The role of ghrelin, leptin and insulin in foetal development

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▌**Abstract**

Introduction and objective. The growing epidemic of childhood obesity has forced scientists to search for methods to prevent feeding disorders. Increasing interest in appetite regulating hormones has revealed their influence on energy homeostasis after birth or even in *utero*.
State of knowledge. The presence of ghrelin in the stomach of human foetuses and the distinctive production in the pancreas of neonates suggests the role of ghrelin in pre- and post-natal development. The neonatal period appears to be a critical time for the formation of adipose tissue-hypothalamus circuits, thus the amount of adipocytes in foetal life may be a major regulator of food intake. Insulin's orexigenic effect in the arcuate nucleus of the hypothalamus can be a major modulator of foetal development.
Objective. This review, based on available literature, aims to analyses the role of appetite regulating hormones in foetal development.
Summary. Different concentrations of hormones, such as ghrelin, leptin and insulin during foetal life raises the question whether or not they can be modulated, thereby avoiding obesity before birth. Children with pancreas agenesis showed smaller body size at birth, which emphasises the probable role of insulin in foetal growth. Study of sheep foetuses with IUGR confirmed these finding. Appetite-regulating hormones show different roles in foetal development and seem to be essential in the perinatal period.

▌**Key words**
ghrelin, leptin, insulin, fetal growth

INTRODUCTION

The growing epidemic of childhood obesity has forced scientists to search for prevention methods that could affect the regulation of energy homeostasis during foetal life and lead to early avoidance of feeding disorders. The increasing popularity of appetite regulating hormones has revealed their influence on energy homeostasis at birth or even *in utero* [1, 2, 3], which suggests they can play a role in the regulation of foetal development [4, 5, 6]. After Barker noticed in 1998 that newborns with low birth weight were more likely to have heart disease or diabetes in adulthood, scientists began searching for an answer to the question whether or not the factors which predispose newborns to reach too low or too high birth weight could be modulated to prevent diseases such as obesity and diabetes. Barker's theory of foetal programming highlights the influence of the mother's lifestyle during pregnancy on the birth weight and susceptibility to diseases. Another study by Barker and Hale confirmed the 'thrifty phenotype' hypothesis: maternal under-nutrition during pregnancy can affect foetal metabolism [7]. There is some speculation about energy homeostasis regulating hormones modulating feeding behaviours directly after birth

[8, 9]. All peripheral signals about the energy status reach the centre for hunger and satiety regulation – the hypothalamus [10]. Arcuate nucleus (Arc), paraventricular nucleus (PVN) lateral hypothalamic area (LHA) and the perifornical area (PFA) are structures in the hypothalamus involved in the regulation of energy homeostasis by communication with nucleus of the solitary tract (NTS), resulting in termination or initiation of feeding. The vagal nerve is also involved in transmission of peripheral signals concerning nutritional status [11].

Ghrelin – hypothalamic regulation of energy homeostasis. Ghrelin was discovered in 1999 by Kojima et al. and many studies have confirmed its additional roles beside the orexigenic effect [12]. It was discovered as a new endogenous ligand for the growth hormone secretagogue(GHS) receptor. Ghrelin modulates GH secretion by GHS-R 1a receptor, different from somatoliberin and somatostatin(main controllers of GH secretion). When ghrelin binds to GHS-R 1a, phospholipase C is activated, phopshoinosytol concentration rises and protein kinase C is activated, resulting in the release of calcium ions from the endoplasmic reticulum. Activation of kinase C stimulates GH secretion [1, 13]. Beside its direct effect, ghrelin stimulates GH secretion by modulating the expression of Pit-1 transcription factor in the anterior pituitary gland. Ghrelin's indirect effect on GH secretion is realised by an increased expression of GH gene in somatotroph cells [13, 14, 15]. Researchers confirmed

350

Annals of Agricultural and Environmental Medicine 2014, Vol 21, No 2

Magdalena Warchoł, Hanna Krauss, Małgorzata Wojciechowska, Tomasz Opala, Beata Pięta, Wioletta Żukiewicz-Sobczak et al. The role of ghrelin, leptin and insulin…

the time- and dose-dependent effect of ghrelin activation of Pit-1 in GH cells cultures. The regulation of growth hormone secretion, both direct and indirect, is independent of the ghrelin orexygenic role in the hypothalamus [16].

The largest number of ghrelin-producing cells in adults (X/A-like cells) are in the corpus gastricum and fundus gatricus. The synthesis of ghrelin, but in smaller quantities, also occurs around the hypothalamic arcuate nucleus [17, 18, 19, 20], pituitary gland [21], gut [22], cells of the immune system [23], lungs [21, 24, 25, 26], kidneys, gonads, placenta and pancreas [3, 21, 27]. Ghrelin produced in the stomach and nuclei of the hypothalamus plays the main role in regulating the body's energy status [28].

The discovery of ghrelin receptor afferent neurons around the vagus nerve in an animal model gave rise to speculations that the hypothalamic effect is made possible by signals sent from the stomach through the nerve, rather than through a direct transfer of peripheral ghrelin to the hypothalamus [29]. Date et al. studied rats after vagotomy and their results confirmed reduction of the ghrelin orexygenic effect after the surgery [30]. Furthermore, subdiaphragmatic vagotomy caused complete elimination of hunger-induced increased ghrelin levels. Studies in humans confirmed this relationship. People after vagotomy treated with ghrelin did not show increased food intake [31]. Other studies indicate a lack of participation of the vagus nerve afferent fibres in the stimulation of food intake in response to peripheral ghrelin [32]. It is possible that there are some compensatory mechanisms which enable the communication of peripheral

ghrelin with hypothalamic arcuate nucleus after vagotomy. Some studies suggest that only some of the ghrelin functions are implemented via the vagus nerve [33]. The food intake regulation mechanism is shown in Figure 1. The signal sent by ghrelin from the stomach reaches the nucleus of the solitary tract (NTS). The production of ghrelin in the hypothalamic arcuate nucleus area is probably also stimulated by the vagus nerve. Binding of ghrelin to its receptor around NPY/AgRP/GABA neurons increases the expression of genes encoding neuropeptide Y (NPY) and agouti-related protein (AgRP). NPY and AgRP inhibit the activity of anorexygenic neurons in the paraventricular nucleus (PVN), at the same time stimulating orexygenic neurons in the LHA. In addition, ghrelin causes an inhibition of POMC/CART neurons (stimulators of anorexygenic response) through γ – aminobutyric acid (GABA). This results in the orexygenic neurons sending signals to the NTS and generating a food-seeking behaviour [21, 22, 35].

Leptin – anorexigenic factor in hyphothalamus. Leptin is produced by white adipose tissue, and in less quantity by the placenta, ovaries, testicles, stomach, pituitary gland, vascular endothelium, brown adipose tissue and skeletal muscle [8, 36]. Pleiotropic effects of leptin, both central and peripheral, are due to the multiple isoforms of the receptor, whose presence is confirmed not only in the hypothalamus. Studies have shown that leptin receptor (Ob-R) is present, among others, in the intestine, pancreas β cells, liver, skeletal muscle cells, pituitary, lymphocytes and monocytes, ovary, and endometrium, as well as in adipocytes [37, 38, 39]. However, the most important effect of leptin involves the control of body weight by acting on the hypothalamus. When leptin reaches hyphothalamus it binds to Ob-R around Arc (Figure 1). Receptor Ob-R is present around POMC/CART neurons (anorexigenic response) and AgRP/Npy/GABA neurons (responsible for orexigenic response). As a result of the stimulation of POMC/CART neurons by leptin, α-melanocortin is released which binds to its receptor (MC4-R) around PVN where anorexigenic neurons generate a fasting behaviour signal in NTS. POMC/CART neurons inhibit orexigenic neurons. Leptin suppresses expression of AgRP/NpY/GABA neurons whose products are released in periods of energy deficiency. As a consequence of all these actions, an anorexigenic signal arises and reaches NTS causing satiety [11, 35]. The effect of leptin on growth is also linked to its effects on GH secretion by inhibiting hypothalamic somatostatin production, and by direct effects on the pituitary somatotroph cells [11, 40].

Hypothalamic action of insulin. Hypothalamic action of insulin in the control of energy homeostasis is one of the most significant roles of this hormone. Insulin diffuses directly around Arc and binds to its receptor located on POMC/CART neurons and AgRP/NpY/GABA neurons. POMC/CART neurons, through enhanced release of α-melanocortin, stimulate anorexigenic neurons. In addition, insulin increases the inhibitory effect of POMC/CART neurons on orexigenic neurons. Binding insulin to AgRP/NpY/GABA neurons stimulates release of AgRP and NpY, the role of which is to actuate orexigenic neurons and inhibit anorexigenic neurons. The result of insulin action in the area of Arc is a signal sent from the stimulated anorexigenic neurons to the NTS, which causes satiety [11, 35].
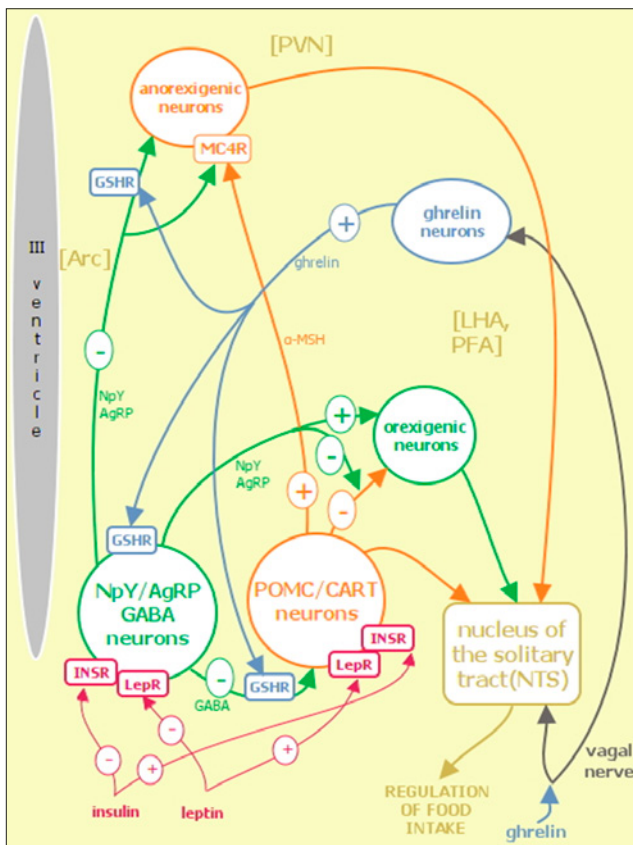

Figure 1. Regulation of food intake at the level of arcuate nucleus in the hypothalamus. Orexigenic effect of ghrelin, anorexigenic effect of leptin and insulin through stimulation or inhibition of neurotransmitters secretion resulting in food seeking or fasting behaviour.
Adapted from: [34]

Annals of Agricultural and Environmental Medicine 2014, Vol 21, No 2

351

Magdalena Warchoł, Hanna Krauss, Małgorzata Wojciechowska, Tomasz Opala, Beata Pięta, Wioletta Żukiewicz-Sobczak et al. The role of ghrelin, leptin and insulin…

Presence of ghrelin in human foetuses. Ghrelin was found for the first time in the human foetal circulation by Cortelazzi et al. In this study, 25 samples of foetal blood between 20–39 weeks of gestation were analysed. From all foetuses, nine had IUGR (Intrauterine Growthe Restriction), while the weight of the other 16 was appropriate for gestational age. Ghrelin was detected in all examined samples of blood from foetuses in various periods of gestation. No gender differences were found in neonatal ghrelin concentrations [41]. Other studies suggested the placental origin of ghrelin in foetus. Despite these findings, ghrelin was not present in placental cells during the last trimester of pregnancy, thus it was considered there are other sources of fetal ghrelin. Ghrelin gene expression and ghrelin concentration in foetal rat pancreas and stomach were compared in other studies. It was confirmed that the total amount of foetal ghrelin levels were higher in comparison to the amount in adults, both pregnant and non-pregnant. Higher gene expression of ghrelin in foetal pancreatic cells than in the stomach was confirmed, in contrast to adult rats, where the ghrelin gene expression was higher in the stomach. Ghrelin mRNA and total ghrelin concentration in pancreatic cells was six to seven times higher than the in cells of the stomach. The results of these studies suggest that the main source of ghrelin can be the foetal pancreas and emphasises its relationship with the development of this organ *in utero* [42]. To confirm the concept of the origin of ghrelin from the stomach of foetuses and newborns, two embryos, 38 foetuses in various stages of development, and 3 infants were examined. Samples were collected from the antrum and corpus. In the embryonic cells, no ghrelin-producing cells (P/D1) were found, but their presence was confirmed in the eleventh week of gestation in a significant quantity in the corpus of stomach (107.7 cells/mm^2 mucosal epithelium).

It was found that ghrelin levels were lower in the third trimester of pregnancy; this level then increased in newborns, compared to the level in foetuses. The number of ghrelin-producing cells in foetuses (107.7 cells/mm^2) was higher compared with the levels in healthy adults measured in another study (60 cells/mm^2) [9]. Expression of ghrelin was also shown in foetal pituitary gland somatotropes between 18–37 weeks of gestation. Despite these findings, the hypothalamic pituitary axis is activated probably in the first week of the extra-uterine life [43].

Foetal leptin and growth of the foetus. The **p**resence of leptin receptor Ob-R in the gastric mucosa, oesophagus and intestine cells between the 7th–9th week of gestation and identification of leptin mRNA in gastric mucosa suggest the possible role of leptin in the development of the neonatal gastrointestinal tract. Leptin added to cultures of cells of the digestive tract, from 11-week-old fetuses, affects the DNA synthesis and consequently may increase the efficiency of the leptin receptor [44]. Beside effects on the gastrointestinal tract development *in utero*, leptin is probably also involved in the development of the brain, as in patients with genetic mutation, resulting in leptin deficiency, treated by leptin injections, in whom an increased brain mass concentration was shown [45]. Similar results were obtained by examining cells from mouse embryos, with the same defect in 16th–18th week of foetal life. The addition of leptin resulted in the growth of neuroepithelium cells [46]. Disrupted formation of pathways between Arc and PVA or LHA can be associated

with abnormal brain apprising the the body's energy status. In leptin deficient (ob/ob) mice, the neuronal pathways from Arc are permanently disrupted and leptin treatment in adulthood did not reverse this defect. Interestingly, the intervention in newborns led to the reconstruction of pathways between Arc and other parts of the hypothalamus, and mice growth showed no obesity [47]. The appearance of leptin between the 6th–10th week of gestation with the beginning of lipidogenesis raises the claim that it may have a part in the formation of adipose tissue: the neonatal period is considered to be a critical time for formation of brain-pituitary adipose tissue axis. Neonatal leptin may be an initiator and modulator of hypothalamic arcuate nucleus response [48].

Insulin role in foetal growth. The presence of foetal insulin in the 8th week of gestation was confirmed. Foetal pancreas is the source of insulin as the transfer of maternal insulin through placenta is not possible. Study of children with pancreas agenesis confirmed the role of insulin in foetal growth as they showed smaller body size at birth. Undoubtedly, the degree of foetal nutrition depends on the amount of nutrients supplied by the mother and her metabolic status [49]. According to the Pedersen's theory about foetal macrosomia in diabetic mothers, it is connected with an excessive amount of glucose penetration to the foetus, resulting in high levels of insulin in the foetus and thus increased growth [50]. The insufficient amount of glucose delivered to the foetus can result in very low birth weight. This mechanism involves a compensatory increase of gluconeogenesis, leading to the growth restriction because of insufficient amount of essential amino acids. Another study of sheep foetuses with IUGR showed a reduced by 76% of pancreatic β-cell mass in the perinatal period due to reduced proliferation of these cells during the development of the foetus [51].

CONCLUSIONS

Appetite-regulating hormones, besides their hypothalamic action, may have an influence on foetal growth. Ghrelin is involved in the differentiation and proliferation of somatotrophes, which confirms its impact on growth and development. The presence of ghrelin and its receptor in various cells and organs shows its broad activity in the body. The total amount of foetal ghrelin levels are higher in comparison to the amount in adults, which affirms its importance in prenatal life. Identification of leptin mRNA in the gastric mucosa of the foetus suggests a possible role of leptin in the development of the neonatal gastrointestinal tract. Leptin is probably involved in the development of the brain and pathways between arcuate nucleus and other parts of hypothalamus. Children with pancreas agenesis showed smaller body size at birth, which emphasises the probable role of insulin in foetal growth. A study of sheep foetuses with IUGR confirmed these finding. Appetite regulating hormones showed different roles in foetal development and seem to be essential in the perinatal period. Different concentrations of this hormones during foetal life raises a question about whether or not they can be modulated and thereby avoid obesity before birth.

352

Annals of Agricultural and Environmental Medicine 2014, Vol 21, No 2

Magdalena Warchoł, Hanna Krauss, Małgorzata Wojciechowska, Tomasz Opala, Beata Pięta, Wioletta Żukiewicz-Sobczak et al. The role of ghrelin, leptin and insulin…

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