Ghrelin influence on metabolism and fertility

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Abstract
Ghrelin is a 28 amino-acid orexigenic hormone originally identified in the stomach. The effect of ghrelin on hunger is most probably indirect and exerted through its interaction with blood glucose level and insulinemia shortly after consumption and just prior to the next meal. Apart from its stimulating effect on growth (GH), ghrelin stimulates the secretion of prolactin (PRL), adrenocorticotropin hormone (ACTH) and cortisol. Its effect on luteinizing hormone (LH) secretion is not unequivocal. The tests carried out on animals and humans so far are inconsistent and it cannot be unequivocally stated whether ghrelin’s activity is stimulating or inhibitory. It has been suggested that ghrelin decreases LH pulse frequency. In this mechanism ghrelin may play a significant physiological role in reproductive function. In patients with polycystic ovary syndrome (PCOS) a lower serum concentration of ghrelin was noted in comparison to healthy women, however the reports are not consistent. Ghrelin is a hormone, that is strictly related to energy balance, obesity, insulin resistance and most probably to the reproductive function. As PCOS predisposes to obesity and metabolic changes such as insulin resistance it may be assumed that ghrelin’s activity is linked with pathogenesis of polycystic ovary syndrome. Role of ghrelin in reproductive functions is very interesting and requires further research studies.

Key words: Ghrelin, PCOS, fertility

Introduction
Ghrelin is an orexigenic hormone which was originally identified in the stomach. It is a 28-amino-acid peptide that constitutes an endogenic ligand for the growth hormone secretagogue receptor (GHS-R) for substances provoking growth hormone secretion (GHSs) [1].

The discovery of this endogenic ligand, a natural hormone acting through GHS-R, was made by a group of Japanese researchers and the results of their first research work were published in “Nature” in December 1999 [2].

Octaacylation of ghrelin molecule residing at 3rd position turned out to be a basis of a new structure activating polypeptides. It contains n-octanoyl modification on the serine 3 residue which is significant for the stimulation of growth hormone (GH) release induced by ghrelin, whereas des-acyl ghrelin in des-n-octanoyl form is biologically inactive. This presents new possibilities for the synthesis of biologically active peptides and peptidomimetics. The biological effect of ghrelin and GHS was confirmed in the year 2000 by Bowers, who discovered GHS and proved that the compound worked out by Kojima et al. is a long sought new hormone inducing the release of growth hormone [3].

Human ghrelin gene was described and identified at chromosome 3 (3p25-26). Ghrelin is subject to other mechanisms of negative feedback than GHRH and its activity is synergic with and complementary to this neurohormone. 3Ghrelin is activated by plasma proteases and tissue esterases. Its half-life period is 30 minutes. The expression of this peptide has been described mainly in the cells of the true endocrine glands of the stomach. However, it has been proven that after gastrectomy the peptide secretion decreases by 65%. This indicates, that it has to be released also by other organs cells. Ghrelin is released from the cells of small and large intestine, hypothalamic arcuate nucleus, pituitary gland, kidneys and placenta [4]. It has also been found in testes, ovaries, thyroid gland, pancreas [5]. Human ghrelin is homologous to rat ghrelin apart from two amino acids. Ghrelin which is produced and released in the stomach has different regulatory effects in the brain and periphery of the body. By peripheral or central administration to rodents ghrelin induces fast increase of food intake and body weight gain. Moreover, it increases motor activity and secretion activity of stomach acid secretion [6].

The closest homologue of the ghrelin receptor is the receptor for motilin which is released in similar locations
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Stimulating effect
Inhibitory effect

Leptin
Arcuate nucleus
Periventricular nucleus
Ghrelin
Stomach
Adipose tissue
MCH, TRH, CRH
NPY
POMC
uMSH

and also affects the autonomous nervous system. In rodents the ghrelin level in the blood rises when starving and declines after feeding or inserting food into the stomach. Water does not have such an effect [7].

In the serum of an adult there is 100-260 pmol/ml of ghrelin. This means that in the stomach ghrelin is not released into the gastrointestinal tract, like other enzymes, but into the blood vessels [5].

The content of ghrelin in the central nervous system is low. By means of immunohistochemical analysis it has been found that nerve cells containing ghrelin receptors are located only in hypothalamic arcuate nucleus. This area is responsible for regulating appetite which may prove that ghrelin participates in the regulation of this process. Moreover, neurons secreting gonadotropin-releasing hormone (GnRH) are located in hypothalamic arcuate nucleus [8].

The most potent impulse provoking the secretion of ghrelin is food deprivation. During food deprivation ghrelin secretion takes place mainly within stomach which leads to the feeling of hunger in the brain. Earlier tests showed that elevated level of the hormone intensifies the pleasure felt when taking cocaine or drinking alcohol [4].

It has been experimentally checked if mice that ate to their fill would choose the room in which they earlier found fatty food or the room where they had ordinary food available. It turned out that rodents did not have any preferences. However, when they had ghrelin injected they were choosing the room associated with fatty food, despite the fact that it was completely empty. Ghrelin stimulated mice to search for fatty food because they remembered how great pleasure it caused. They associated the room where the food was placed with something pleasurable even when it was already empty. Researchers noted that animals deprived of food in which the activity of ghrelin was blocked did not spend as much time in the place where they had been given fatty food. In other tests it was verified how long mice are able to wait in order to get fatty food. It turned out that the animals that did not get ghrelin resigned much quicker than the ones that had the hormone injected. These observations indicate that ghrelin intensifies the feeling of pleasure connected with tasty food. It is because of the effect of this hormone that after eating a full dinner we still have a craving for different tidbits e.g. a high calorie dessert [9]. Ghrelin affects the so called reward system in the brain. It is responsible for the feeling of pleasure and becoming addicted to things that cause pleasure. That is why ghrelin is a powerful motivating factor which conditions the achievement of the thing that will cause pleasure and constitutes memorization of the way to achieve the goal. Ghrelin stimulates food intake and affects energy balance in the body (Fig. 1)[10].

Through numerous interactions with neurotransmitting system in hypothalamic arcuate nucleus it affects the regulation of appetite and broadly defined hormone management in the body. Ghrelin induces increase in the production of neuropeptide Y (NPY) and agouti-related peptide (AgRP) by hypothalamic arcuate nucleus which subsequently leads to increased production of orexin and melanin-concentrating hormone (MCH) in the hypothalamus. It is also known, that orexin and MCH transmit the signal about increased energy demand of the organism in the hypothalamus. Ghrelin inhibits the formation of alpha melanocyte-stimulating hormone (αMSH) and this in turn inhibits the secretion of corticoleiberin (CRH), which results in storing adipose tissue [10].

The rise in ghrelin concentration was noted before a meal and the drop was noted after a meal. In patients with anorexia and in people who have fasted for a short period of time elevated ghrelin concentration was observed, whereas in obese people ghrelin concentration was lowered [11]. In experimental tests it has been observed that prompt administration of ghrelin stimulates food intake in many animal species as well as in people, whereas lengthy administration results in body mass growth not only because of constant stimulation of appetite but also because of decreased lipid catabolism and
energy consumption [12]. Blocking the transmission of signal by ghrelin, by means of genetic techniques, using the receptors antagonists for ghrelin and anti-ghrelin antibodies, resulted in the decrease in food consumption and in the drop of body mass. Low body mass and loss of body mass are related to the elevated concentration of ghrelin, whereas high body mass and its increase are related to concentration decrease [13]. These observations suggest that ghrelin transmits the signal about the organism’s state of nutrition to the hypothalamus [14]. Prader-Willi syndrome is also characterized by high fasting levels of ghrelin; here the ghrelin levels are associated with high food intake.

Ghrelin regulates broadly defined energy balance and many other processes taking place in the human body. Its effect on appetite is most probably indirect and exerted through ghrelin’s interaction with blood glucose level and insulinemia shortly before and after a meal. Several significant correlations between insulinemia and blood glucose level have been found at individual stages of Oral Glucose Tolerance Test (OGTT). It is thought, that the drop in ghrelin’s concentration after administration of glucose and after a meal in case of healthy people is most probably determined by insulinemia [15]. It is suggested that leptin and insulin may regulate the production of ghrelin. It is however unclear in what way ghrelin, as a hormone secreted by the digestive tract, may be responsible for changes in storing energy [16]. Insulin concentration in persons with insulin resistance is high. It is typical for obesity and changes in insulin concentration before and after a meal reflect the fluctuations of ghrelin’s concentration. It seems that insulin is the factor that regulates ghrelin’s secretion. In humans administration of insulin results in the decrease of ghrelin’s concentration which is consistent with the possible inhibiting effect of insulin on ghrelin’s secretion [17]. Contrary to ghrelin, the concentration of insulin rises together with the rise of fat mass and declines during starving and when body mass is lost. Thus, it may be assumed that insulin may play the role of a mediator between body mass and ghrelin concentration. Moreover, hyperinsulinemia and insulin resistance as indices of overeating may inhibit orexigenic concentration of ghrelin, but the reports made so far on the issue have not been consistent [18]. The effect of ghrelin on insulin is not fully known as there are contradictory reports on the issue. However, most probably it is ghrelin that inhibits the secretion of insulin by stimulating the secretion of NPY in pancreas [19]. Ghrelin induces the rise of glucose concentration in humans. This most likely results from its inhibitory effect on insulin secretion. Adipogenic and stimulating effect on appetite is independent from GH, but most likely depends on the leptin system. Leptin and ghrelin have opposing effects [20].

**Mechanism of ghrelin’s activity in ovary**

Ghrelin is an endogenic ligand for GHSR receptor (Growth Hormone Secretagouge Receptor), whose activity consists mainly in stimulating the secretion of growth hormone and increasing appetite as well as inducing obesity. Recent research indicates the significant role of this hormone in regulating reproductive functions in broad aspect. Ghrelin is a hormone that is secreted by the ovarian follicle and regulates the processes of steroidogenesis in the ovary stimulating the proliferation of cells and showing antiapoptotic effect. Compounding ghrelin with a functional receptor GHSR-1a activates protein G and the signal transduction proceeds through activation of protein kinase C and phospholipase C which hydrolyzes phosphatidylinositol 4,5-bisphosphate into 1,2-diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3). These changes lead to intracellular calcium rise. A number of researches point to the possibility of the existence of alternative signal transmission paths e.g. activation of protein kinase A dependent on cAMP or the kinase activated by mitogen (MAPK) [21]. Taking into account the possibility of ghrelin acting through the activation of phosphatidylinositol path, the researches focus on the mechanism of the hormone’s activity by specifying the effect of ghrelin on enzymatic activity of phosphatidylinositol kinase (IP3), which catalyzes the formation of IP3 and protein expression for IP3 kinase. Another stage being tested is the specification of IP3 participation in ghrelin’s effect on proliferation and apoptosis of the ovarian follicle’s cells. Ghrelin significantly stimulates the enzymatic activity of IP3 kinase. However, ghrelin’s effect on protein expression for IP3 kinase has not been proven. It has been noted that ovarian follicle’s cells’ proliferation decreases upon stimulation with ghrelin down to the control level after adding the inhibitor of IP3 kinase. In parallel, the reversal of antiapoptotic activity of ghrelin has been observed. The conclusion drawn from these researches constitutes the confirmation of the activation of phosphatidylinositol kinase path being one of the mechanisms of ghrelin’s activity affecting proliferation and apoptosis of ovarian follicle’s cells [20, 22]. In vitro researches suggest that ovaries may be important locations of ghrelin’s activity as high concentration of ghrelin is found in human ovaries [23, 24]. Negative correlation
between ghrelin’s concentration and androgens in females’ serum has also been noted. The research carried out so far on animals showed that estrogens are engaged in the regulation of ghrelin secretion. Many of these researches also confirmed that estrogens affect the size of meals consumed. Estradiol inhibits food intake in mice and ghrelin acts as a signal initiating food intake. Specific mechanism of estadiol’s effect on food intake has not been fully understood yet, but most probably it plays the role of a modulator of ghrelin’s activity [20].

**Ghrelin’s effect on fertility**

Apart from its stimulating effect on GH secretion, ghrelin increases the secretion of PRL, ACTH and cortisol [25, 26]. Ghrelin’s effect on LH secretion has not been unanimously identified yet. Researches carried out so far on animals and humans are inconsistent and it cannot be unanimously stated whether ghrelin’s effect is stimulating or inhibitory. It is suggested that ghrelin decreases the frequency of LH pulses. In this mechanism ghrelin may play a significant physiological role in the reproductive function (Fig. 2) [27-29].

Research on rats proved that ghrelin stimulates the production of testosterone by inhibiting key steroidogenesis enzymes [30]. Ghrelin deficiency leads to disorders in the activity of genes responsible for proper development of the uterus as well as for providing appropriate conditions for the development of embryo in the mature uterus. Such a finding may explain the problems with fertility and with getting pregnant observed in obese women because they have a low level of ghrelin [18].

Subsequent observations point to even stronger and more lasting relationship between ghrelin and fertility. It is thought that ghrelin deficiency during pregnancy may negatively affect a female child’s fertility. The research was carried out on mice which, due to genetic modifications, had ghrelin deficiency. It turned out that the female mice gave birth to offspring with decreased fertility which was manifested in the smaller number of their offspring. Ghrelin deficiency caused a drop in the activity of HOXA 10 gene which is decisive in a proper development of uterus in female embryos. This gene is necessary to provide the embryo with conditions appropriate for development. This finding may explain the fertility problems in very obese women. Paradoxically, these women have a low level of ghrelin. Mother’s obesity may negatively affect the fertility of next generations [17]. Significantly overweight women face increased risk of pregnancy complications. Excess weight during pregnancy has a negative effect on the woman’s body as well as on the child. The risk of hypertension for a pregnant woman rises by 6-8 times and the risk of diabetes by 7-20 times. The fetus in turn faces a greater risk of developing serious abnormalities especially cardiological and neurological. In the long run the child of an obese mother is much more susceptible to diabetes or has increased tendency to be overweight. Moreover, the research also indicates that the additional weight of the adipose tissue lowers fertility causing ovulation irregularities 3 times more often than in women with body weight within normal range. Also the cases of successful treatment of infertility in obese women are fewer and rarer [31]. It has been found that the concentration of ghrelin in pregnant women increases as the pregnancy develops, with the gain of body mass and growth of insulin resistance, which may be the evidence of ghrelin’s participation in inducing insulin resistance. Ghrelin may also have an indirect effect on fetus development and the metabolical condition of a newborn baby shortly after the birth. No size differences have been noted between the new born babies of obese mothers and mothers with proper weight. There were no differences between the direct dependency neonatal anthropometric parameters and serum concentrations of ghrelin in cord blood and maternal serum in the final stages of pregnancy.

No mutual relationships have been found between the concentration of ghrelin and the concentration of insulin in umbilical cord blood [31]. Polycystic ovary...
syndrome (PCOS) is an important endocrine disorder related to hyperandrogenism and oligo- or anovulation. In PCOS patients a lower serum level of ghrelin has been noted than in healthy women, although the reports on this issue are not unanimous. Ghrelin is a hormone that is strictly related to the energy balance, obesity, insulin resistance and most probably to the reproductive function. As PCOS predisposes to obesity and metabolic changes such as insulin resistance it may be assumed that ghrelin’s function is connected with the polycystic ovary syndrome [20, 22].

Presented data about role of ghrelin in reproductive functions are very interesting, however this topic requires further studies.

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References

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