebroventricular or intravenous routes. Thus, peripheral NPY plays a clear inhibitory role in glucose-induced insulin secretion. It is also possible that the duration of treatment, and not just the route of administration, may be a relevant factor.

Several appetite-regulating genes (MCH, CRH, NPY, cholecystokinin, etc.) as well as their corresponding receptors, are expressed in the adipose tissue. The coexistence of locally produced NPY and NPYR-2 suggests a NPY autocrine/paracrine system of regulation of adipocyte function. Kos et al. reported that NPY is not only expressed but also secreted by human adipose tissue and insulin increases NPY secretion [110]. Direct effects of NPY on adipocyte function are also described. Thus, NPY was as potent as insulin in increasing both leptin and resistin secretion from pre-adipocytes from visceral fat in vitro [105]. Treatment of human subcutaneous adipocytes with recombinant human NPY downregulates leptin receptor [110], exerts an anti-lipolytic effect probably mediated by adenylate cyclase inhibition [111], and promotes the proliferation of pre-adipocytes [105, 112]. Probably, the enhanced local expression of NPY within visceral adipose tissue may contribute to the molecular mechanisms underlying increased visceral adiposity. The anti-lipolytic action on NPY can promote an increase in adipocyte size in hyperinsulinaemic conditions, such as abdominal obesity and metabolic syndrome.

As compared to the numerous experimental and genetic studies, the clinical studies on circulatory NPY in obesity are not so many. It is interesting that significant alteration of NPY circulatory levels is not found in adults after weight reduction [113] as well as in adolescents [114] besides the progressive decrease of leptin levels. Probably, the leptin control on hypothalamic production of NPY cannot be estimated by the levels of the latter in peripheral circulation.

In one of our recent studies on different morphological types of obesity [115], NPY levels in obese women were lower than those of the normal weight controls, the differences being significant when comparing the obese group as a whole and the subgroup with android obesity only (Table 1). There was a reverse correlation between NPY and body weight, and percentage body fat. In analogy with the comparisons regarding NPY, leptin levels did not differ significantly between the two groups of obese women. Our data are in accordance with the data of Zahorska-Markiewicz et al. in obese women and in women with normal weight [113]. Notwithstanding the absence of statistically significant differences in leptin

and NPY levels between our obese patients, we observed that at relatively highest leptin levels NPY had relatively lowest levels, and vice versa. This was supported by the ascertained negative correlation between the two hormones. In the control group, significantly lower leptin levels were associated with significantly higher NPY levels as compared to the obese group. We can suggest that the decrease of NPY concentration in obesity may play a role of a counter-regulatory factor intended to prevent further weight gain. In this and previous study of ours [116] we did not find significant differences in circulatory levels of resistin and TNF $\alpha$  between lean women and women with both gynoid and android obesity. The latter were insulin-resistant with significantly higher basal insulinaemia and HOMA-index, respectively (Table 1).

Hormones	Leptin (ng/ml)	Resistin (ng/ml)	TNF $\alpha$ (pg/ml)	NPY (ng/ml)	Insulin ( $\mu$ IU/ml)	HOMA index
<b>Android obesity (n=32)</b>	21.28 $\pm$ 11.14*	2.35 $\pm$ 0.59	15.75 $\pm$ 6.79	4.59 $\pm$ 1.13*	20.13 $\pm$ 8.17*#	4.34 $\pm$ 1.68*#
<b>Gynoid obesity (n=27)</b>	17.14 $\pm$ 9.05	2.24 $\pm$ 0.76	18.18 $\pm$ 6.07	5.21 $\pm$ 1.19	10.47 $\pm$ 5.24	2.18 $\pm$ 1.34
<b>Controls (n=24)</b>	10.02 $\pm$ 5.98	2.09 $\pm$ 1.19	19.17 $\pm$ 9.08	5.99 $\pm$ 1.18	8.03 $\pm$ 3.22	1.69 $\pm$ 0.98

(All data are expressed as mean $\pm$ SD.\* - significant difference as compared to the control group; # - significant difference as compared to the group with gynoid obesity)

**Table 1.** Hormonal parameters and HOMA-index in the women with obesity and normal weight women [115].

The NPY levels were found similar in a group of patients with gestational diabetes mellitus and in pregnant women with normal glucose tolerance in a study of Ilhan et al [117]. Notably, the NPY concentration correlated positively with insulin levels in patients with type 2 diabetes mellitus [117]. These data suggest a potential involvement of circulating NPY in diabetes pathology that needs further purposeful studies.

#### *NPY and reproductive function*

Having in mind the fact, that NPY secretion is increased in response to metabolic challenges that inhibit luteinizing hormone releasing hormone (LHRH) secretion (e.g., fasting) and decreased by treatments that restore the metabolic deficit and reinstate reproductive function (e.g., re-feeding) [20], several studies have focused on the role of NPY in reproductive processes.

A modulating action of NPY on the gonadotropic and somatotrophic systems in experimental animals has been reported. NPY affects luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release from anterior pituitary cells *in vitro* and enhances LHRH-induced LH secretion [118]. In female rats NPY decreased LH release in pituitary cell culture *in vitro* [119]. Barb et al. [120] conducted 2 experiments in ovariectomized prepubertal gilts to test the hypothesis that NPY stimulates appetite and modulates LH and growth hormone (GH)

secretion, and that leptin modifies such acute effects of NPY on feeding behavior and LH and GH secretion. In the first one, gilts received icv injections of NPY. In the second one gilts received icv injections of leptin, NPY or NPY + leptin, and feed intake was measured. The authors found that NPY suppressed LH secretion and the 100 µg dose stimulated GH secretion. NPY reversed the inhibitory effect of leptin on feed intake and suppressed LH secretion, but serum GH concentrations were unaffected [120]. In another experiment in prepubertal gilts, Barb et al. [121] demonstrated that NPY did not alter basal LH secretion nor 10(-8) M LHRH-induced increase in LH secretion but 10(-9) M LHRH-stimulated LH secretion was reduced by NPY and was not different from control or LHRH alone. At the same time NPY increased basal GH secretion and enhanced the GH response to growth hormone releasing factor (GRF) at the level of the pituitary gland [121]. These data support the modulating role of NPY on GH and LH secretion. Experimental evidence in rodents and monkeys suggests that NPY preferentially exerts inhibitory effects on LHRH-LH secretion when estrogen levels are low [122, 123]. In primates, the role of NPY as a regulator of gonadotropin secretion is complicated by the observation that age may influence the effects of NPY (inhibitory or stimulatory), as does the site of exogenous NPY administration [124, 125]. An important physiological role for NPY as a modulator of neuroendocrine activity which culminates in the preovulatory surge of LH is discussed [118]

All above mentioned and many similar results support the hypothesis that NPY modulates feed intake, and LH and GH secretion and may serve as a neural link between metabolic state and the reproductive, as well as the growth axis.

Clinical evidence suggests that NPY exerts primarily an inhibitory effect on the hypothalamic-pituitary-ovarian (HPO) axis in humans. Thus, a role for NPY in hypothalamic amenorrhea is inferred from the observation that NPY levels in the cerebrospinal fluid and serum are elevated in underweight amenorrheic women, and are returned to normal after long-term weight restoration in women who resumed normal menstrual cycling [126-128]. Starvation-induced alterations of neuropeptide activity probably contribute to neuroendocrine dysfunctions in anorexia nervosa. Kaye et al. [126] made the conclusion, that in girls with anorexia nervosa a disturbance of CNS corticotropin releasing hormone (CRH) activity is likely to be responsible for hypercortisolemia, while a disturbance of CNS NPY may contribute to amenorrhea [126]. In addition, disturbances of these neuropeptides could contribute to other symptoms such as increased physical activity, hypotension, reduced sexual interest, depression, and pathological feeding behavior [129].

Similarly, a role for NPY in the initiation of puberty is suggested by the observation that concentrations of NPY in girls with delayed puberty are higher than in girls matched for weight and body composition who exhibited normal pubertal development [130]. Higher concentrations of NPY in girls with constitutional delay of puberty (CDP) may be responsible for the disorder and reduced levels of IGF-I. Correlation of NPY with % body fat suggests an involvement of this neuropeptide in the process of fat accumulation associated with CDP [130].

Of great interest is to focus on the role of NPY in one of the most common endocrine-metabolic diseases, affecting up to 10% of women of reproductive age, the polycystic ovary syndrome (PCOS) [131, 132]. It is widely accepted that PCOS is a prototype of a sex specific metabolic syndrome [132-134]. Obesity is present in 30–70% of affected women depending on the setting of the study and the ethnical background of the subjects, and it is characterized by central distribution of fat [133, 135, 136]. In women with PCOS, hyperinsulinemia, dyslipidemia, and/or hypertension are highly dependent on obesity, which worsens all of the clinical manifestations of PCOS [133, 136-138]. At present there is an increasing body of evidence of high levels of atherogenic adipocytokines and low levels of adiponectin in women with PCOS that change according to variations of fat mass [139]. Endocrine function of the adipocytes is regulated mainly by nutritional status, and both these factors are complexly interweaved in the energy storing mechanism in the adipose tissue [140]. It is still not fully elucidated if there are consistent differences in the levels or in the effects of appetite-regulating hormones as is NPY in PCOS.

Manneras et al. [141] demonstrated an enhanced mesenteric (visceral) adipose tissue expression of NPY in a rat model of PCOS in comparison with normal rats. Exercise reduced adiposity and adipose NPY expression and additionally normalized ovarian cyclicity [141].

Women with PCOS may exhibit altered leptin sensitivity of the hypothalamic NPY neurons to leptin inhibition, and higher plasma NPY levels have been observed in women with PCOS compared to nonPCOS controls; this may perturb LHRH secretion [142]. Thus, Baranowska et al. [143] found elevated NPY levels in both lean and overweight women with anovulatory PCOS. The increase in NPY in their study was independent of the increase in BMI. In obese women with PCOS, plasma leptin was increased compared to lean women [143]. Bidzińska-Speichert et al. [144] also found higher leptin and NPY levels and lower galanin levels in PCOS women as compared to healthy controls [144]. These data are in conformity with observations from our recent on-going study where we found significantly higher NPY and leptin levels in obese insulin-resistant PCOS women as compared to nonPCOS weight matched women [Orbetzova, unpublished data]. It can be suggested that the feedback system in the interaction between leptin and NPY is disturbed in PCOS.

In contrast, Romualdi et al. [145] demonstrated that in basal conditions, obese PCOS women exhibited lower NPY levels than obese controls. Ghrelin injection markedly increased NPY in controls, whereas PCOS women showed a deeply blunted NPY response to the stimulus. Metformin treatment induced a significant decrease in insulin levels and the concomitant recovery of NPY secretory capacity in response to ghrelin in PCOS women. Leptin levels, which were similar in the two groups, were not modified by ghrelin injection; metformin did not affect this pattern. The authors conclude that hyperinsulinaemia seems to play a pivotal role in the alteration of NPY response to ghrelin in obese PCOS women. This derangement could be implicated in the pathophysiology of obesity in these patients [145]. The limitations of this very interesting study on the ghrelin–NPY relationship in PCOS is the small number of patients (seven obese, hyperinsulinaemic subjects with PCOS and seven obese control women) and the data need further purposeful investigation.

Interventions that influence reproductive and metabolic function in PCOS may also affect levels of the adipose tissue hormones and regulators of appetite, such as NPY. It has been postulated that some of the effects of insulin-sensitizing agents in PCOS may be mediated through changes in adipocytokines levels. Some authors demonstrated that treatment of women with PCOS with insulin-sensitising agents induces a reduction in serum leptin levels [146-149]. In this context our recent data from a study comprising of 2 groups of overweight insulin-resistant PCOS women [150] showed that a 3-month treatment with metformin (Group 1) and rosiglitazone (Group 2), added to an oral hormone contraceptive (OHC) (a standard combination of ethynil oestradiol 35 µg plus cyproterone acetate 2 mg) resulted in decrease of atherogenic adipocytokines (leptin, resistin, and TNFα) (Table 2) that may have beneficial effects in the future prevention of atherosclerosis and cardiovascular diseases in this risk cohort of young women. But the serum concentrations of NPY also decreased that is in support of some our previous [151] and other authors [143, 144] data for impaired NPY-leptin link in PCOS. The change of NPY and adipocytokines was associated with weight loss only in the metformin group that is an expected effect of the drug and in conformity with other studies [150].

Parameters \ Groups	Group 1 (n=32) Metformin + OHC		Group 2 (n= 34) Rosiglitazone +OHC	
	0 months	3 months	0 months	3 months
Leptin (ng/ml)	13.17±3.42	6.40±1.40**	15.54±4.49	7.17±1.59**
Resistin (ng/ml)	2.19±0.67	1.63±0.45**	2.61±0.79	1.68±0.41**
TNFα (pg/ml)	12.52±5.78	8.47±3.09***	13.45±4.30	9.60±4.16****
NPY (ng/ml)	4.51±1.18	3.64±0.46*	4.54±1.47	3.21±1.25***
Weight (kg)	78.24±20.14	75.50±18.66**	82.41±16.17	82.23±15.80
BMI (kg/m <sup>2</sup> )	28.45±4.38	27.45±3.73**	29.27±4.24	29.22±4.27
Waist (cm)	88.69±7.72	86.63±5.92*	88.97±8.05	88.24±7.63
Fats (%)	35.56±10.10	33.98±8.77	35.55±12.40	34.72±13.09
Fats (kg)	29.77±15.20	27.23±13.39*	32.30±17.86	31.33±17.94

\* - p<0.05 – vs basal; \*\* - p<0.01 – vs basal; \*\*\* - p=0.001 – vs basal; \*\*\*\* - p<0.001 – vs basal

**Table 2.** NPY, adipose tissue hormones, and some clinical characteristics of the groups before and after treatment [150]

Having in mind that the decrease in NPY and adipocytokines was not in parallel with changes in body weight and composition in the rosiglitazone group and was associated with only slight and non significant influence on hyperinsulinaemia, resp. insulin resistance, additional direct adipose tissue and/or disease specific effects of the treatment may come into consideration that needs further elucidation.

### 3. Ghrelin

Ghrelin was discovered by Kojima et al. [152] in rat stomach extracts in 1999. This peptide has been identified in many species, including mammals, avians, amphibians, reptiles, and

fish [153-159] and the sequence of first seven amino acids of the N-terminal region of ghrelin are highly conserved between species [160].

Ghrelin is an orexigenic factor released primarily from the oxyntic cells of the stomach, but also from duodenum, ileum, caecum and colon [161, 162]. Gastric ghrelin cells had been classified as X/A-like cells by their round, compact, electron-dense secretory granules that distinguish them electron-microscopically from other previously characterized gastric endocrine cell types before the discovery of ghrelin [161, 163]. Ghrelin has also been detected in many other organs, such as the bowel, pancreas, kidney, placenta, lymphatic tissue, gonads, thyroid, adrenal, lung, pituitary and hypothalamus, and in different human neoplastic tissues and related cancer cell lines, such as gastric and intestinal carcinoids, lymphomas and thyroid, breast, liver, lung and prostate carcinomas. Levels of ghrelin expression in these normal and tumoral tissues or cell lines are lower than in the stomach, and although the potential physiological role of ghrelin as an autocrine/paracrine factor in these tissues is still under investigation [164].

In mice, rats and humans, ghrelin is an acyl-peptide consisting of 28 amino acids, sharing a 36% structural resemblance to motilin [165]. A hydroxyl group of serine at position 3 of the ghrelin molecule is esterified with an octanoic acid. The esterification increases the hydrophobicity of the ghrelin molecule, and is essential for most of its biological activities [152,166-168]. An enzyme that catalyses the acyl-modification of ghrelin was discovered in 2008 by Yang et al. [169], was renamed ghrelin O-acyltransferase (GOAT). In vivo studies showed that GOAT gene disruption in mouse models completely abolished ghrelin acylation [170, 171]. GOAT inhibition leading to weight reduction and beneficial metabolic effects [172] is therefore a useful target for future development of therapeutic compounds for obesity and metabolic syndrome.

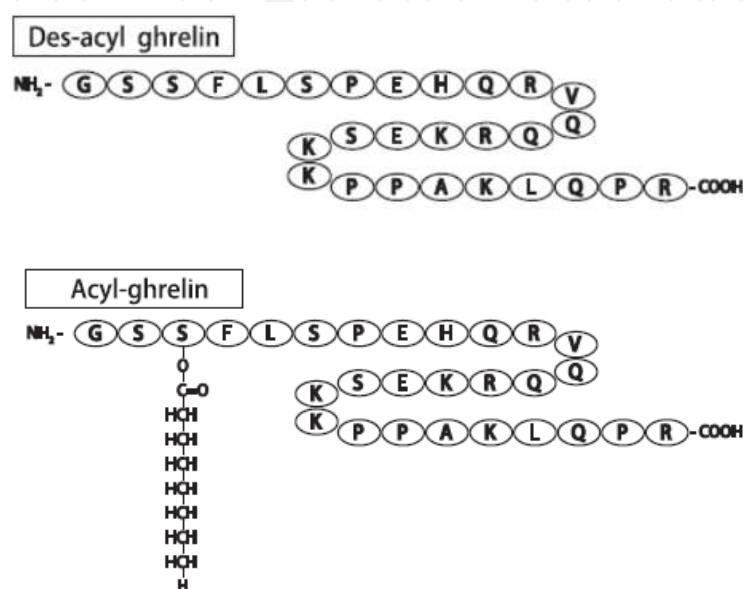
#### *Ghrelin receptor*

Ghrelin was discovered via its growth hormone releasing effect as an endogenous agonist of the GHS-R, that is still the only receptor so far described [152, 173, 174]. The GHS-R was first identified in 1996 as a seven transmembrane domain peptide totaling 366 amino acids. It is a G protein-coupled receptor (GPCR) that is linked to both Gq and Gs signaling pathways. It generates intracellular signaling through its Gα11 subunit, although the specific intracellular pathways elicited by this receptor are dependent on the tissue type in which it is expressed [175].

There are two splice variants - GHS-R type 1a that is the receptor to which ghrelin binds and through which it exerts its stimulatory effects on growth hormone release [152, 161, 176, 177], and GHS-R type 1b, which is a COOH-terminal truncated form of the type 1a receptor, and is physiologically inactive [178]. Ghrelin administration does not increase food intake in mice lacking GHS-R type 1a, suggesting that the orexigenic effects may be mediated by the above receptor; however, these mice have normal appetite and body composition [173, 179].

Ghrelin exists as two different molecular forms in both gastric ghrelin-producing cells and circulation: 1) acylated ghrelin (with the n-octanoic acid at the serine-3 position), which is

essential for activation of GHS-R1a and modulation of neuroendocrine and orexigenic effects; and 2) nonacylated ghrelin (des-acyl ghrelin), which is the most abundant form in the stomach and circulation but is unable to activate GHS-R1a, and to exhibit further GH-releasing activity [180] (Figure 3). Nonetheless, food intake is induced by des-acyl ghrelin, administered by icv injection, to the same extent as ghrelin [181]. Nonacylated ghrelin exerts some cardiovascular and antiproliferative actions. Because the genome database does not contain another GPCR that resembles GHS-R, probably des-acyl ghrelin acts by binding different GHS-R subtypes or as yet unidentified receptor families [178, 182].



**Figure 3.** Structure of nonacylated and acylated ghrelin

GHS-R1a is widely distributed in the body with high expression levels in the hypothalamus and in all three components of the dorsal vagal complex, including the area postrema, the nucleus of the solitary tract (NTS), the dorsal motor nucleus of the vagus and parasympathetic preganglionic neurons [183]. Low expression is detected in other brain areas and in numerous other tissues including the myocardium, stomach, small intestine, pancreas, colon, adipose tissue, liver, kidney, lung, placenta and peripheral T-cells [152, 161, 182, 184-187].

The ghrelin receptor is well conserved across all vertebrate species examined, including a number of mammals, bird and fish. This strict conservation suggests that ghrelin and its receptor serve essential physiological functions [188]. Some studies have also described ghrelin analogues which show dissociation between the feeding effects and stimulation of GH, suggesting that GHS-R type 1a may not be the only receptor mediating the effects of ghrelin on food intake [189].

The gene encoding ghrelin also encodes another peptide, called *obestatin*. The administration of obestatin reduces food intake and weight gain in rats via activation of GPR3, an orphan G-protein coupled receptor [190, 191]. Therefore, one gene produces two products with opposing metabolic effects, which are exercised through different receptors [192].

*Ghrelin as a member of the 'gut-brain axis'*

The human body is endowed with a complex physiological system that maintains relatively constant body weight and fat stores despite the wide variations in daily energy intake and energy expenditure. With weight loss, compensatory physiological adaptations result in increased hunger and decreased energy expenditure, while opposite responses are triggered when body weight increases. This regulatory system is formed by multiple interactions between the gastrointestinal tract (GIT), adipose tissue, and the CNS and is influenced by behavioural, sensorial, autonomic, nutritional, and endocrine mechanisms [2, 3].

The hypothalamus (particularly the ARC) and the brain (particularly the NTS) are the main sites of convergence and integration of the central and peripheral signals that regulate food intake and energy expenditure [193, 194]. There are mechanisms of short-term regulation (satiety signals) which determine the beginning and the end of a meal (hunger and satiation) and the interval between meals (satiety) [195], and long-term regulatory factors (signals of adiposity) which help the body to regulate energy depots. Thus, meal-generated satiety signals from the GIT do interact with longer-term adiposity signals, such as insulin and leptin in maintaining energy balance. Satiety signals from the GIT are transmitted primarily through vagal and spinal nerves to the NTS. There is, however, a large integration and convergence of these signals by neural connections between the ARC nucleus, NTS, and vagal afferent fibres. The nervous system, in turn, influences gastric and pancreatic exocrine secretion, gastrointestinal motility, blood supply, and secretion of gut hormones [191].

The GIT contributes with several peptides that have incretin-, hunger-, and satiety-stimulating actions, such as ghrelin, glucagon-like peptide 1 (GLP-1), peptide YY (PYY), oxyntomodulin (OXM), and cholecystikinin (CCK) and that are considered as members of the 'gut-brain axis'. Many of the GIT hormones that affect food intake are also synthesized in the brain, such as CCK, GLP-1, apolipoprotein A-IV, gastrin-releasing peptide, PYY, and ghrelin. Generally, peptides that reduce (or increase) food intake when administered systemically usually have the same action when administered centrally. This has been demonstrated for CCK, GLP-1, apolipoprotein A-IV, gastrin-releasing peptide, neuromedin B, and ghrelin [5, 6, 191].

Ghrelin is expressed in a group of neurons adjacent to the third ventricle, between the DMN, VMN, PVN and ARC. These neurons terminate on NPY/AgRP, POMC and corticotrophin-releasing hormone neurons, and are able to stimulate the activity of ARC NPY neurons, forming a central circuit which could mediate energy homeostasis [17]. The central ghrelin neurons also terminate on orexin-containing neurons within the LHA [18], and icv administration of ghrelin stimulates orexin-expressing neurons [18, 196]. The feeding response to centrally administered ghrelin is attenuated after administration of anti-orexin antibody and in orexin-null mice [18].

Ghrelin reaches the hypothalamus through the circulation, and the brain stem through vagal innervation. The integrity of the vagus nerve is crucial for ghrelin effects since vagotomy prevents its orexigenic effect in animal models and humans. Ghrelin is thought to exert its orexigenic action via the ARC in a pattern representing a functional antagonism to leptin. c-

Fos expression increases within ARC NPY-synthesizing neurons after peripheral administration of ghrelin [197], and ghrelin fails to increase food intake following ablation of the ARC [198]. Studies of knockout mice demonstrate that both NPY and AgRP signalling mediate the effect of ghrelin, although neither neuropeptide is obligatory [179]. GHS-R are also found on the vagus nerve [185], and administration of ghrelin leads to c-Fos expression in the area postrema and NTS [196, 199], suggesting that the brainstem may also participate in ghrelin signalling. The orexigenic action of ghrelin occurs independently of its stimulatory effects on GH secretion [176, 198, 200]. It is more likely that the physiological role of ghrelin is to prepare the body for an influx of metabolic energy [201-203].

Administration of ghrelin, either centrally or peripherally, increases food intake and body weight and decreases fat utilization in rodents [176, 204]. Furthermore, central infusion of anti-ghrelin antibodies in rodents inhibits the normal feeding response after a period of fasting, suggesting that ghrelin is an endogenous regulator of food intake [199]. Human subjects who receive ghrelin intravenously demonstrate a potent increase in food intake of 28% [205], and rising pre-prandial levels correlate with hunger scores in humans initiating meals spontaneously [202]. Chronic administration increases body weight, not only by stimulating food intake, but also by decreasing energy expenditure and fat catabolism [165, 176, 199].

In summary, the orexigenic effect of hypothalamic ghrelin is regulated through a neuronal network involving food intake. Fasting results in increased release of ghrelin from the stomach (the exact mechanism of this remains obscure) leading to increased plasma ghrelin levels, which reach the hypothalamus either via the blood stream directly in areas with no blood-brain barrier, or by crossing the blood-brain barrier via a saturable transport system or via the vagus nerve and the NTS [206]. Ghrelin's effect on appetite is mediated by an effect both on the hypothalamus and the NTS. To stimulate the release of the orexigenic peptides, ghrelin-containing neurons send efferent fibers onto NPY/AgRP-expressing neurons. On the other hand, to suppress the release of the anorexigenic peptide, ghrelin-containing neurons send efferent fibers onto POMC neurons [17]. Leptin directly inhibits appetite-stimulating effects of NPY and AgRP, whereas hypothalamic ghrelin augments NPY gene expression and blocked leptin-induced feeding reduction. Thus, ghrelin and leptin have a competitive interaction in feeding regulation [188].

#### *Regulation of ghrelin secretion*

Serum ghrelin concentrations vary widely throughout the day. The most known factor for the regulation of ghrelin secretion is feeding [201] - ghrelin decreases after food intake, and increases when fasting with higher values during the night sleep [180, 201, 207]. In people on a fixed feeding schedule, circulating ghrelin levels are thought to be regulated by both calorie intake and circulating nutritional signals [162, 176]. Thus, blood glucose levels may play an important role in the regulation of ghrelin secretion: oral or intravenous administration of glucose decreases plasma ghrelin concentration [208]. Ghrelin levels fall in response to the ingestion of food, but not following gastric distension by water intake suggesting that mechanical distension of the stomach alone clearly does not induce ghrelin

release [176, 209, 210]. In healthy subjects, a longer fasting period during the day (i.e. irregular meal pattern typical for several eating disorders) increases ghrelin concentration, but does not affect postprandial ghrelin response to a mixed meal [211]. The described pattern of secretion raised the concept of ghrelin as a hunger hormone, responsible for meal initiation. However, one study has failed to show a correlation between the ghrelin level and the spontaneous initiation of a meal in humans [212], and an alteration of feeding schedule in sheep has been shown to modify the timing of ghrelin peaks [213]. Recently Schüssler et al. showed that ghrelin levels increased significantly during a 30-min. interval following a presentation of pictures with food in healthy volunteers and suggested that in addition the sight of food can elevate ghrelin levels [214].

The most remarkable inhibitory input on ghrelin secretion is represented by the activation of somatostatin (SS) receptors as indicated by evidence that native SS, its natural analog cortistatin, and a synthetic analog such as octreotide lower circulating ghrelin levels in humans [215]. Ghrelin secretion in humans is under the stimulatory control of the cholinergic, namely muscarinic receptors, and acetylcholine is the first stimulatory neurotransmitter shown to play a stimulatory role on ghrelin secretion in humans [216].

In rats, ghrelin shows a bimodal peak, which occurs at the end of the light and dark periods [217]. In humans, ghrelin levels vary diurnally in phase with leptin, which is high in the morning and low at night [201].

It should be considered that ghrelin secretion may be a conditioned response which occurs to prepare the metabolism for an influx of calories. But, whatever the precise physiological role of ghrelin, it appears not to be an essential regulator of food intake, as ghrelin-null animals do not have significantly altered body weight or food intake on a normal diet [218].

#### *Relationship between ghrelin and glucose-insulin homeostasis*

Current extensive study data of ghrelin's role in metabolic processes indicate its unambiguous relation with control on glucose homeostasis and  $\beta$ -cell function. Both GHS-R1a and GHS-R1b are present in animal and human endocrine pancreas [219, 220]. Ghrelin is also present in pancreas, and epsilon pancreatic cells have been suggested to be a putative ghrelin-expressing cell type [221]. Moreover, a specific receptor able to bind both acylated and nonacylated ghrelin has also been demonstrated within the human pancreas; this is therefore a non-GHS-R1a [168, 222]. Ongoing studies support the hypothesis that ghrelin, independently of its acylation, modulates glucose metabolism at the hepatic level [223].

Exogenous ghrelin *short-term effects* induce hyperglycaemia in experimental rodents via an GH-independent mechanism of action [224]. In contrast, ghrelin-receptor antagonists may improve glucose tolerance in rats, with no weight gain due to increased insulin secretion [225]. Acute administration of ghrelin to humans increases plasma glucose levels and amplifies the hyperglycaemic effect of arginine [226]. This hyperglycaemic effect might result from the endocrine effects of ghrelin as well as from direct effects on hepatocytes in which it modulates glycogen synthesis and gluconeogenesis [227]. Although data of ghrelin *long-term effects* are insufficiently clarified, a tendency of an increase in plasma glucose levels

appears to be presented [224]. Many of the studies in patients with type 1 diabetes show low ghrelin levels, probably as a manifestation of a compensatory mechanism against hyperglycaemia [225].

Numerous studies indicate a negative association between systemic ghrelin and insulin levels [184, 228]. Thus, ghrelin is found to inhibit insulin secretion both *in vitro* and *in vivo* in most human and animal studies [226, 229]. In humans the acute administration of ghrelin inhibits spontaneous and arginine-stimulated insulin secretion but does not affect the insulin response to the oral glucose tolerance test (OGTT) [226, 230, 231]. In addition, the regulation of insulin secretion by ghrelin is closely related to the blood glucose level. Date et al. [232] reported that ghrelin stimulates insulin release in the presence of high levels of glucose (8.3mM) that could independently cause insulin release from cultured islet cells. In contrast, ghrelin had no effect on insulin release in the context of a basal level of glucose (2.8 mM) [232]. Antagonism of the pancreatic ghrelin can enhance insulin release to meet increased demand for insulin in high-fat diet-induced obesity of mice [233].

Ghrelin might influence some of the peripheral effects of insulin. Thus, it is found to stimulate hepatic glucose production [227], reinforce the action of insulin on glucose disposal in mice [234], inhibit adiponectin secretion [235] and stimulate secretion of the counter-regulatory hormones, including GH, cortisol, adrenaline [236] and possibly glucagon [237]. In healthy subjects, in the absence of GH and cortisol secretion, ghrelin acutely decreased peripheral, but not hepatic, insulin sensitivity together with stimulation of lipolysis. These effects occurred without detectable suppression of AMP-activated protein kinase phosphorylation (an alleged second messenger for ghrelin) in skeletal muscle [238]. So, ghrelin also exerts direct metabolic effects towards induction of insulin resistance independent of the regulation by counter-regulatory hormones.

Insulin in turn decreases ghrelin levels, regardless of changes in glucose concentrations [239]. Broglio et al. [240] have found that both oral and intravenous insulin suppress ghrelin, although they exhibit opposite effects on glucose levels. The same authors have shown that protein-induced inhibition of ghrelin is enabled by oral administration, while intravenous arginine does not lead to ghrelin reduction regardless of insulin elevations, which is a fact of interest and of relation to protein diets [240].

Given all the above data, it is proposed that ghrelin could have an important function in glucose homeostasis and insulin release, independent of GH secretion [241]. Data of administration of GHSR1a antagonists suggest that these compounds improve long-term glucose tolerance and insulin resistance. Since there are some differences about the role of ghrelin on insulin secretion [188], further research on ghrelin-insulin interrelationship is expected. At least theoretically, ghrelin and/or its signalling manipulation could be used for the treatment or prevention of diseases of glucose homeostasis.

#### *Ghrelin in obesity, diabetes mellitus and metabolic syndrome*

In addition to a probable role in meal initiation, ghrelin seems to be an adiposity-related hormone that is involved in the long-term regulation of body weight. Plasma ghrelin levels are inversely correlated with body mass index and current evidence strongly suggests that

ghrelin could contribute to obesity and the metabolic syndrome [225]. Variations within the ghrelin gene may contribute to early-onset obesity [242, 243] or be protective against fat accumulation [225], but the role of ghrelin polymorphisms in the control of body weight continues to be controversial [244, 245].

It has been shown that ghrelin secretion differs between lean and obese subjects. Thus, plasma ghrelin concentration is found to be low in obese people and high in lean people in some studies [207, 208, 246]. The expression of ghrelin receptors in the hypothalamus increases markedly with either fasting or chronic food restriction [247], as does the hypothalamic response to a ghrelin-receptor agonist [248], which is consistent with a feed-forward loop that enhances ghrelin-mediated stimulation of appetite during energy deficit. Anorexic individuals have high circulating ghrelin which falls to normal levels after weight gain [249]. The suppressed plasma ghrelin levels in obese subjects normalize after diet-induced weight loss [250]. The postprandial falls of serum ghrelin concentrations are proportional to energy intake in lean subjects, but not in obese subjects. Unlike lean individuals, obese subjects do not demonstrate the same rapid post-prandial drop in ghrelin levels [251]. Moreover, obesity is associated with much lower overall reduction of postprandial ghrelin levels and an absence of nocturnal elevations as seen in subjects of normal weight [194, 195, 210]. This may result in increased food intake and contribute to obesity. The fall in plasma ghrelin concentration after bariatric surgery, despite weight loss, is thought to be partly responsible for the suppression of appetite and weight loss seen after these operations [252].

The severe hyperphagia seen in Prader–Willi syndrome is associated with elevated ghrelin levels [253] that is in contrast to other forms of obesity, and it has been hypothesized that ghrelin might contribute to the nature of this syndrome. Moreover, there are similarities between the clinical features of Prader–Willi syndrome and those predicted from overstimulation of NPY by ghrelin (e.g. hyperphagic obesity, hypogonadotropic hypogonadism and dysregulation of GH) and the correlation between ghrelin levels and hyperphagia and excessive obesity, in these patients [254]. Indeed, the high ghrelin levels in obese people with Prader–Willi syndrome make the carriers of the syndrome logical first-line candidates for testing the weight reducing effects of ghrelin-blocking agents

Recently, the role of ghrelin in diabetes mellitus has been investigated: polymorphisms of the ghrelin gene are associated with the risk of diabetes [255], ghrelin promotes regeneration of b-cells in streptozocin-treated newborn rats, preventing the development of diabetes in disease-prone animals after b-cell destruction [256], and ghrelin antagonists partially reverse hyperphagia in uncontrolled, streptozocin-diabetic rats [257]. It has been found that fasting ghrelin concentrations are lower in people with type 2 diabetes mellitus than in non-diabetic people, even after adjusting for BMI. It has also been shown that the decrease in circulating ghrelin is proportionate to the degree of insulin insensitivity. We also found significant negative correlation between ghrelin and fasting insulin, and HOMA-index, respectively, in insulin resistant women with type 2 diabetes mellitus [258]. These observations suggest that ghrelin and insulin sensitivity are linked. All the data indicate that ghrelin might have a role in the pathogenesis and therapy of diabetes, contributing to either the impairment of insulin

sensitivity or to the restraint of body-mass gain. Nonetheless, because of the controversy about the cause-and-effect relationship between ghrelin levels and diabetes mellitus, further investigations are needed to elucidate the precise role of ghrelin (and its variants) in the development and treatment of this disease.

Low plasma ghrelin levels are associated with metabolic cluster per se, which indicates that ghrelin might be a useful biomarker for the metabolic syndrome [228, 259]. Thus, conditions of severe metabolic syndrome due to insulin resistance, such as in obese Pima Indians, are related with reduced fasting ghrelin plasma levels [207]. In a study on the relation between metabolic parameters, ghrelin, leptin and IGF-1 in a cohort of 1,045 individuals, Ukkola et al. [260] have found that low ghrelin levels are associated with metabolic syndrome and type 2 diabetes mellitus only in presence of insulin and leptin resistance. At high leptin levels, ghrelin concentrations decrease linearly with increasing the number of metabolic syndrome components [260]. In patients on haemodialysis, fasting ghrelin levels negatively correlate with metabolic syndrome manifestation, ghrelin shows a tendency to decrease with increasing the number of the metabolic syndrome components, and the waist circumference appears to be an independent predictor of its levels [261].

In patients with the metabolic syndrome and low ghrelin levels, intraarterial administration of ghrelin rapidly improves endothelial function [262]. Similar to insulin, ghrelin stimulates an increased nitrogen oxide (NO) production in cultured bovine aortic endothelial cells in a dose- and time-dependent manner. It has been found that ghrelin-induced NO production in human aortic endothelial cells is arrested by their pre-treatment with a NO-synthase inhibitor, phosphatidylinositol synthase (PI 3)-kinase inhibitor, selective GHSR-1a antagonist or “exclusion” of these receptors. On the other hand, ghrelin has been found to stimulate enhanced phosphorylation of Akt (Ser<sup>473</sup>) and endothelial NO-synthase in human aortic endothelial cells, as well as phosphorylation of mitogen-activated protein (MAP) kinase, but not of MAP-kinase-dependent production of the vasoconstrictor endothelin-1 in bovine aortic endothelial cells. With regard to these data it may be concluded that ghrelin exhibits characteristic, rapid vascular effects, presented as stimulated NO production in the endothelium via signal pathways including the GHSR-1a, PI 3-kinase, Akt and endothelial NO-synthase, which may be taken into consideration for the development of innovative therapeutic strategies for endothelial dysfunction in diabetes and insulin resistance [262].

Vlasova et al. have found that peripheral injection of a ghrelin antagonist in experimental animals (rats) increases arterial pressure and pulse rate via at least partial activation of the sympathetic nervous system [263]. These findings direct our attention to eventual cardiovascular adverse effects, when administering ghrelin antagonists as a therapeutic strategy for reducing food intake, particularly in patients at a high cardiovascular (CV) risk (e.g., patients with metabolic syndrome).

#### *Ghrelin's role in processes of reproduction and PCOS*

Presently not so much is known of ghrelin effects on processes of reproduction. Experimental models in rats have shown that ghrelin plays a role at different levels of the hypothalamic-pituitary-ovary axis regulation. Its central route of administration in female

rats results in suppression of the LH secretion at various stages of estrus [264]. In *in vitro* settings, ghrelin also inhibits gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus [264]. At a pituitary level, ghrelin exhibits either stimulating or inhibiting action on basal LH secretion, depending on the menstrual cycle stage. However, the *in vitro* GnRH-stimulated LH release is inhibited by ghrelin, regardless of the steroid medium [265]. In rhesus monkeys, the confirmed inhibitory effect of ghrelin on the GnRH-LH system suggests that in primates, ghrelin exhibits a central regulatory effect on processes of reproduction [266].

It was shown by Kluge et al. that ghrelin suppresses the secretion of LH and FSH in healthy women [267]. Ghrelin levels have been found to be higher in anovulatory women with excessive physical loading-induced anorexia nervosa and amenorrhea, as well as in normal weight-women with hypothalamic amenorrhea [268-270]. In normal-weight women with amenorrhea, the increased ghrelin levels have been associated with disturbed dietary habits and regimen [271]. It is not clear whether disturbances in ghrelin secretion play a direct role in neuroendocrine regulation of the hypothalamic-pituitary-ovary axis or present a marker of the metabolic status itself.

In males, ghrelin has an additional inhibitory role, decreasing human chorionic gonadotropin (hCG)- and cAMP-stimulated testosterone secretion [272] and the expression of the gene encoding stem cell factor that is a key mediator of spermatogenesis and a putative regulator of Leydig-cell development [273]. In hypogonadal males, a positive correlation between ghrelin and androgens persists after testosterone replacement therapy [274].

There is no consensus on whether alterations in levels of appetite-regulating hormones, such as ghrelin, are associated with PCOS. Fasting ghrelin levels were found decreased in most [275-279], but not in all studies [280, 281] in women with PCOS. Thus in 2002, Pagotto et al. [275] first demonstrated that ghrelin levels were lower in obese women with PCOS, compared with these in weight-matched healthy controls. Ghrelin has been inversely correlated with insulin resistance markers. These correlations have persisted even after therapy (hypocaloric diet plus metformin or placebo) for improving insulin sensitivity. In both groups, weight reduction has resulted in minimal changes of plasma ghrelin levels. The observed negative correlation between ghrelin and androstenedione, but not between ghrelin and testosterone or other androgens, is interesting [275]. In PCOS, Schofl et al. have confirmed lower ghrelin levels that are in a close correlation with insulin resistance rates [276]. After therapy with metformin in insulin-resistant women, ghrelin levels have increased, but in insulin-sensitive women with PCOS, ghrelin levels have been comparable to these in controls. Furthermore, the authors have found no correlation between ghrelin and the body mass index (BMI) [276], which suggests a ghrelin-insulin resistance interrelation apart from ghrelin activity in controlling appetite, body weight, respectively. Panidis et al. [282] have reported that women with PCOS and hyperandrogenaemia have significantly lower ghrelin levels compared to healthy controls and PCOS carriers with clinical hyperandrogenism but normal androgen levels. Ghrelin levels in the latter are lower than these found in the control group, but the differences are not statistically significant. The

authors have concluded that PCOS-associated hyperandrogenaemia results in reduced ghrelin concentrations [282]. Although PCOS-associated hyperandrogenaemia and 17-OH-progesterone levels are inversely related to ghrelin levels, anovulation and polycystic ovary morphology are associated with higher ghrelin concentrations [283]. Thus, it has been hypothesized that different clinical and biochemical manifestations of the syndrome might be associated with different concentrations of ghrelin.

In support of the relation between PCOS and ghrelin, there are data of increased ghrelin levels after a 3-month treatment with an oral contraceptive containing both ethinyl oestradiol and drospirenone in women with PCOS [284]. Similar to other studies, ghrelin is negatively correlated with the BMI, waist/hip ratio, insulin, homeostatic model assessment (HOMA) index and free testosterone [284]. According to a study conducted by Fusco et al., ghrelin administration in normal weight-women and obese PCOS patients has exerted glucose-enhancing and insulin-lowering effects, the latter absent in the normal weight-controls [285], which supports the relation between ghrelin and hormonal/metabolic disorders in PCOS.

One of studies that have not confirmed changes in ghrelin levels in women with PCOS is this conducted by Orio et al. [280]. The authors have found no correlation between ghrelin and either of the hormonal or biochemical parameters (including insulin and insulin resistance markers), but only a correlation between ghrelin and the BMI [280]. These data support the relation determined between ghrelin and the body weight only and exclude the effects of the disease itself. These findings are in a sense similar to those observed by Bik et al., who have not found significant differences in ghrelin levels between a group of normal weight PCOS women and a group of normal weight healthy women; however, ghrelin was significantly lower in healthy obese women compared to lean women with PCOS [286].

Impaired ghrelin suppression after a test meal and increased feeling of hunger and decreased feeling of satiety (according to visual analogue scales) have been described in a small group of obese women with PCOS, even after weight reduction [287], which has been also confirmed by another study, comparing lean and obese women with PCOS and relevant, weight-matched controls [288]. Romualdi et al. [289] gave more detailed evaluation of ghrelin and polypeptide YY responses following oral load with a test meal (527 kcal, distributed by contents in 24.1% fats, 54.4% carbohydrates and 21.5% proteins) in women with PCOS. Low baseline ghrelin levels and reduced suppression after meals, more pronounced in the obese than in the lean patients was shown. The authors have found no correlation between ghrelin and the androgens; however, a negative correlation has been established between ghrelin and the HOMA-index. Compared to controls, PCOS women had a significantly suppressed neuropeptide Y response to injected ghrelin, as the response has restored after treatment with metformin and significant insulin reduction. In this experimental setting, leptin has undergone no significant changes [289]. Obviously, hyperinsulinaemia is the factor which exerts effect on the ghrelin-neuropeptide Y relation.

We found significantly lower ghrelin levels in women with PCOS compared to healthy controls ( $21.78 \pm 2.12$  ng/ml versus  $34.67 \pm 3.57$  ng/ml;  $p = 0.04$ ), as ghrelin was inversely correlated with insulin levels at a degree similar to these of insulin resistance markers.

Negative correlations were also found with the BMI, waist measurement and waist-to-hip ratio, in conformity with most of the studies at present. Furthermore, the observed negative correlation between ghrelin and testosterone ( $r = -0.315$ ;  $p < 0.05$ ), and this between ghrelin and leptin ( $r = -0.306$ ;  $p < 0.05$ ) disappear after the exclusion of BMI, waist-to-hip ratio and HOMA-index [290]. In a comparative study on ghrelin levels in insulin-resistant women with PCOS and women with type 2 diabetes and higher insulin resistance, ghrelin levels have been significantly lower in syndrome carriers versus diabetics [291]. Based on our data we consider that ghrelin levels in women with PCOS reflect both the metabolic and hormonal disturbances, typical for the syndrome.

A recent study conducted by Panidis et al. [292] has confirmed that alterations in ghrelin secretion are intrinsic for the disease itself and has demonstrated that active (acetylated) ghrelin/total ghrelin ratio is decreased in normal weight-women with PCOS, as the anomalies are most pronounced in the severe forms of the syndrome, including all diagnostic criteria: hyperandrogenia, chronic anovulation and morphologically polycystic ovaries [292]. Based on the proven changes in women with PCOS and various phenotype manifestations, some authors have even suggested ghrelin to be used as a predictive marker of PCOS and have found through a plot-analysis of the receiver operating characteristic curve (ROC) a sensitivity of 70% and specificity of 86% with a cut-off value of 34.1 ng/ml, below which the diagnosis of PCOS is likely, while a cut-off ghrelin value below 9 ng/ml is highly specific for PCOS [293].

In conclusion, there is, probably, an anomaly in ghrelin regulation in PCOS, related not only to overweight and insulin resistance. The mechanisms associating abnormal ghrelin regulation with the disease are still to be elucidated. However, the pathological and therapeutic importance of this association is unclear. Independent effects of ghrelin on the hypothalamic-pituitary-gonadal axis with an inhibitory effect on the LH secretion and a decreased LH response to GnRH, typical for the syndrome, may also be taken into consideration.

#### 4. Conclusion

Given the growing epidemic of obesity, it has become increasingly important to understand the physiological processes that regulate body weight. Regulation of food intake and metabolism is maintained by complex pathways and neuronal circuits which themselves receive peripheral signals such as gut hormones. Metabolically important abdominal obesity with an excess of visceral fat accumulation results in altered release of adipokines, leading to CNS mediated skeletal muscle and hepatic insulin resistance. The central regulation of energy balance has become even more fascinating and complex with the characterization of mechanisms of action of NPY, the most abundant hypothalamic orexigenic factor. Much attention has recently centered on ghrelin, the only known circulating orexigen. Insulin resistance and compensatory hyperinsulinemia are independently associated with suppression of ghrelin that furthers our understanding of the variable expression of ghrelin in humans.

With continued research, it should be possible to elucidate exactly how the associations among insulin resistance, hyperinsulinemia, and orexigens (NPY and ghrelin) participate in the more intricate web of factors that regulate body weight. Better understanding of the mechanisms involved in the regulation of energy metabolism will become a background for development of new therapeutic approaches against obesity, insulin resistance, metabolic syndrome, and other nutritional disorders.

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