SPANDIDOS PUBLICATIONS

Leptin and its receptors in the course of pregnancy in the rat

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Abstract. Control of processes responsible for food intake and regulation of energy homeostasis during pregnancy is crucial for mother as well as for fetus development. Leptin is one of the main hormonal factors involved in regulation these processes in organisms. During pregnancy leptin regulates mother's energy balance and may also affect fetus growth and development, particularly via receptors in hypothalamus arcuate nuclei (ARC), pituitary and placenta. In the present study, serum leptin levels and expression of both short (ObRs) and long (ObRb) form of leptin receptor in the hypothalamus, pituitary and placenta were measured in the course of pregnancy. The results of these studies indicate that leptin concentration in serum increases during pregnancy and decreases 24 h after the delivery. The expression of both short and long forms of the leptin receptor in the hypothalamus decreases in the course of pregnancy and increases after the delivery. In the pituitary, however, a decrease of leptin receptor mRNA during pregnancy was observed only for ObRb. Analysis of placental leptin receptor expression demonstrated an increase of ObRb and constant high levels of ObRs mRNA. Our results suggest that changes in leptin level and its receptor expression may influence the energy homeostasis during pregnancy. In addition, changes in ObR expression are suggestive for: i) leptin resistance in the hypothalamus and pituitary; and ii) an increased leptin-dependent signaling in the placenta.

Introduction

Leptin, the hormone of adipose tissue, is also produced in placenta (1) and acts via receptors, which belong to the family of class I cytokine receptors. To date six splice variants of these receptors have been described (2,3). The long form of the leptin receptor - ObRb (contains a long intracellular domain) is involved in signal transduction, whilst five short forms (contain a short or no intracellular domain), are inactive or act as leptin transporters in the blood and through the blood-brain barrier (2,4-6). It was found that leptin receptors are expressed in the hypothalamus especially in arcuate (ARC), paraventricular (PVC) and dorsomedial (DMN) nuclei, as well as in the lateral hypothalamic area (7,8). These hypothalamic regions are most important for the regulation of food intake and energy balance (9). Through the ARC leptin may also regulate functions associated with reproduction (10). Mice without endogenous leptin are infertile and they have disruption in gonadotrophins (LH, FSH) secretion. However, leptin injection restored fertility and normal LH and FSH levels (11-14). Moreover, Morash *et al* (15,16) described leptin receptors (both, ObRb and ObRs) in the rat pituitary. Expression of leptin receptors was also found in the placenta (17,18). These data suggest that leptin may regulate mother's metabolism, fetus growth and development via pituitary, hypothalamic and placental receptors. On the other hand, leptin hormone is also produced in the placenta, indicating that leptin may be involved in fetal development.

The aim of our current study is the characterization of: i) serum leptin levels; and ii) expression of its receptors in the hypothalamus, pituitary and placenta during the course of pregnancy in the rat.

Materials and methods

Animals. The experiments were carried out using adult cycling female Wistar rats. Fertilization was confirmed by the presence of sperm in the vaginal smear and such state was defined as the first day of pregnancy. Pregnant females were transferred to the metabolic cages (Techniplast). Animals were kept there under 12 h:12 h light-dark cycles at 22±1˚C. The body weight, fodder (rat chow Labofeed H, A. Morawski, Kcynia, Poland) and water consumption were measured every day. Food and water were consumed *ad libitum*. Rats were sacrificed by decapitation at different stages of pregnancy as described by Altman and Dittmer (19). Decapitation was performed on the 4th (before implantation, group B), 13th (embryonic period, group C) and 18th day (fetal period, group D) of pregnancy. An additional group of animals (group E) was sacrificed 24±5 h after the delivery. Rats in the diestrus phase of the estrous cycle served as controls (group A). The Local Ethics Committee for Experiments on Animals approved the experiment protocol.

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Table I. Condition of PCR reaction for ObRb, ObRs and 18S.

	ObRb	ObRs	18S
Steps of PCR			
Initial denaturation	94˚C - 2.30 min	94˚C - 2.30 min	94˚C - 2.30 min
Denaturation	94˚C - 45 sec	94˚C - 45 sec	94˚C - 45 sec
Annealing	50˚C - 45 sec	57˚C - 45 sec	57˚C - 45 sec
Elongation	72˚C - 1.30 min	72˚C - 1 min	72˚C - 1 min
Terminal elongation	72˚C - 10 min	72˚C - 11 min	72˚C - 1 min
Number of cycles in:			
Hypothalamus	35	35	13
Pituitary	39	33	12
Placenta	40	32	12

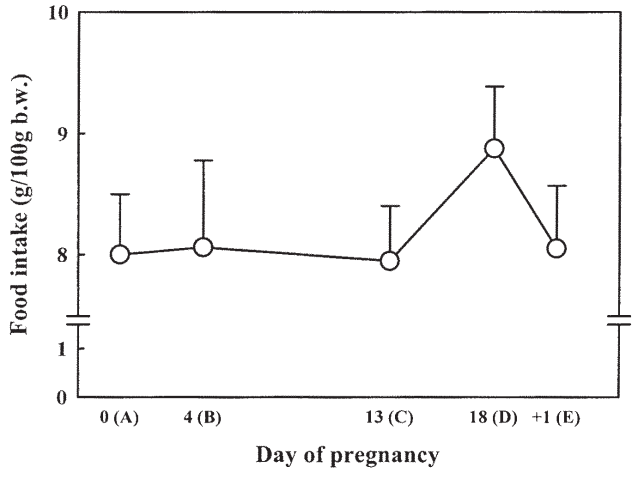

Figure 1. Food intake changes in the course of pregnancy. Results are expressed as mean ± SEM (n=6).

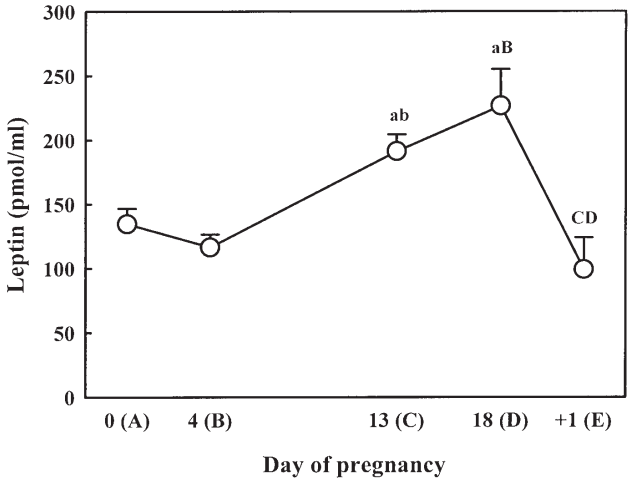

Figure 2. Changes of leptin concentration in blood serum of the pregnant rats. Statistically significant differences are marked with letters corresponding to the groups (small letters, P≤0.05; capital letters, P≤0.01).

Radioimmunoassay. Serum leptin concentration was measured by RIA using specific rat leptin kit (Linco Res., Inc., St. Charles, MO, USA). Intra- and interassay variations were 5% and 8%, respectively.

RNA isolation and semi-quantitative RT-PC. Hypothalamus, pituitary and placenta were isolated, crushed, transferred to a 2 ml tube (Eppendorf Biopur; Eppendorf AG, Hamburg, Germany) with RNA Later (Ambion, Inc., Austin, TX, USA) and frozen at -80˚C. Total RNA was isolated using TRIzol LS Reagent (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's protocol. Reverse transcription was performed using ImProm-II Reverse Transcription System (Promega Corp., Madison, WI, USA). The cDNA was amplified in PCR using primers for short (ObRs) and long (ObRb) forms of leptin receptors published by Morash *et al* (20) with some modifications (Table I). In the PCR reaction *Taq* DNA polymerase (Promega Corp.) was used. The expression of the PCR end-products was normalized against the internal standard 18S (Classic 18S primers; Ambion, Inc.). Amplification reaction was performed within the linear range of the reaction for short and long form of leptin receptor and 18S (Table I). The PCR products were separated on a standard 1.5% ethidium bromide agarose gel, photographed and analyzed using Gel Logic 100 Imaging System and Kodak 1D Analysis Software v.3.6 (Estman Kodak Co., New Haven, CT, USA). Data are shown as a ratio of leptin receptors expression to 18S.

Statistical analysis. Results were averaged per experimental group and the SEM was calculated. The statistical comparison of the data was done by ANOVA followed by the multiple range test of Duncan.

Results

As shown in Fig. 1 no statistical differences in food intake in the course of pregnancy were observed, however, it was found that rats were eating more food per 100 g of body weigh (b.w.) in late pregnancy.

Leptin level was significantly higher on the day 13th and 18th of pregnancy than on the day 4th or in the diestrus phase. Decrease of serum leptin was observed after the delivery, and this result was statistically significant in comparison to day 13th and 18th (Fig. 2).

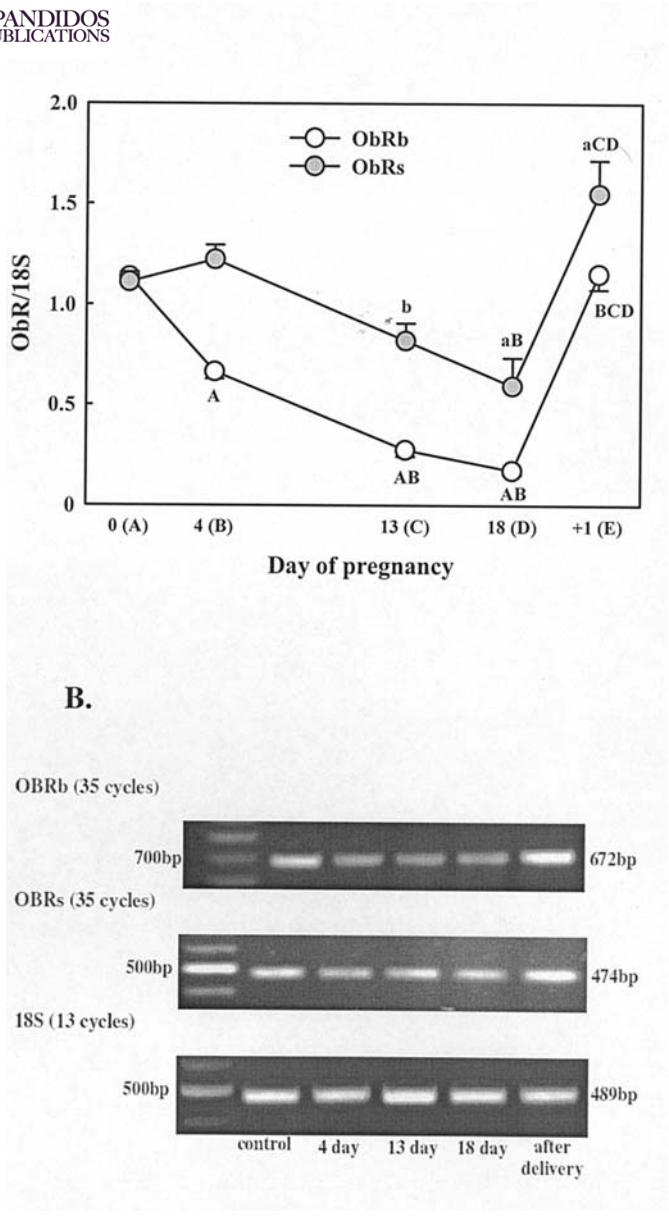

Figure 3. Changes in hypothalamic expression of leptin receptors in the course of pregnancy. (A) Expression of long (ObRb) and short (ObRs) forms of leptin receptor. (B) Example analysis of ObRb, ObRs and 18S in the course of pregnancy. Statistically significant differences are marked with letters corresponding to the groups (small letters, P≤0.05; capital letters, P≤0.01).

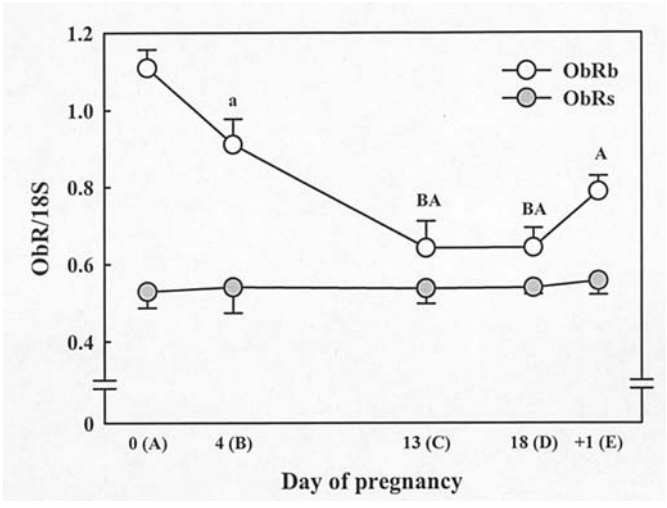

Figure 4. Changes in pituitary expression of leptin receptors (ObRb and ObRs) in the course of pregnancy. Statistically significant differences are marked with letters corresponding to the groups (small letters, P≤0.05; capital letters, P≤0.01).

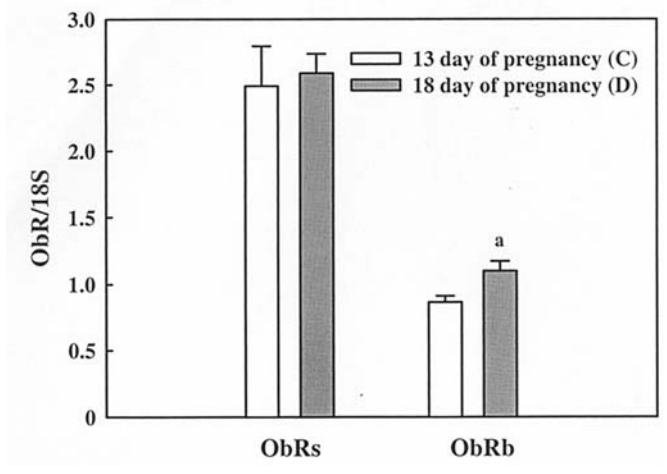

Figure 5. Changes in expression of leptin receptors (ObRb and ObRs) in the rat placenta. Statistically significant differences are marked with letters corresponding to the groups (small letters, P≤0.05; capital letters, P≤0.01).

On the other hand, leptin receptors expression tended to decrease in the hypothalamus and these changes were statistically significant for both long and short forms (Fig. 3). Decrease of the leptin receptors expression was observed during pregnancy until the delivery. Afterwards an increase of both forms of the leptin receptor was observed.

As presented in the Fig. 4 only ObRb mRNA decreased significantly in the pituitary on day 4, 13 and 18 of the pregnancy. Expression of short form of leptin receptor in the pituitary remained unchanged during the study.

Leptin receptors expression increased in the placenta (Fig. 5) but this change was statistically significant only for ObRb on the 13 and 18 day of pregnancy.

Discussion

Increase of leptin level in human in course of pregnancy correlates with a higher expression of the leptin gene in placenta (21). On the other hand, the main source of leptin during pregnancy in mice and rats is rather adipose tissue, because lack of leptin expression in placenta was reported (22,23). However, some contrary results on leptin expression have been reported. Hoggard *et al* (24) confirmed leptin gene expression in the mouse placenta. Similarly, Garcia *et al* (25) demonstrated leptin expression in rat placenta using RT-PCR and their results show that leptin mRNA levels increased during pregnancy. These data indicate that placenta may be a source of leptin during pregnancy and may explain the decrease of leptin levels after the delivery. We found that leptin concentration in serum significantly increased during

pregnancy and declined after the delivery. Increase of leptin concentration was observed on the day 13 and 18, when the animals have placenta. These data are in concert with the results reported by Amico *et al* (1). On the other hand, Terada *et al* (26) did not observe any changes in blood serum level up to the day 20 of pregnancy, after which leptin levels decreased.

In presented study, we found that during pregnancy the expression of the long form of leptin receptor decreases in the hypothalamus and increases after the delivery. Similar results were published by Garcia *et al* (25). These results indicate that during pregnancy leptin resistance occurs which is consistent with the previous reports (25,27). Moreover, we observed a decrease of the short form of the leptin receptor in the hypothalamus ($P \leq 0.05$). Such data may indicate that small amounts of leptin cross the blood-brain barrier thereby contributing to the leptin resistance. Contrary to our results Garcia *et al* (25) did not find any changes in the expression of short forms (ObRa, ObRc, ObRe, ObRf) of the leptin receptor in the hypothalamus.

One of the recent studies by Ladyman and Grattan (28) showed, that ObRb mRNA levels in ventromedial nucleus (VMH) decreased during pregnancy whilst in arcuate nuclei (ARC) they remained relatively constant. They also found that number of leptin-responsive cells, measured by leptin-induced phosphorylation of STAT3, decreased in VMH upon leptin treatment of pregnant rats whilst in ARC it was invariable. However, previous data of the same group indicate that ARC and VMH had reduced leptin-induced activation of STAT3 during pregnancy (29) that was related to leptin resistance during pregnancy. This inconsistent data may indicate that hypothalamic targets of leptin are differentially regulated to control the energy homeostasis during pregnancy. In the present study, we also found that leptin changes do not influence the food intake during pregnancy, which additionally confirms leptin resistance.

It was found that leptin and its receptor, both long and short forms, are expressed in the rat pituitary (15,16). Leptin receptors have been localized in GH secreting pituitary cells (30), which suggests that leptin possible contributes to the increased GH secretion during pregnancy (31). Shimon *et al* (32) found that leptin stimulates GH secretion from the pituitary of human fetus. Moreover, leptin was a strong regulator of LH and less potent of FSH secretion from the pituitary (33). However, we found that the expression of the long form of leptin receptor generally decrease during pregnancy and increase after the delivery. This suggests that leptin probably does not change the secretion of the pituitary directly. We also found, that short form of the leptin receptor in the pituitary remain relatively constant during pregnancy, suggesting that leptin availability in the pituitary does not change during pregnancy.

Our data indicate that placental leptin receptor expression increases during pregnancy in particular ObRb, which is in agreement with the study reported by Chien *et al* (34). Similarly, Kawai *et al* (17) using Northern blot and immunocytochemistry found that leptin receptor expression increases in rat placenta from day 17 to day 21. By RT-PCR they showed that long, short and soluble (ObRe) forms of leptin receptor are expressed in placenta. Kawai *et al* (17) demonstrated the expression of leptin receptors on day 19 and day 21 of pregnancy, whilst we detected ObR mRNA as early as on the day 13 and day 18.

Smith and Waddell (18) showed, that short forms of leptin receptor, ObRa and ObRe, increased in the labyrinth zone of rat placenta from day 16 to day 22 of pregnancy whilst in the basal zone the expression of these forms was unchanged. Expression of the long form leptin receptor mRNA was stable in the labyrinth and the basal zone but the protein concentration of the receptor significantly decreased. Increase of ObRe may contribute to leptin resistance through hormone binding and subsequent decrease of the accessible fraction in the circulating blood.

All these data may indicate that leptin resistance during pregnancy occurs rather in hypothalamic and pituitary cells than in placenta. Higher levels of leptin in late pregnancy play probably an important role in fetal growth and development. Leptin crosses into placenta, acting as information on mother's energy state. On the other hand, because short forms of receptor may act as leptin transporter (5), Hoggard *et al* (24) suggested that receptors expression in placenta might inform mother about fetal energy state. We found that the expression of the long form of receptor in placenta increases, indicating that mother's leptin influences the fetus via placenta or may regulate placenta development. On the other hand, the expression of short forms of leptin receptor in placenta is very high and rather invariable. These data suggest the possibility of cross-talk between mother's and fetal energetic state via short forms of leptin receptor. However, Kastin *et al* (35) suggested that in spite of short form of leptin receptor another transporter(s) may exist which may participate in leptin transport across blood-brain barrier. However, these data require further analysis.

Our results suggest that changes in leptin level and its receptor expression may influence the energy homeostasis during pregnancy. We observed changes in ObR, suggestive for: i) leptin resistance in the hypothalamus and pituitary; ii) and increase of leptin signaling in placenta. Leptin resistance developed on the hypothalamic level by decrease of ObRb and ObRs forms, thereby diminishing leptin binding and availability. On the other hand, on pituitary level we observed a decrease only of the long form of leptin receptor whereas the availability of leptin was unchanged. On the contrary, placental expression of ObRb increased during pregnancy while ObRs levels remained high and relatively constant. These data suggest the possibility of a cross-placental communication concerning energetic status of fetus and mother.

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