

Leptin Gene Expression In Rabbits During Pregnancy And Fetal Life

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ABSTRACT

Leptin may act as the critical link between adipose tissue and reproduction. Although considerable progress has been achieved in understanding the reproductive actions of leptin, much work is needed for understanding its physiological role. Till now, no data has been published about the distribution and expression levels of leptin in the rabbit maternal adipose tissue, placenta, and various fetal rabbit tissues during pregnancy and postpartum. Our results indicated that circulating leptin levels in rabbit serum during pregnancy were significantly higher than that in postpartum and non pregnant rabbits. Furthermore, leptin showed positive correlations with body weight in pregnant and postpartum rabbits. RT-PCR verified the presence of RNAs encoding leptin in the rabbit maternal perirenal adipose tissue, placenta, and several fetal tissues; including brain, liver, adipose tissue, and bone. The relative abundance of leptin RNA in rabbit maternal adipose tissue was significantly higher at 20th day of pregnancy than that of non pregnant rabbits, while it was significantly decreased at 2nd day after parturition. No significant changes in the placental leptin RNA levels were noticed in pregnant rabbits at 10th, 20th, and 30th day of pregnancy. The relative abundance of fetal leptin transcripts at day 30th of pregnancy was in the order of liver > bone ≥ adipose tissue > brain. The present study provides new evidence for the distribution and expression levels of leptin in the rabbit maternal and fetal tissues during pregnancy and supports the importance of leptin in reproductive physiology and fetal development.

INTRODUCTION

Leptin is a 16-kDa cytokine encoded by the *obese* gene and primarily secreted by adipocytes (1). Leptin, upon binding to specific receptors in different areas of the hypothalamus (2), is involved in the control of body weight through its effects on food intake and energy expenditure by negative feedback at the hypothalamic nuclei (1). Giving that leptin is a ubiquitous molecule, it has been implicated in several key points of the mammalian reproductive functions (3) such as ovulation (4) and pregnancy (5). Although considerable progress has been achieved in understanding the reproductive actions of leptin over the past few years, much work is needed before we can arrive at a complete understanding of its physiological role, if any.

In fact, leptin has been identified in the adult human (6) and rat (7) and mouse (8).

In rabbits, leptin receptor (Ob-R) has been detected in endometrial cells (9) and the ovary at day 9 of pseudo-pregnancy by immunohistochemistry and Western blot analysis that supports a permissive luteolytic role for leptin in rabbit corpus luteum (10). Recently, Koch et al (11) identified the leptin gene cloning and expression in the rabbit mammary epithelial cells during pregnancy and lactation. Up till now, no data has been published concerning the expression and distribution of leptin in the rabbit adipose tissue and placenta as well as the different fetal rabbit tissues.

Pregnancy is a hyper metabolic state in which a great increase in maternal body fat and weight occurs and associated with relevant endocrine changes. In addition, energy needs are increased during pregnancy. These increased energy needs may be met through partitioning of nutrients for energy utilization which is under hormonal control. Therefore, in order to verify to what degree pregnancy influences leptin in rabbits, we intended to measure the leptin hormone in the blood and estimate its *in vivo* expression level in the maternal adipose tissue and placenta at different stages of pregnancy in rabbits. Moreover, to assess whether leptin is involved in the rabbit fetal growth and development, we aimed to investigate the expression level and distribution of RNAs encoding leptin in the 10-day post-coitus rabbit fetal tissues using reverse transcription-PCR.

MATERIALS AND METHODS

Animals and experimental designs

A total of 25 sexually mature healthy New Zealand White female rabbits weigh between 2700-3000 g and of ~ 6 months old were used. Rabbits were housed in individual stainless steel cages provided with automatic drinkers. All rabbits were raised on balanced diet (20% protein, 2.62% fat, 11% fiber, and 2600 kcal/kg energy). Rabbits were housed in an animal room which maintained on a 12-h light-dark cycle at an ambient temperature of 25 ± 2 °C and 35-50% humidity for two weeks before starting the experiment for adaptation.

After quarantine period, rabbits were distributed into two groups; the control group (5 rabbits) which represent the non pregnant rabbits and the experimental group (20 rabbits) that were introduced into the male's cages. The mated ratio is identically one male one female (for mating on a 1:1 basis). The female was considered mated when the sperms were found in the vaginal washing and that day was designed the first day of pregnancy. It

was expected that a female would give birth 30 days following an observed copulatory episode. These mated rabbits were divided equally into 4 groups (5 rabbits each) which designated at pregnancy day 10, 20, 30, and after parturition. The experimental protocol used in the present study was approved by the Laboratory Animal Control Guidelines of Faculty of Veterinary Medicine, Zagazig University.

Blood and tissue samples

Blood samples were collected during sacrificing the rabbits in each group into sterile test tubes without anticoagulant. For serum collection, samples were kept at room temperature for 30 min, and then centrifuged at 3000 rpm for 15 min. The serum samples were stored at -20°C until used for leptin analysis.

After scarifying on days 10, 20, and 30 of pregnancy as well as days 2 and 7 after parturition, tissue samples from the maternal perirenal adipose tissue and placenta as well as different fetal tissues such as (brain, liver, bone, and perirenal adipose tissue) were immediately collected from the rabbits and washed in ice-cold 0.9% (w/v) NaCl. These tissue samples were immediately snap-frozen in liquid nitrogen and subsequently stored at -80°C until use for RT-PCR analysis.

Hormonal analysis

Serum leptin was measured using DRG Leptin ELISA kit (DRG Instruments GmbH, Germany) according to the manufacturer protocol.

Molecular analysis

Leptin gene expression was determined in the female and fetal rabbit's tissues by using the semi-quantitative RT-PCR.

Total RNA isolation

RNA was isolated from the tissue samples by using Easy-RED™ Total RNA Extraction Kit (iNtRON Cat. No. 17063) following the manufacturer instructions.

Reverse transcription

Total RNA (5 ug per sample) was reverse transcribed into cDNA using Revert Aid H minus reverse transcriptase (Fermentas, #EP0451, Thermofisher Scientific, European Union) and a mix of oligo (dT) (0.5 ug/reaction, 4 ul 5x reaction buffer, 0.5 ug RNase inhibitor, and 2 ul deoxynucleotide triphosphate (dNTP) mix) in a 12.5 ul total reaction volume at 42°C for 60 min. To terminate the reaction, the tubes were heated at 70°C for 10 min and then stored at -80°C.

Polymerase chain reaction (PCR)

The specific oligonucleotide primers used for PCR to amplify the leptin gene were designed using primer 3.0 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) based on the published leptin sequences of rabbits (GenBank accession no. 001163069.1) (12). The forward primer was 5'-GTCGTCGGTTTGGACTTCATC-3' and the reverse primer was 5'-CGGAGGTTCTCCAGGTCGTTG-3'. As a control, PCR amplification with a pair of GAPDH primers based on published sequences with GenBank accession number 001082253.1 (The forward primer 5'-GGAGCCAAAAGGGTCATC-3' and reverse primer 5'-CCAGTGAGTTTCCCGTTC-3') were used. The oligonucleotide primers were synthesized by Metabion International AG, Planegg/Martinsried, Germany.

The isolated cDNA were amplified using Maximo Taq DNA Polymerase 2X-preMix kit (GeneOn GmbH, Deutschland, Germany) following the manufacturer protocol. In brief, the PCR was carried out in a reaction volume of 50 µl, containing 3.0 ul cDNA template (50 ng), 25 ul 2x Taq master mix, 0.5 ul (10 µM) forward primer, 0.5 ul (10 µM) reverse primer, and 21 ul sterile nuclease free water. The thermal cycling parameters were an initial denaturation at 94°C for 3 min, 38 cycles of amplification in a Techne, TC-3000 thermal cycler (Bibby Scientific Ltd, Staffordshire, UK) were performed under the following conditions: 94°C for 1 min, 55–60° C for 1

min, and 72°C for 80 sec followed by a final extension at 72°C for 10 min.

The PCR products (146 and 346 bp for leptin and GAPDH, respectively) were electrophoresed on 1.5% agarose gel, stained by ethidiumbromide, and visualized under UV light. Homology searches of the cDNA sequences were carried out against previously identified genes by using the Basic Local Alignment Search Tool (BLASTx) program (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) of the GenBank database (National Center for Biotechnology Information, Washington, D.C., USA).

The relative abundance of leptin RNA was normalized with respect GABDH. Densitometric analysis for the leptin bands was quantified using ImageJ gel analysis program (NIH, Bethesda, MD, USA). (<http://rsb.info.nih.gov/ij/index.html>).

Statistical analysis

Results are expressed as means ± S.E.M. All statistical analyses were performed using one-way analysis of variance (ANOVA) (SPSS 17.0; SPSS, Inc., Chicago, IL, USA) or Dunnett's test. The diagrams were drawn by GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Differences were considered significant at P values ≤ 0.05 .

RESULTS AND DISCUSSION

The present study, for the first time, estimated the serum leptin levels in the non-pregnant, pregnant, and lactating rabbits (Fig. 1). Furthermore, the study proved that serum leptin level was correlated positively to the body weight (Fig. 3) along the stages examined. Additionally, to the best of our knowledge, this is the first report to describe the existence of leptin transcripts in the adipose and placental tissues of rabbits during different pregnancy stages (Fig. 4 and 5). Our

quantitative RT-PCR data confirmed that leptin expression level in the maternal perirenal adipose tissue during lactation was lower than in pregnancy but higher than non-pregnant rabbits (Fig. 4). Our findings, which were similar to those obtained in other species, support a direct role of leptin in the regulation of several reproductive functions. Additionally, the present study demonstrates, for the first time, that leptin gene expression was present in a number of tissues in the fetal rabbits (Fig. 6). This expression of leptin in the rabbit fetal tissues suggests that the leptin may be involved in the growth and development of the fetus.

Serum leptin levels throughout pregnancy and after parturition in rabbits

Figure (1) showed the significant increase in serum leptin level (2.40 ± 0.08 ng/ml and 2.32 ± 0.04 ng/ml) at 20th and 30th day of pregnancy in rabbits, respectively when compared to the non-pregnant control (1.88 ± 0.04 ng/ml) group. Interestingly, after parturition, the serum leptin level in rabbits was significantly lower, as detected on the 2nd day post-partum (2.06 ± 0.02 ng/ml) than that detected on the 20th and 30th day of pregnancy in rabbits (2.40 ± 0.08 ng/ml and 2.32 ± 0.04 ng/ml, respectively). Whereas, the serum leptin level after parturition (2.06 ± 0.02 ng/ml) was significantly ($P < 0.05$) higher than that of rabbits at 10th day of pregnancy and the non-pregnant group (1.76 ± 0.02 ng/ml and 1.88 ± 0.04 ng/ml, respectively). Additionally, it is apparent from the data in figure (1) that there was no significant changes in serum leptin levels between non pregnant control rabbits

(1.88 ± 0.04 ng/ml) and at the 10th day of pregnancy (1.76 ± 0.02 ng/ml), also there was no significant changes in serum leptin levels at days 20 (2.40 ± 0.08 ng/ml) and 30 (2.32 ± 0.04 ng/ml) of pregnancy.

Increased leptin level appears to be a ubiquitous feature of pregnant mammals. Most reports indicated a substantial increase that occurs in the rat from midgestation, and this is followed by a clear pre-partum decline (7,13). Additionally, the maternal plasma leptin concentrations remain relatively stable during the first half of rodent pregnancy but then increase dramatically from midgestation in the mouse (8) and humans (6). The high levels of maternal leptin serum throughout pregnancy and its decline drastically postpartum suggests a functional importance of leptin during pregnancy. On the other hand, another study reported that the increased plasma leptin during pregnancy are not evident if compared to the non pregnant control rats (14).

The rise in plasma leptin from mid pregnancy appears to be due to the combined effects of increased plasma leptin binding activity (13) and enhanced maternal adipocyte expression of leptin mRNA (7,8). Since leptin is cleared by the kidney, at least partially by filtration (15), binding of the leptin to the binding protein should decrease its clearance. Thus hyperleptinemia during pregnancy is probably due to reduced renal clearance of bound leptin. Nonetheless, the prepartum decline in plasma leptin levels in the rat may reflect reduced fat mass in late pregnancy (16).

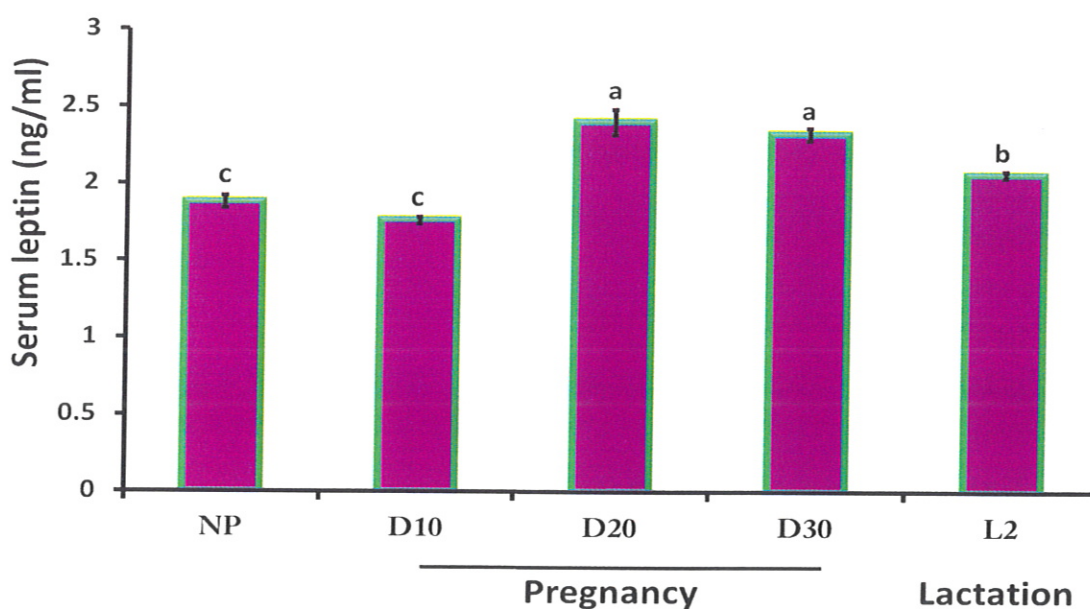


Fig. 1. Changes in circulating leptin concentrations during pregnancy and lactation in rabbits. Leptin concentrations (ng/ml) were measured in the serum samples obtained from rabbits at 10th, 20th, 30th day of pregnancy (D10, D20, and D30, respectively), lactation day 2 (L2), and non pregnant (NP). Different letters denote significant differences ($P \leq 0.05$). Data are expressed as means \pm S.E.M. of five animals each group.

Correlation between leptin level and body weight during pregnancy and lactation in rabbits

Gestational weight gain is a unique and complex biological phenomenon that supports the functions of growth and development of the fetus (17). Consistent with this concept, the results of the present study (Fig. 2) revealed that the body weight in rabbits was significantly increase ($P \leq 0.05$) at 30th day of pregnancy (3150 ± 220.22 g) in comparison to rabbits at 10th day of pregnancy (2700 ± 141.42 g) and the non-pregnant control group (2675 ± 79.84 g) (Fig. 2). Moreover, there was significant increase in the body weight of rabbits at 20th day of pregnancy (2875 ± 58.09 g) in comparison to the non-pregnant control group (2675 ± 79.84 g) (Fig. 2). Besides, (Fig. 2) showed increase in the body weight of pregnant rabbits at 30th day of pregnancy (3150 ± 220.22 g) more than that of the 20th (2875 ± 58.09 g) day of pregnancy but such

increase was not significant ($P > 0.05$). Whereas, there was significant decrease ($P \leq 0.05$) in body weight of rabbits at 2 days postpartum (2656 ± 213.69 g) compared to the rabbits at 30th day of pregnancy (3150 ± 220.22 g) (Fig. 2).

Pregnancy is the only normal physiologic process where body weight increases by ~20 percent and additional fat stores for lactation are deposited during the pregnancy period (18). Gestational weight gain has been reported to be the primary and most important determinant of weight change from preconception to postpartum (postpartum weight change). Postpartum weight change is made up of the sum of gestational weight gain and the post-delivery weight loss (17).

Moreover, our study estimated the correlation matrix to detect the degree of correlation between serum leptin level and body weight during pregnancy and postpartum

in rabbits. The results showed that serum leptin was positively correlated with body weight ($P \leq 0.05$) (Fig. 3).

This data was in agreement with those reported in human and rodent (6,19). Maffei et al (20) revealed that leptin may play an important role in regulating body weight by signaling the size of the adipose tissue mass.

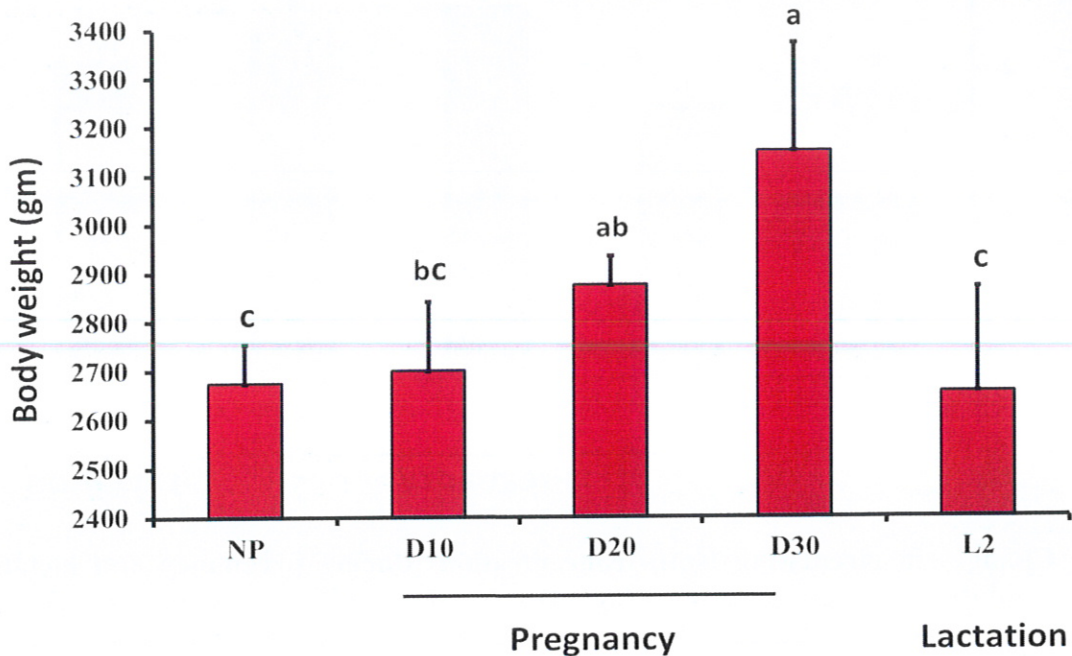


Fig. 2. Body weight of rabbits during pregnancy and lactation. Body weights were estimated at 10th, 20th, 30th day of pregnancy (D10, D20, and D30, respectively), lactation day 2 (L2), and non pregnant (NP) rabbits. Different letters denote significant differences ($P \leq 0.05$). Data are expressed as means \pm S.E.M. of five animals each group.

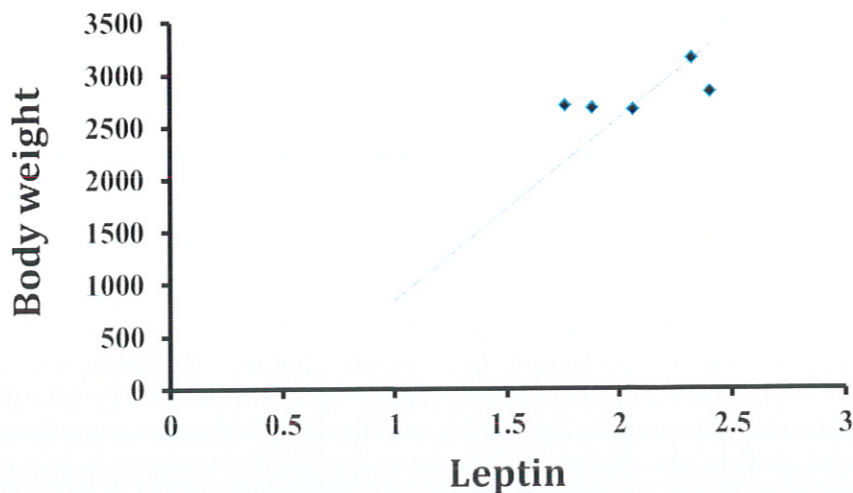


Fig. 3. Correlation between serum circulating leptin levels and body weight in rabbits during pregnancy and lactation.

In all mammals thus far examined, serum leptin levels increase during pregnancy and then decline shortly before or after parturition (6). The elevated maternal concentrations of leptin are not associated with decreased food intake or increased metabolic rate, as might be expected from known actions of leptin in non-pregnant mammals (5). Leptin resistance is a state in which there is an excess of serum leptin but the body does not effectively respond to these increased levels by reducing food intake or body weight (21). This suggests that a pregnancy-associated state of partial leptin resistance may exist within the hypothalamus.

Recently, resistance to the central effects of leptin during pregnancy and its effect on food intake has been verified in mice (22). The inconsistent hyperphagic behavior of pregnant rodents during pregnancy in the presence of rising leptin levels supports a leptin resistant state to the satiety adipokine. However, whether the suggested purpose of such resistance to leptin during pregnancy is to preferentially transport nutrients and/or promote fetal growth is not fully recognized. Tessier et al (23) has been reported that leptin resistance in healthy pregnancy seems to be central and beneficial for mobilizing energy stores to support adequate fetal growth. One possible mechanism by which leptin resistance might occur is down regulation of the leptin receptor in the hypothalamus. In rats, hyperleptinemia in maternal circulation during healthy pregnancy leads to central leptin resistance, by down regulation of the OB-Rb in the ventromedial nuclei of the hypothalamus and increased circulating OB-Re, both leading to attenuated leptin signaling in the regulatory appetite center of the brain (24). This central leptin resistance may act as a compensatory mechanism to meet the developing fetal energy needs. In later stages of a healthy pregnancy, central leptin resistance occurs to allow increased nutrient availability for the fetus (23).

Expression of leptin RNA in the adipose tissue of non pregnant, pregnant, and postpartum rabbits

To examine the leptin expression and its levels in perirenal adipose tissue of rabbits, RT-PCR was used to detect the RNA of leptin in perirenal adipose tissue of non pregnant, pregnant (pregnancy day 10, 20, 30), and parturated (post-partum day 2) rabbits (Fig. 4). As demonstrated in figure (Fig. 4), leptin was expressed in all adipose tissue samples. The relative abundance of leptin RNA were significantly increased ($P \leq 0.05$) in rabbit maternal adipose at 10th, 20th and 30th day of pregnancy as compared to the control non pregnant group (Fig. 4). The highest expression level was observed at 20th day of pregnancy and the lowest was noticed at the 10th day of pregnancy (Fig. 4). However, leptin RNA was significantly decreased at 2nd day after parturition as compared to the control group ($P \leq 0.05$) (Fig. 4). The elevated circulating leptin levels during pregnancy in rabbits, suggested that maternal leptin may play a role in maintenance of pregnancy and preparation for parturition and lactation.

Leptin is secreted by adipose cells, and its circulating concentration normally correlates with body adiposity (19). Leptin RNA is expressed in mice adipocytes, as shown using *in situ* hybridization, cell fractionation, and immunohistochemistry (20). The plasma levels of leptin are highly correlated with adipose tissue mass and fall in both humans and mice after weight loss (20).

Kronfeld-Schor et al (25) directly demonstrated that leptin secretion rate from mouse adipose tissue *in vitro* are decreased during early pregnancy and up-regulated during late pregnancy and lactation and these changes in leptin secretion rates *in vitro* paralleled those of circulating leptin *in vivo* during pregnancy. On the other hand, the increase in circulating leptin levels during mid to late pregnancy do not consistently correlate with adiposity in rats (7), mice (8), and humans (6). A clear increase in adipose leptin mRNA, however, has been observed during pregnancy in mice (8), suggesting that adipose tissue may be a major source of elevated serum leptin during pregnancy.

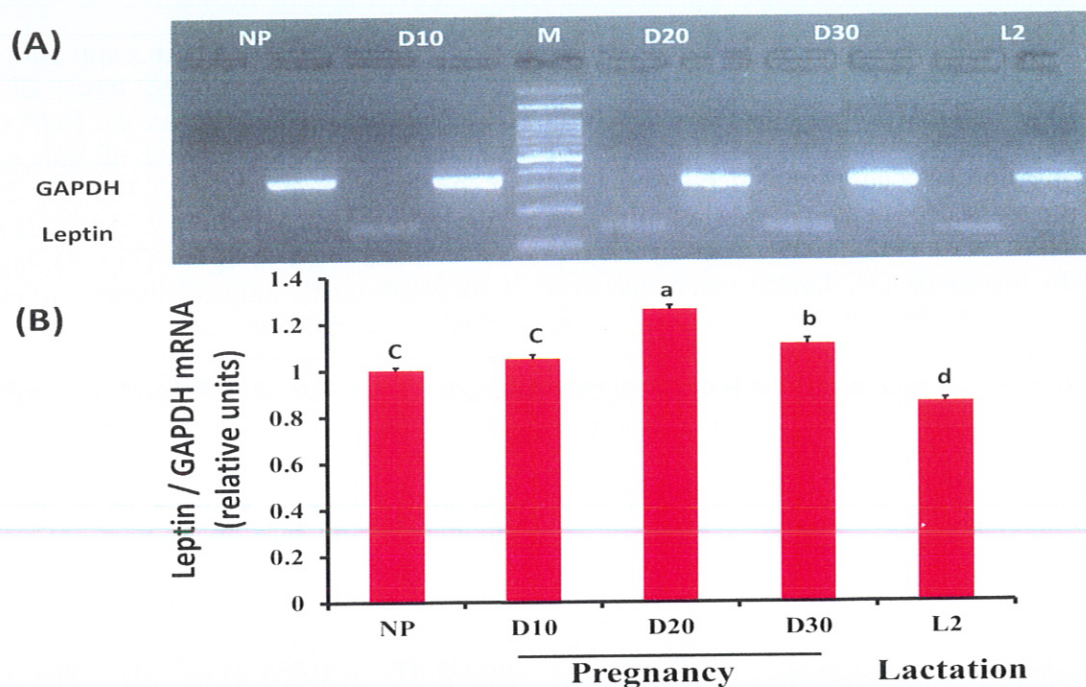


Fig. 4. RT-PCR analysis of leptin in the rabbit maternal perirenal adipose tissue. (A) representative RT-PCR of leptin RNA expression in perirenal adipose tissue of rabbits during pregnancy and lactation. RNA from perirenal adipose tissue of non pregnant (NP), pregnant day 10, 20, and 30 (D10, D20, and D30, respectively), and lactating day 2 (L2) rabbits, were reverse transcribed and amplified using the PCR method and specific primers for leptin. A 100 base pair (bp) DNA ladder was included as a reference for fragment size. (B) Bar graph for the relative expression of leptin after normalization to GAPDH. Different letters denote significant differences ($P \leq 0.05$). Data are expressed as means \pm S.E.M. of five animals each group.

Expression of leptin RNA in the placenta during pregnancy in rabbits

The non adipose sources of leptin also might involve in the changes of circulating leptin during pregnancy. Several works have shown that in addition to adipose tissue, leptin is present in substantial amounts in the placentas of several species. Most available information describing placental leptin has been obtained from studies of rodents and humans. Our RT-PCR data proved the presence of RNA transcripts for leptin in the placenta of the pregnant rabbits (Fig.5). Even though no significant change in the placental leptin RNA expression levels was noticed among pregnant rabbits at 10th (1.0 ± 0.045), 20th (0.91 ± 0.058), and 30th (0.99 ± 0.040) day of pregnancy (Fig.5).

The human placenta contains substantial amounts of leptin mRNA and protein (26). Comparable amounts of immunoreactive leptin occur in the rodent placenta, although expression of rodent placental leptin mRNA appears to be much lower than in humans (7,8,27). Importantly, the rodent placenta appears to contribute little, if any, leptin to the maternal circulation (7,27). In agreement with our results (Fig. 1 and 5), Gavrilova et al (28) have reported a dramatic increase of circulating leptin levels during mouse gestation with, however, no detectable increase in placental leptin production. On the other hand, Amico et al (29) revealed that as gestation advanced in rats, the serum leptin concentrations increased significantly and that was concomitant to a significant increase of

placental leptin production. The benefit of placental leptin to the fetus may be for growth and angiogenesis, as leptin is considered an important growth factor in intrauterine and neonatal development.

On the contrary with the suggestion that placenta provides a major contribution to

circulating leptin during pregnancy, the plasma leptin levels do not return to baseline until several days after parturition and expulsion of the placenta (28). One possible explanation for this is that adipose tissue continues to secrete leptin for a brief time after parturition (25).

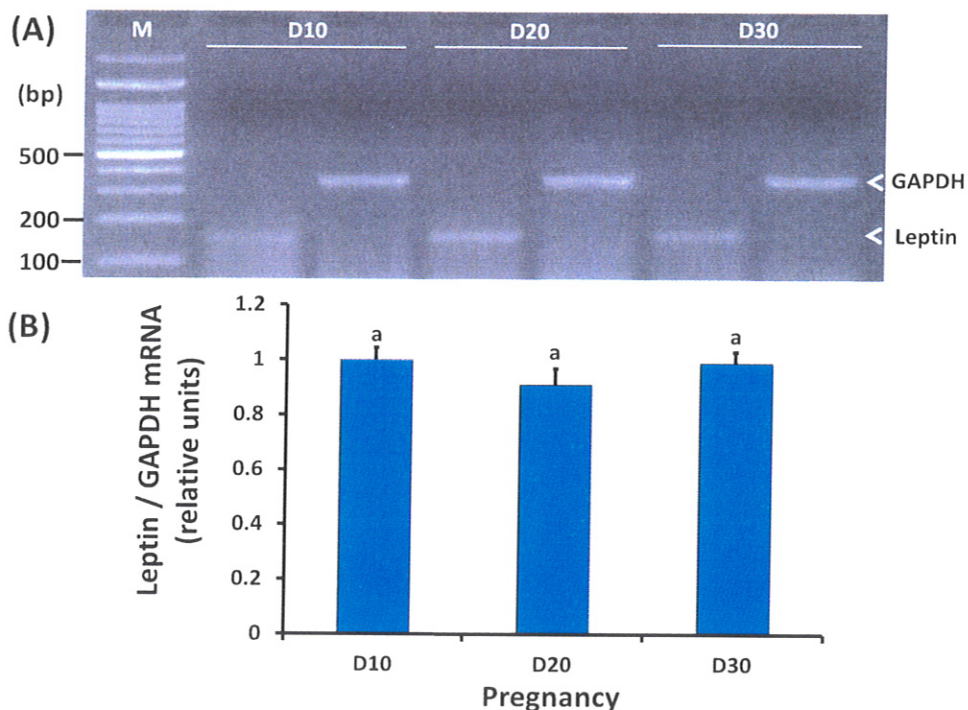


Fig. 5. RT-PCR analysis of leptin in the rabbit maternal placenta. (A) representative RT-PCR of leptin RNA expression in placentas of rabbits during pregnancy and lactation. RNA from perirenal adipose tissue of non pregnant (NP), pregnant day 10, 20, and 30 (D10, D20, and D30, respectively), and lactating day 2 (L2) rabbits, were reverse transcribed and amplified using the PCR method and specific primers for leptin. A 100 (bp) DNA ladder was included as a reference for fragment size. (B) Bar graph for the relative expression of leptin after normalization to GAPDH. Different letters denote significant differences ($P \leq 0.05$). Data are expressed as means \pm S.E.M. of five animals each group.

Distribution and expression levels of leptin RNA in various fetal tissues of rabbits

The only indications that leptin may have a biologic function in the fetus is based on data obtained in mice and rats showing the expression of leptin receptor mRNA in several

fetal tissues including cartilage, bone, lung, kidney, testes, and hypothalamus (27). To assess whether leptin is involved in the rabbit fetal growth and development, we have examined the distribution and relative expression levels of RNAs encoding leptin in

various fetal tissues during rabbit pregnancy using RT-PCR analysis (Fig. 6).

In the present study, RT-PCR verified the existence of RNAs encoding leptin in the whole rabbit fetus at day 10 of pregnancy and also in the brain, liver, perirenal adipose tissue, and bone of the rabbit fetus at 30th day of pregnancy (Fig. 6). Additionally, the relative

abundance of leptin transcripts, as measured by the densitometric analysis, showed significant differences ($P \leq 0.05$) among the different regions of the fetal rabbit tissues (Fig. 6). Leptin was most abundantly in the liver, at intermediate levels in adipose tissue and bone, and low level in the brain of fetuses on day 30th of pregnancy (Fig. 6).

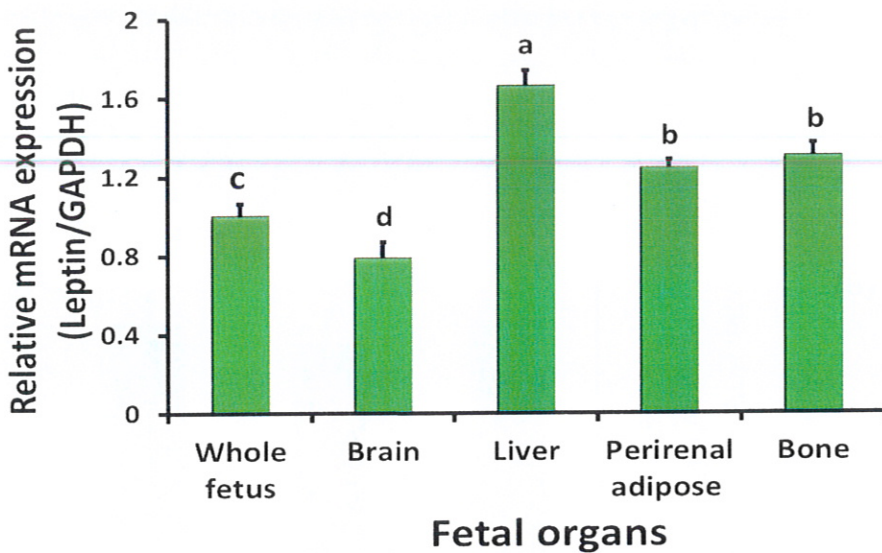


Fig. 6. Detection of leptin expression in various rabbit fetal tissues by RT-PCR analysis. RNA from whole rabbit fetus at 10th day of pregnancy as well as the rabbit brain, liver, perirenal adipose, and bone of fetus at 30th day of pregnancy, were reverse transcribed and amplified using the PCR method and specific primers for leptin. The bar graph represents the relative expression of leptin after normalization to GAPDH. Different letters denote significant differences ($P \leq 0.05$). Data are expressed as means \pm S.E.M. of five animals each group.

On the contrary, the study by Hoggard et al (27) revealed that no leptin receptor (OB-R) gene expression either on the mRNA or protein level was identified in the heart, kidney, liver, adrenal, or pancreatic primordium of the 14.5-day postcoitus murine fetus. High levels of RNA expression for leptin and its receptor, however, were seen in the cartilage/bone, hair follicles, and lung as well as the leptomeninges and choroid plexus of the brain of the 14.5-day postcoitus murine fetus, suggesting a possible function of leptin as an autocrine or paracrine regulator in the fetus (27).

The expression of leptin in the rabbit fetal tissues (Fig. 6) suggests that leptin may be involved in the growth and development of the fetus with one possible function being a fetal growth factor or a signal to the fetus of maternal energy status. Alternatively, fetal leptin could provide a signal to the mother of fetal growth and development. This implies a role for leptin in fetal bone and/or cartilage development that may be linked to its influence on hematopoiesis in the adult (30). The importance of SNAT (System A sodium dependant neutral amino acid transport) in fetal growth regulation has been well

demonstrated (31). Leptin has been shown to enhance SNAT activity, suggesting a role for leptin as a mediator of amino acid delivery to the fetus via the placenta (31).

Conclusion

To the best of our knowledge this is the first report on the distribution and expression levels of leptin in rabbit maternal adipose tissue and placenta as well as in different fetal tissues including; brain, liver, bone, and perirenal adipose tissue during pregnancy. Results of our study imply a fundamental role of leptin in the maintenance of pregnancy and preparation for lactation. The present data strongly reinforce the idea that circulating leptin levels may provide a growth-promoting signal for fetal development during late pregnancy. Overall, the present work supports the importance of leptin in reproductive physiology and fetal development. Although great progress has been made in the last few years towards understanding the physiological roles of leptin, several fundamental questions still need to be answered.

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الملخص العربي

التعبير الجيني لهرمون اللبتين في الأرانب أثناء الحمل والحياة الجنينية

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يعتبر هرمون اللبتين بمثابة حلقة الوصل بين الأنسجة الدهنية والتناسل. وعلى الرغم من أنه تم إحراز تقدم كبير في فهم دور اللبتين في التناسل، إلا أنه هناك حاجة إلى الكثير من العمل لفهم دوره الفسيولوجي. حتى الآن لم يتم نشر أية دراسات حول مستويات التوزيع والتعبير الجيني لهرمون اللبتين في الأنسجة الدهنية والمشيمة للأرانب وكذلك الأنسجة المختلفة لأجنحتها. تشير نتائجنا إلى أن مستوى هرمون اللبتين في دم الأرانب خلال فترة الحمل كانت أعلى بكثير مما كانت عليه في ما بعد الولادة وكذلك في الأرانب غير الحوامل. أيضا أظهرت الدراسة إلى أنه هناك علاقة ايجابية بين مستوى هرمون اللبتين في الدم ووزن الجسم في الأرانب أثناء فترة الحمل. كذلك باستخدام تقنية RT-PCR تبين وجود RNA لهرمون اللبتين في الأنسجة الدهنية والمشيمة للأرانب وأيضا في العديد من أنسجة الأجنة للأرانب مثل المخ والكبد والأنسجة الدهنية والعظام. وأخيرا تقدم هذه الدراسة أدلة جديدة لأول مرة للتعبير الجيني ومستويات توزيع هرمون اللبتين في الأرانب وأجنحتها. وهذا يدعم أهمية هرمون اللبتين في فسيولوجيا التناسل ونمو الأجنة.