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Possible Role of Placental Leptin in Pregnancy

A Review

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Leptin was initially identified as an adipocyte-derived hormone that decreases food intake and body weight via its receptor in the hypothalamus. Subsequent animal studies revealed various physiologic functions of leptin. Leptin plays an essential role in reproduction by regulating gonadotropin-releasing hormone secretion from the hypothalamus. It also modulates glucose metabolism by increasing insulin sensitivity and activates the sympathetic nervous system. In humans, leptin is also produced by placental trophoblasts and is secreted into both the maternal and fetal circulation. Leptin production in the placenta is increased in pregnancies complicated with several pathologic conditions. Leptin gene expression in the placenta is augmented in severe preeclampsia, and maternal plasma leptin levels in severe preeclampsia are significantly higher than those in normotensive pregnant women. Leptin production in the placenta is also increased in diabetic pregnancy with insulin treatment. Furthermore, leptin is proposed to play a functional role in implantation by virtue of its stimulatory effect on matrix metalloproteinase expression in cytotrophoblast. Dysregulation of leptin metabolism and/or function in the placenta may be implicated in the pathogenesis of various disorders during pregnancy, such as recurrent miscarriage, gestational diabetes, intrauterine growth retardation, and preeclampsia. In this review, possible roles of placental leptin are discussed.

Key Words: Fetal growth; insulin sensitivity; leptin; placenta; preeclampsia; pregnancy.

Introduction

Leptin was initially introduced as an adipocyte-derived messenger of energy metabolism *(1)*. It decreases food intake via its receptor in the hypothalamus *(2,3)*. Subsequent animal studies revealed various physiologic functions of leptin. Leptin plays an essential role in reproduction by regulating gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus *(4,5)*. It also modulates glucose metabolism by increasing insulin sensitivity *(6)* and activates sympathetic nervous system *(7,8)*. We previously reported that leptin is produced in the human placenta and is secreted into both maternal and fetal circulation *(9)*. Since leptin receptor is abundantly expressed in various maternal tissues, placenta and fetal tissues, physiological and pathophysiological roles in pregnancy are expected *(10–15)*.

Dramatic changes in energy metabolism are well recognized in pregnant women. Increased food intake, decreased insulin sensitivity, and hyperlipidemia are major features during pregnancy *(16)*. These changes are beneficial in providing energy to the fetus and preparing the mother for nursing. It is proposed that maternal adaptation to the pregnant state is mainly owing to placental hormones, such as prolactin (PRL), placental lactogen, and steroid hormones *(16)*. However, the role of leptin in pregnancy has not been fully elucidated to date, because production and metabolism of leptin during pregnancy are different among species.

In this review, we first introduce leptin as an adipocyte-derived signal to neuroendocrine and reproductive system. Then we overview reports on leptin production in the human placenta and discuss possible roles of placental leptin in maternal glucose metabolism and fetal growth. We also discuss the roles of other placental hormones in the fetomaternal energy metabolism, including our recent findings on the production of resistin in the human placenta. Resistin is the newly found adipocyte-derived peptide hormone that regulates insulin resistance and development of type II diabetes mellitus *(17)*. We finally introduce the possible roles of placental leptin in the maintenance of pregnancy.

Functions of Adipocyte-Derived Leptin

Leptin as a Messenger of Energy Metabolism

Leptin is a peptide hormone that consists of 146 amino acids and is expressed abundantly and specifically in the adipose tissue *(1)*. Plasma leptin concentrations show a positive correlation with fat mass or body mass index (BMI). Leptin decreases food intake by inhibiting neuropeptide Y secretion

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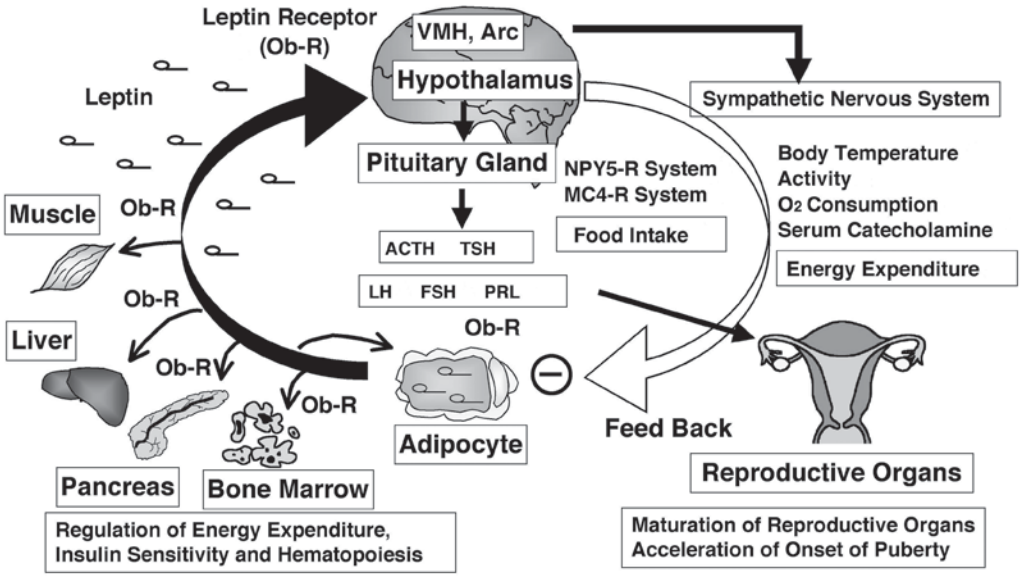

Fig. 1. Physiologic functions of adipocyte-derived leptin. Leptin receptor is expressed in the various tissues, such as hypothalamus, muscle, liver, pancreas, bone marrow, and adipocyte itself. Leptin may directly act on these tissues and modulate food intake, the sympathetic nervous system, energy expenditure, and insulin sensitivity. Leptin also increases GnRH secretion from the hypothalamus, which stimulates FSH and LH secretion from the pituitary. Thus, leptin plays a crucial role in reproductive function.

and stimulating proopiomelanocortin (POMC) via its cognate receptor (Ob-R) in the hypothalamus *(2,3)*. In addition, it activates the sympathetic nervous system (SNS) and increases energy expenditure *(18)*. For example, urinary catecholamine secretion is significantly increased in transgenic (Tg) mice overproducing leptin *(6,19)*. Thus, leptin decreases body weight and adiposity as a messenger of peripheral energy metabolism.

Leptin as a Peripheral Signal to Neuroendocrine and Reproductive Systems

Following the discovery of genes for leptin *(1)* and its receptor *(20)*, a number of in vivo animal studies and in vitro studies revealed various functions of leptin *(11)*. Figure 1 schematically illustrates the physiologic functions of adipocyte-derived leptin.

In addition to the satiety action and activation of the SNS, leptin acts as a peripherally produced metabolic signal to the neuroendocrine and reproductive systems and plays a crucial role in reproduction. In leptin-deficient *ob/ob* mice, reproductive function is impaired owing to deficient GnRH secretion *(4)*. The reproductive function of *ob/ob* mice is recovered by either administration of follicle-stimulating hormone (FSH) or leptin supplementation *(4)*. Administration of leptin accelerates the onset of puberty in normal mice *(21,22)*. On the other hand, in Tg mice overexpressing leptin, the onset of puberty is accelerated, but these mice later exhibit infertility owing to hypogonadotropic hypogonadism *(5)*. Prolonged exposure to hyperleptinemia might modulate leptin receptor and/or the postreceptor signaling system in the hypothalamus since the administration of FSH can cause those mice to ovulate *(5)*. Leptin directly modulates secretion of FSH, luteinizing hormone (LH), adrenocorticotropic hormone (ACTH), and PRL from the hypothalamus in addition to the indirect action through POMC or GnRH secreted from the hypothalamus *(23)*. In in vitro experiments, leptin suppresses progesterone production in the ovarian granulosa cells *(24)*. Thus, leptin modulates reproductive function at various stages of the hypothalamic-pituitary-ovarian axis.

Leptin as a Placenta-Derived Hormone

Gene Expression of Leptin and its Receptor in Human Placenta

During pregnancy, women encounter dramatic changes in the body, such as weight gain, increased fat deposit, and glucose metabolism. Plasma leptin levels in pregnant women are significantly higher than those in nonpregnant women *(9,25,26)*. To elucidate the site of leptin production during pregnancy, leptin levels in maternal and cord plasma were measured.

Irrespective of BMI, plasma leptin levels in pregnant women were found to be significantly higher than in nonpregnant women. When plasma leptin levels were measured consecutively during 40 normal pregnancies and puerperium, plasma leptin levels in the first trimester were twice as high as the nonpregnant levels. In the second and third trimester, plasma leptin levels further increased to approx 35 ng/mL and returned to normal nonpregnant levels within 24 h of

placental delivery, suggesting that the major source of leptin in maternal plasma is the placenta *(9)*.

Leptin gene expression in human pregnant uteri were examined. Northern blot analysis identified in the placental chorionic tissue a single leptin mRNA species of the same size (~4.5 kb) as in mature adipocytes. Expression of the leptin gene was found abundantly in the first-trimester chorionic villi, and slightly in the third-trimester chorion laeve, and amnion. Immunohistochemically, both syncytiotrophoblasts and cytotrophoblasts were stained positively for leptin *(9)*. All these results indicate that leptin is synthesized in the human placenta and is secreted to maternal circulation.

On the other hand, plasma leptin levels in umbilical artery and umbilical vein were significantly lower than those in paired maternal plasma. However, leptin levels in umbilical vein were significantly higher than those in paired umbilical artery, suggesting that leptin is secreted from placenta into fetal circulation *(27)*.

Recent in vitro studies confirmed that most of the leptin produced by the placenta is secreted into maternal circulation, but that a considerable proportion of leptin is also released into fetal circulation *(28–30)*. Some investigators *(28,30)* reported that a relatively low proportion (<5%) of placental leptin is secreted into fetal circulation. Lepercq et al. *(30)* suggested that umbilical leptin levels can be taken as a marker of fat tissue in human fetuses since leptin mRNA was expressed in the fetal adipose tissue by reverse transcriptase polymerase chain reaction (RT-PCR). By contrast, Hoggard et al. *(29)* reported that a higher proportion (13.6%) of leptin is released into fetal circulation. Thus, the origin and physiologic significance of leptin in the fetal circulation are an interesting aim of future investigation.

Augmented Leptin Gene Expression in Placenta of Complicated Pregnancy

Preeclampsia is a hypertensive disorder that develops in late pregnancy and is usually associated with fetal growth retardation owing to placental dysfunction. The pathophysiologic significance of leptin in preeclampsia has been investigated by measuring plasma leptin levels and placental leptin mRNA expression in pregnant women with preeclampsia *(31)*.

In a study by Mise et al *(31)*, women with preeclampsia were divided into two subgroups, mild and severe preeclampsia, according to the severity of hypertension defined by The American College of Obstetricians and Gynecologists. No significant differences in plasma leptin levels were observed between the mild preeclampsia group and its gestational age–matched control group. By contrast, plasma leptin levels in the severe preeclampsia group were approximately threefold higher than those in its gestational age–matched control group. Plasma leptin levels in the severe preeclampsia group were also significantly higher than those in the mild preeclampsia group *(31)*. The elevated plasma leptin levels returned to those of healthy nonpregnant women within 24 h of delivery *(31)*.

Leptin mRNA expression was markedly augmented in the placental tissue from women with preeclampsia compared with that of gestational age–matched healthy pregnant women. Leptin mRNA levels in severe preeclampsia were markedly higher than those in mild preeclampsia. Placental leptin mRNA levels were roughly parallel to plasma leptin levels in all the women with preeclampsia who were examined *(31)*.

Plasma leptin levels showed a positive correlation with the mean arterial blood pressure in pregnant women with preeclampsia, but not with BMI. Plasma leptin levels showed a negative correlation with ΔSD of neonatal body weight, the degree of fetal growth restriction. On the other hand, plasma leptin levels in women with preeclampsia who delivered small-for-date newborns were significantly higher than those in women who delivered appropriate-for-date newborns. Moreover, the incidence of small-for-date newborns in pregnant women with plasma leptin levels higher than the mean +1.5 SDs was significantly higher than that in women with normal leptin levels. These findings suggest a close correlation between fetal growth restriction and the elevated plasma leptin levels.

It has been reported that leptin synthesis and secretion are increased during the course of forskolin-induced cellular differentiation from cytotrophoblasts to syncytiotrophoblasts in the human choriocarcinoma cell line BeWo cells, suggesting that BeWo cells are a useful in vitro model system with which to access the regulation of leptin production in placental trophoblasts *(9,31,32)*. Treatment of BeWo cells with 20 μ*M* forskolin induced dose- and time-dependent increases in leptin and human chorionic gonadotropin (hCG) secretions. When BeWo cells were exposed for 72 h to hypoxic stimulation with 5% oxygen condition under treatment with froskolin, leptin secretion was increased approximately threefold relative to those cultured under 20% oxygen. By contrast, hCG levels in the culture media of BeWo cells cultured under 5% oxygen were decreased significantly compared with those cultured under 20% oxygen *(31)*.

Figure 2 shows a hypothetical relationship between augmented leptin secretion from placenta and fetal growth restriction in severe preeclampsia. In severe preeclampsia with hypertension, maternal uteroplacental blood flow is impaired. The impaired placental circulation causes chronic disturbance of nutrient supply and finally results in fetal growth restriction. The impaired placental perfusion also produces local hypoxia, which, consequently, augments leptin gene expression in the placenta. It is plausible that the elevated leptin levels in the maternal circulation may aggravate hypertension, since leptin activates the SNS and stimulates catecholamine secretion *(7,8)*. Thus, the maternal plasma leptin level is a possible marker of the fetoplacental milieu in pregnancy complicated by fetal growth restriction.

Augmented placental leptin mRNA expression has also been reported in pregnancies complicated with diabetes mellitus, especially in pregnant women treated with insulin *(33,*

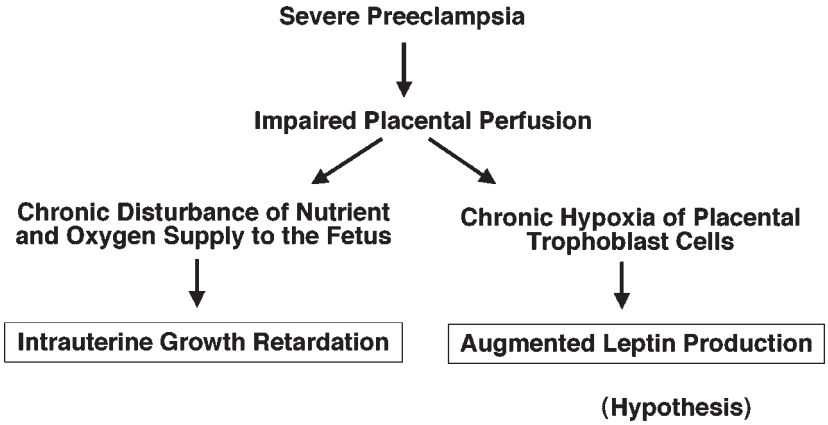

Fig. 2. Relationship between augmented leptin secretion and fetal growth restriction in severe preeclampsia (a hypothesis). Impaired placental perfusion causes both augmentation of placental leptin production and fetal growth retardation. Hyperleptinemia be associated with fetal growth retardation, as is discussed in the experiment with Tg mice overexpressing leptin.

34). Leptin concentration in both maternal plasma and placental tissue is increased in these patients. The physiologic role of insulin in the regulation of leptin production in human placenta has been proposed *(33)*. On the other hand, plasma leptin levels in pregnant women with mild gestational diabetes mellitus were lower compared with women with normal glucose tolerance after adjusting for BMI and fasting insulin levels *(35)*. Leptin gene expression in adipocyte has been reported to be stimulated by insulin *(36,37)*.

Leptin gene expression is also augmented in hydatidiform mole *(38)*. Augmented leptin expression in the placenta may stimulate the proliferation of trophoblast, since trophoblast expresses functional leptin receptor *(39,40)*.

Analysis of the promoter region of leptin gene in mouse and human adipose tissue demonstrated that CCAAT/enhancer binding protein (C/EBP)α is involved in the adipocyte-specific transcription of the mouse and human leptin genes *(41–44)*. Miller et al. *(44)* reported that only 217 bp of 5' sequence are required for basal adipose tissue–specific expression of the leptin gene as well as enhanced expression by C/EBPα. On the other hand, Ebihara et al. *(45)* reported that DNA sequences between −1885 and −1830 of human leptin 5'-flanking sequences are involved in the trophoblast-specific transcription of human leptin gene. By electrophoresis mobility shift assay, this sequence is not blocked by oligonucleotide encompassing the consensus C/EBP-binding site *(45)*. Moreover, leptin gene expression in trophoblast was enhanced by activation of protein kinase C *(32)*, whereas leptin gene expression in adipose tissue was inhibited *(46)*. These findings together suggest that regulation of leptin gene expression in trophoblast is different from that in adipose tissue. Elucidation of the precise mechanism and physiologic significance of augmentation of leptin gene expression in the placenta of complicated pregnancy requires further investigation.

Possible Effect of Hyperleptinemia on Mother and Fetus

We further examined the effects of hyperleptinemia on the course of pregnancy by using Tg mice overexpressing leptin. In mice, the leptin gene is not expressed in the placenta. Therefore, we generated Tg mice overexpressing leptin under the control of the liver-specific human serum amyloid P component (SAP) promoter. The human SAP promoter is highly specific to the liver and is active only after birth *(6,19)*. Plasma leptin levels in nonpregnant Tg mice at 12 wk of age were 10 times higher than those in control non-Tg mice. Daily urinary norepinephrine excretion in Tg mice was significantly higher than in non-Tg mice, indicating activation of the SNS by heperleptinemia *(8)*. Pregnant Tg mice were obtained by mating normal male non-Tg mice to female Tg mice (10–12 wk of age).

In mice, placenta secretes soluble leptin receptor into maternal circulation, which binds to leptin and inhibits leptin clearance from plasma *(47)*. Thus, in non-Tg mice, plasma leptin levels increased 20-fold during pregnancy. On the other hand, before pregnancy, plasma leptin levels in Tg mice overexpressing leptin were 10 times higher than those in non-Tg control mice, and, interestingly, they further elevated sevenfold, to approx 600 ng/mL in late pregnancy.

In pregnant non-Tg mice, the elevated plasma leptin levels did not suppress the amount of food intake throughout pregnancy, suggesting the so-called leptin resistance in pregnant mice. Leptin resistance in rodents and obese humans is proposed to be related to decreased cerebrospinal fluid/serum leptin ratio, which is regulated by the transport of leptin across the blood-brain barrier *(48,49)*. However, in pregnant Tg mice overexpressing leptin, food intake was significantly suppressed in late pregnancy compared with pregnant non-Tg mice, suggesting the possibility that even

in the pregnant state, leptin can be functional when its level is high enough *(50)*.

Blood pressure in the nonpregnant Tg mice was significantly higher than that in control nonpregnant non-Tg mice *(8)*. The systolic blood pressure of Tg mice at d 18 of pregnancy (113 ± 5.0 mmHg, $n = 4$) showed a higher tendency than that of non-Tg mice (102.6 ± 2.6 mmHg, $n = 4$), although the difference was not statistically significant. The litter size of Tg mice overexpressing leptin at d 19 of pregnancy (7.5 ± 1.29; mean ± SD, $n = 4$) was similar to that of non-Tg control mice (7.25 ± 1.26, $n = 4$). When the mean fetal body weight of each litter at d 19 of pregnancy was used for statistical analysis, the mean fetal body weights of Tg mice overexpressing leptin (0.95 ± 0.03 g, $n = 4$) were significantly ($p < 0.001$) less than those of non-Tg control mice (1.22 ± 0.01, $n = 4$) *(50)*. These results suggest that hyperleptinemia may affect fetal growth by modulating maternal food intake and vascular tone.

Another major function of leptin is regulation of glucose metabolism and insulin sensitivity *(6,19,51)*. In an experimental animal with mutation of leptin receptor, pregnancy spontaneously induced gestational diabetes *(52)*. Interestingly, administration of leptin prevented development of gestational diabetes *(52)*. Although these data are based on rodent experiments, it is possible that hyperleptinemia may also affect fetal growth through this pathway, since the maternal plasma glucose level is one of the essential nutrients for fetal growth. Further study on the regulation of maternal glucose metabolism by leptin is necessary to confirm the role of placental leptin in fetal growth in humans.

Other Placental Regulators of Maternal Energy Metabolism

Maternal insulin sensitivity is regulated by various factors including placenta-derived hormones (Fig. 3). Several placenta-derived hormones, such as PRL, human placental lactogen (hPL), and steroid hormones, are considered to decrease insulin sensitivity *(16)*. Placental production of these hormones is increased in accordance with the increasing size of the placenta.

Resistin is a newly identified adipocyte-derived hormone that decreases insulin sensitivity and increases plasma glucose concentration, thus contributing to the development of type II diabetes mellitus *(17)*. Resistin is proposed to link obesity to insulin resistance *(53)*. Adipocyte secretes various substances that modulate insulin sensitivity, such as free fatty acid, tumor necrosis factor-α, and leptin. Resistin was recently cloned by Steppan et al. *(17)* as a substance whose expression in adipose tissue decreases with treatment with thiazolidinedione, an antidiabetic drug. Thus, resistin is proposed to be a major factor that induces insulin resistance and hyperglycemia in obese individuals.

In experiments with mice, pretreatment with recombinant resistin significantly increased plasma glucose levels after glucose injection and blunted the suppression of plasma glucose levels by insulin injection *(17)*. Thus, resistin interferes with insulin action and increases plasma glucose levels in vivo. Therefore, we examined the resistin gene expression in the human placenta.

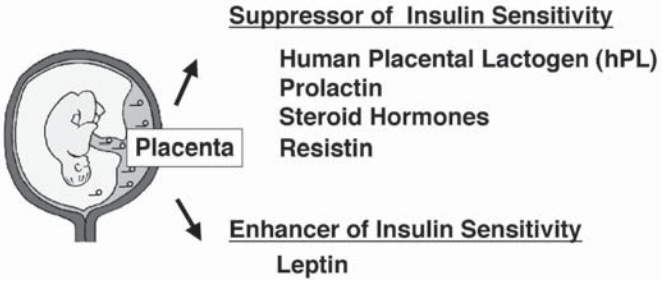

Fig. 3. Regulation of maternal insulin sensitivity by placenta-derived hormones. Human placenta secretes various hormones that modulate insulin sensitivity. hPL, PRL, and steroid hormones are proposed to suppress insulin sensitivity. Leptin has been reported to improve insulin sensitivity in several animal studies, as described in the text. Resistin is a newly identified adipocyte-derived hormone that reduces insulin sensitivity in animal studies. Human placenta expresses resistin gene and protein (unpublished data).

Northern blot analysis revealed resistin mRNA expression in term placenta as well as in the amniotic membrane (unpublished finding). Resistin mRNA expression was also detected in a trophoblastic cell line (BeWo cells), and a very faint band was detected in decidua vera tissue. *In situ* hybridization and immunohistochemistry suggest the expression of resistin in placental villi, mainly in syncytiotrophoblast. Resistin protein was secreted from cultured placental tissue (unpublished study). Resistin gene expression in term placental tissue was significantly larger than that in chorionic villous tissue in the first trimester ($p < 0.01$). By contrast, the resistin gene was expressed to a lesser degree in adipose tissue than in term placental tissue. Moreover, resistin gene expression in the adipose tissue of pregnant women at term did not differ from that of nonpregnant women. Because resistin is supposed to induce insulin resistance and increase plasma glucose level *(17,53)*, it is possible that placental resistin may contribute to regulation of maternal glucose metabolism in concert with various placental hormones including leptin.

On the other hand, as mentioned previously, human placenta produces leptin, a well-known adipoctye-derived hormone that increases insulin sensitivity and corrects the hyperglycemia in leptin-deficient *ob/ob* mice *(18)*. Moreover, in the present study, we introduced resistin as a new member of placental hormone in humans.

Other Functions of Placental Leptin

Leptin affects various peripheral functions, such as glucose and lipid metabolism *(6,19)*, insulin sensitivity *(51,54)*,

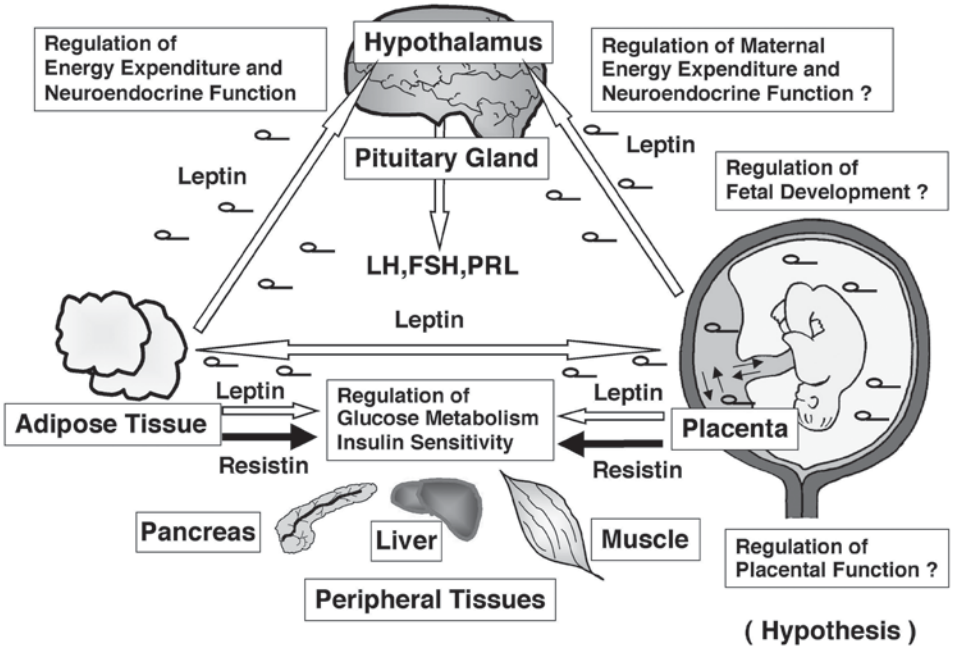

Fig. 4. Physiologic functions of placental leptin. Placenta-derived leptin may act on the hypothalamus and regulate the maternal energy expenditure and neuroendocrine functions, since leptin receptor is expressed in the hypothalamus. Placenta-derived leptin may also affect glucose metabolism in the liver, pancreas, and muscle, since these organs express functional leptin receptors. In addition, placenta secretes leptin into fetal circulation, although the role of leptin in the fetal growth and development has not been fully elucidated. It is possible that placenta-derived resistin may modulate maternal glucose metabolism since administration of resistin decreases insulin sensitivity in mice.

hematopoiesis *(55,56)*, angiogenesis *(57)*, and blood pressure *(7,8)*. Leptin receptor is expressed in various tissues of whole body, such as hypothalamus, muscle, liver, and adipose tissue *(20,24,58)*. Thus, it is plausible that in addition to adipose tissue–derived leptin, placenta-derived leptin may act on the hypothalamus and regulate the maternal energy expenditure and neuroendocrine functions *(4,5)* (Fig. 4). On the other hand, placental leptin may also act on maternal peripheral tissues, such as muscle, liver, or pancreas, and regulate glucose metabolism and insulin sensitivity *(6,19,54)*. In addition, placental leptin is transferred to the fetus *(28–30)* and may regulate fetal development and growth *(58)*. It has also been proposed that leptin stimulates myelopoiesis, erythropoiesis, and lymphopoiesis and probably promotes maturation of the fetal immune system *(59)*. Placental leptin may play a role in implantation since cytotrophoblast expresses leptin receptor and leptin stimulates matrix metalloproteinase expression in cytotrophoblast cells *(60)*. Thus, disorder of leptin metabolism and/or function in the placenta may be implicated in the pathogenesis of recurrent miscarriage *(15)*.

The present study revealed that resistin is expressed in the human placenta, and that the expression in this tissue is higher than that in adipose tissue. Thus, it is plausible that placenta-derived resistin may have physiologic significance in the regulation of maternal glucose metabolism by decreasing insulin sensitivity during human pregnancy. Figure 4 schematically illustrates the hypothesis of physiologic functions of placental leptin and resistin.

Conclusion

Human placenta produces leptin and secretes it into both maternal and fetal circulation. Abundant expression of leptin receptor in whole body suggests the physiologic and pathophysiologic significance of leptin in fetal growth in normal and complicated pregnancy. Human placenta produces various substances that regulate glucose metabolism, such as hPL, PRL, and steroid hormones. Moreover, resistin has been newly introduced as a possible placenta-derived regulator of glucose metabolism during pregnancy. However, the regulatory mechanism of resistin gene expression in the human placenta has not yet been elucidated. Further investigation on the effects of these hormones on the maternal glucose metabolism and insulin sensitivity may provide a better understanding of the placental role in the maternal energy metabolism and fetal growth.

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References

1. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). *Nature* **372,** 425–432.
2. Campfield, L. A., Smith, F. J., Guisez, Y., Devos, R., and Burn, P. (1995). *Science* **269,** 546–549.
3. Halaas, J. L., Gajiwala, K. S., Maffei, M., et al. (1995). *Science* **269,** 543–546.
4. Chehab, F. F., Lim, M. E., and Lu, R. (1996). *Nat. Genet*. **12,** 318–320.
5. Yura, S., Ogawa, Y., Sagawa, N., et al. (2000). *J. Clin. Invest*. **105,** 749–755.
6. Ogawa, Y., Masuzaki, H., Hosoda, K., et al. (1999). *Diabetes* **48,** 1822–1829.
7. Shek, E. W., Brands, M. W., and Hall, J. E. (1998). *Hypertension* **31(Pt. 2),** 409–414.
8. Aizawa-Abe, M., Ogawa, Y., Masuzaki, H., et al. (2000). *J. Clin. Invest*. **105,** 1243–1252.
9. Masuzaki, H., Ogawa, Y., Sagawa, N., et al. (1997). *Nat. Med.* **3,** 1029–1033.
10. Holness, M. J., Munns, M. J., and Sugden, M. C. (1999). *Mol. Cell. Endocrinol*. **157,** 11–20.
11. Ahima, R. S. and Flier, J. S. (2000). *Annu. Rev. Physiol.* **62,** 413–437.
12. Henson, M. C. and Castracane, V. D. (2000). *Biol. Reprod.* **63,** 1219–1228.
13. Ashworth, C. J., Hoggard, N., Thomas, L., Mercer, J. G., Wallace, J. M., and Lea, R. G. (2000). *Rev. Reprod.* **5,** 18–24.
14. Reitman, M. L., Marcus-Samuels, B., and Gavrilova, O. (2001). *Biochem. Soc. Trans.* **29(Pt. 2),** 68–72.
15. Bajoria, R., Sooranna, S. R., Ward, B. S., and Chatterjee, R. (2002). *Placenta* **23,** 103–115.
16. Abrams, B. and Pickett, K. E. (1999). In: *Maternal-fetal medicine*, 4th ed. Creasy, R. K. and Resnik, R. (eds.). W.B. Saunders: Philadelphia.
17. Steppan, C. M., Bailey, S. T., Bhat, S., et al. (2001). *Nature* **409,** 307–312.
18. Pelleymounter, M. A., Cullen, M. J., Baker, M. B., et al. (1995). *Science* **269,** 540–543.
19. Masuzaki, H., Ogawa, Y., Aizawa-Abe, M., et al. (1999). *Diabetes* **48,** 1615–1622.
20. Tartaglia, L. A., Dembski, M., Weng, X., et al. (1995). *Cell* **83,** 1263–1271.
21. Chebab, F. F., Mounzih, K., Lu, R., and Lim, M. E. (1997). *Science* **275,** 88–90.
22. Ahima, R. S., Dushay, J., Flier, S. N., Prabakaran, D., and Flier, J. S. (1997). *J. Clin. Invest.* **99,** 391–395.
23. Yu, W. H., Kimura, M., Walczewska, A., Karanth, S., and McCann, S. M. (1997). *Proc. Natl. Acad. Sci. USA* **94,** 1023–1028.
24. Karlsson, C., Lindell, K., Svensson, E., et al. (1997). *J. Clin. Endocrinol. Metab.* **82,** 4144–4148.
25. Butte, N., Hopkinson, J., and Nicholson, M. (1997). *J. Clin. Endocrinol. Metab.* **82,** 585–589.
26. Schubring, C., Kiess, W., Englaro, P., et al. (1997). *J. Clin. Endocrinol. Metab.* **82,** 1480–1483.
27. Yura, S., Sagawa, N., Mise, H., et al. (1998). *Am. J. Obstet. Gynecol.* **178,** 926–930.
28. Linnemann, K., Malek, A., Sager, R., Blum, W. F., Schneider, H., and Fusch, C. (2000). *J. Clin. Endocrinol. Metab.* **85,** 4298–4301.
29. Hoggard, N., Crabtree, J., Allstaff, S., Abramovich, D. R., and Haggarty, P. (2001). *Placenta* **22,** 347–352.
30. Lepercq, J., Challier, J. C., Guerre-Millo, M., Cauzac, M., Vidal, H., and Hauguel-de Mouzon, S. (2001). *J. Clin. Endocrinol. Metab.* **86,** 2409–2413.
31. Mise, H., Sagawa, N., Matsumoto, T., et al. (1998). *J. Clin. Endocrinol. Metab.* **83,** 3225–3229.
32. Yura, S., Sagawa, N., Ogawa, Y., et al. (1998). *J. Clin. Endocrinol. Metab.* **83,** 3609–3614.
33. Lepercq, J., Cauzac, M., Lahlou, N., et al. (1998). *Diabetes* **47,** 847–850.
34. Lea, R. G., Howe, D., Hannah, L. T., Bonneau, O., Hunter, L., and Hoggard, N. (2000). *Mol. Hum. Reprod.* **6,** 763–769.
35. Festa, A., Shnawa, N., Krugluger, W., Hopmeier, P., Schernthaner, G., and Haffner, S. M. (1999). *Diabet. Med.* **16,** 656–662.
36. MacDougald, O. A., Hwang, C. H., Fan, H., and Lane, M. D. (1995). *Proc. Natl. Acad. Sci. USA* **92,** 9034–9037.
37. Rentsch, J. and Chiesi, M. (1996). *FEBS Lett*. **379,** 55–59.
38. Sagawa, N., Mori, T., Masuzaki, H., Ogawa, Y., and Nakao, K. (1997). *Lancet* **350,** 1518–1519.
39. Henson, M., Swan, K., and O'Neil, J. S. (1998). *Obstet. Gynecol.* **92,** 1020–1028.
40. Bodner, J., Ebenbichler, C., Wolf, H., et al. (1999). *Placenta* **20,** 677–682.
41. He, Y., Chen, H., Quon, M. J., and Reitman, M. (1995). *J. Biol. Chem.* **270,** 28,887–28,891.
42. Hwang, C. H., Mandrup, S., MacDougald, O. A., Geiman, D. E., and Lane, M. D. (1996). *Proc. Natl. Acad. Sci. USA* **93,** 873–877.
43. De la Brousse, F. C., Shan, B., and Chen, J. L. (1996). *Proc. Natl. Acad. Sci. USA* **93,** 4096–4101.
44. Miller, S. G., deVos, P., Guerre-Millo, M., et al. (1996). *Proc. Natl. Acad. Sci. USA* **93**, 5507–5511.
45. Ebihara, K., Ogawa, Y., Isse, N., et al. (1997). *Biochem. Biophys. Res. Commun.* **241,** 658–663.
46. Pineiro, V., Casabiell, X., Peino, R., Garcia-Vallejo, L., Dieguez, C., and Casanueva. F. F. (1998). *Biochem. Biophys. Res. Commun.* **252,** 345–347.
47. Gavrilova, O., Barr, V., Marcus-Samuels, B., and Reitman, M. (1997). *J. Biol. Chem.* **272,** 30,546–30,551.
48. Caro, J. F., Kolaczynski, J. W., Nyce, M. R., et al. (1997). *Lancet* **348,** 159–161.
49. Kastin, A. J. and Pan, W. (2000). *Regul. Pept.* **92,** 37–43.
50. Sagawa, N., Yura, S., Itoh, H., et al. (2002). *Placenta* **23(Suppl. A,** *Trophoblast. Res.* **16**), S80–S86.
51. Kim, Y. B., Uotani, S., Pierroz, D. D., Flier, J. S., and Kahn, B. B. (2000). *Endocrinology* **141,** 2328–2339.
52. Yamashita, H., Shao, J., Ishizuka, T., et al. (2001). *Endocrinology* **142,** 2888–2897.
53. Flier, J. S. (2001). *Nature* **409,** 292–293.
54. Ebihara, K., Ogawa, Y., Masuzaki, H., et al. (2001). *Diabetes* **50,** 1440–1448.
55. Cioffi, J. A., Shafer, A. W., Zupancic, T. J., et al. (1996). *Nat. Med.* **2,** 585–589.
56. Mikhail, A. A., Beck, E. X., Shafer, A., et al. (1997). *Blood* **89,** 1507–1512.
57. Sierra-Honigmann, M. R., Nath, A. K., Murakami, C., et al. (1998). *Science* **281,** 1683–1686.
58. Hoggard, N., Hunter, L., Duncan, J. S., Williams, L. M., Trayhurn, P., and Mercer, J. G. (1997). *Proc. Natl. Acad. Sci. USA* **94,** 11,073–11,078.
59. Gainsford, T., Wilson, T. A., Metcalf, D., et al. (1996). *Proc. Natl. Acad. Sci. USA* **93,** 14,564–14,568.
60. Castellucci, M., Matteis, R., Meisser, A., et al. (2000). *Mol. Hum. Reprod.* **6,** 951–958.