Endocrine, *vol. 27, no. 1, 75–81, June 2005* 0969–711X/05/27:75–81/$30.00

CASE

Secretion of Leptin Throughout Pregnancy and Early Postpartum Period in Japanese Monkeys

Placenta as Another Potential Source of Leptin

Cheng Wang,[1,2] Mohamed S. Medan,[1,3] Keiko Shimizu,[4] Chihiro Kojima,[1] Mariko Itoh,[4] Gen Watanabe,[1,5] and Kazuyoshi Taya[1,5]

[1]Laboratory of Veterinary Physiology, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, 183-8509, Japan; [2]College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, 210095,China; [3]Department of Theriogenology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt; [4]Department of Cellular and Molecular Biology, Primate Research Institute, Kyoto University, Aichi 484-8506, Japan; and [5]Department of Basic Veterinary Science, The United Graduate School of Veterinary Sciences, Gifu University, Gifu, 501-1183, Japan

Leptin is one of the most important factors linking nutrition and reproduction. In the present study, plasma concentrations of leptin during pregnancy and early lactation in the Japanese monkey were determined. Plasma concentrations of gonadotropins, immunoreactive (ir-)inhibin, and steroid hormones were also measured. Plasma concentrations of leptin significantly increased during the second quarter of pregnancy and progressively elevated throughout pregnancy. During the fourth quarter of pregnancy, leptin levels reached up to 89 and 64 times of those during pre-pregancy and first quarter of pregnancy periods, respectively. After parturition, the circulating leptin level abruptly decreased. During the first 10 d of lactation, its average level decreased to the levels of the second quarter of pregnancy. Plasma ir-inhibin and estradiol-17β were elevated throughout the pregnancy and decreased after parturition, and both of them were positively correlated with leptin levels during the whole pregnancy and early lactation. Plasma concentrations of progesterone significantly increased during the first quarter of pregnancy and kept at a higher level compared with pre-pregnancy and sharply decreased after parturition. Placental homogenates contain a large amount of leptin protein. These results suggest that placenta secretes a large amount of leptin and may be another source of leptin during pregnancy in Japanese monkeys. In addition, high correlations among leptin, ir-inhibin, and estradiol-17β during these stages suggest that these hormones may have important regulating roles on leptin secretion during pregnancy in the Japanese monkey.

Received April 4, 2005; Revised May 8, 2005; Accepted June 1, 2005.

Author to whom all correspondence and reprint requests should be addressed: Kazuyoshi Taya, DVM, PhD, Laboratory of Veterinary Physiology, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8, Saiwai-cho, Fuchu, Tokyo 183-8509, Japan. E-mail: taya@cc.tuat.ac.jp 22

Key Words: Leptin; pregnancy; placenta; Japanese monkey; lactation.

Introduction

Leptin, a polypeptide hormone secreted mainly by adipocytes, plays important roles in the regulation of food intake and energy expenditure *(1–4)* and is linked to a variety of reproductive processes in mammals. Previous studies have demonstrated that maternal peripheral leptin levels are enhanced during pregnancy *(5–12)* and they collectively showed that leptin concentrations reached a peak in the second trimester and remained elevated until parturition. Determination of specific leptin mRNA transcripts in human placental tissue by reverse transcription-polymerase chain reaction (RT-PCR) at term *(13,14)* and by Northern analysis in both first and third trimesters *(15)* supports a leptin-producing role for the placenta. Intriguingly, in humans, placenta may be both a source of leptin and a target for its action, as leptin *(13)* and leptin-receptor mRNA *(16,17)* have been detected in placental trophoblasts. Localization of leptin protein in the syncytiotrophoblast by immunohistochemistry *(14,15)* indicates that at least a portion of the increase noted in the maternal circulation with advancing gestation is of placental origin in humans, rodents, and farm animals *(18)*. A significant increase in the maternal leptin concentrations and a decline in leptin mRNA abundance in syncytiotrophoblast are common features of advancing gestational age in human pregnancy. However, in rodents, conflicting results have been reported regarding production of leptin by placenta *(19–23)*. In pregnant mice, although leptin mRNA *(21)* expression and protein secretion from adipose tissues increased, leptin protein did not constitutively secrete in placenta *(24)*. Therefore, it seems that the regulation of leptin secretion during pregnancy differs among the mammalian species. In humans, maternal circulating leptin

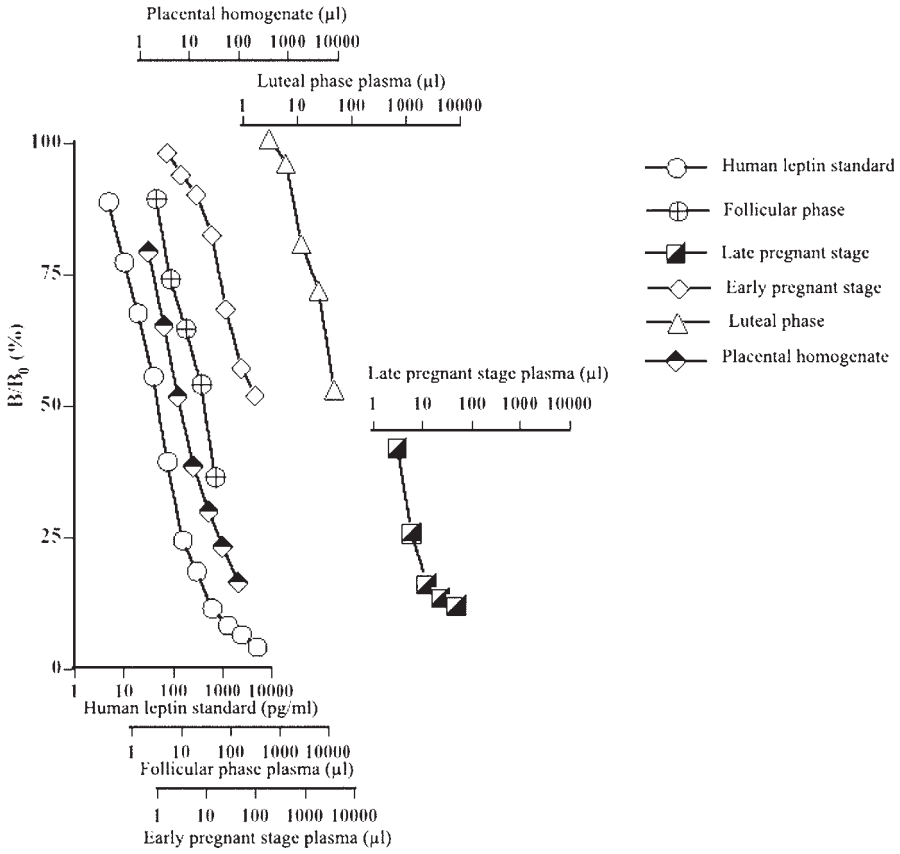

Fig. 1. Dose–response curves of plasma collected during different reproductive stages and placental homogenate of Japanese monkeys with standard in leptin radioimmunoassay. Each value represents the mean of triplicate determinations.

levels are elevated during pregnancy and declined after delivery to prepregnancy levels *(5)*. Circulating estrogen concentrations rise with advancing primate gestation and play key roles in placental progesterone biosynthesis and fetal adrenal maturation *(17,25)*. Previous studies suggested that leptin production is similarly regulated by estrogen *(12,26)*.

Japanese monkey (*Macaca fuscata*) shows seasonal breeding under natural conditions *(27,28)*. Owing to its special breeding characteristics and also owing to the different regulation of leptin secretion during pregnancy, studies on the leptin secretion during pregnancy in Japanese monkey may give us a further understanding on the regulation of leptin secretion during pregnancy. Therefore, the present study was designed to examine the secretory pattern of the leptin in the Japanese monkey and to determine the relationship between leptin, immunoreactive (ir-)inhibin, steroid hormones, and gonadotropins in peripheral plasma during prepregnancy, pregnancy, and early postpartum periods. Placenta-derived leptin secretion was also examined by measuring leptin content in placental homogenate.

Results

Characterization of Leptin Assays

Competition between labeled and unlabeled antigens within peripheral plasma of normal female Japanese monkeys during different reproductive stages produced good dose–response curves and exhibited parallelism with the standard curve. This indicates that it is possible to measure leptin concentrations in both plasma and placental homogenate using the human leptin RIA systems (Fig. 1).

Hormonal Changes During Pre-Pregnancy, Pregnancy, and Early Lactation

The data of individual animals were divided into six groups according to the stage of pregnancy, i.e., pre-pregnancy, first, second, third, and fourth quarter of pregnancy, and post-parturition. During pre-pregnancy and the first quarter of pregnancy, circulating levels of leptin were low (0.511 ± 0.110 and 0.717 ± 0.082 ng/mL, respectively). During the second quarter of the pregnancy, circulating levels of leptin increased significantly compared with that of pre-pregnancy and the first quarter of the pregnancy ($p < 0.01$). After that, circulating levels of leptin sharply increased and reached a peak at the fourth quarter of the pregnancy. Compared with the levels during pre-pregnancy and the first quarter of pregnancy, circulating levels of leptin during the fourth quarter of pregnancy increased 89 times and 64 times (from 0.511 ± 0.110 and 0.717 ± 0.082 ng/mL, respectively, to 45.576 ± 5.162 ng/mL, $p < 0.0001$). After parturition, circulating leptin level sharply decreased, and, during the early

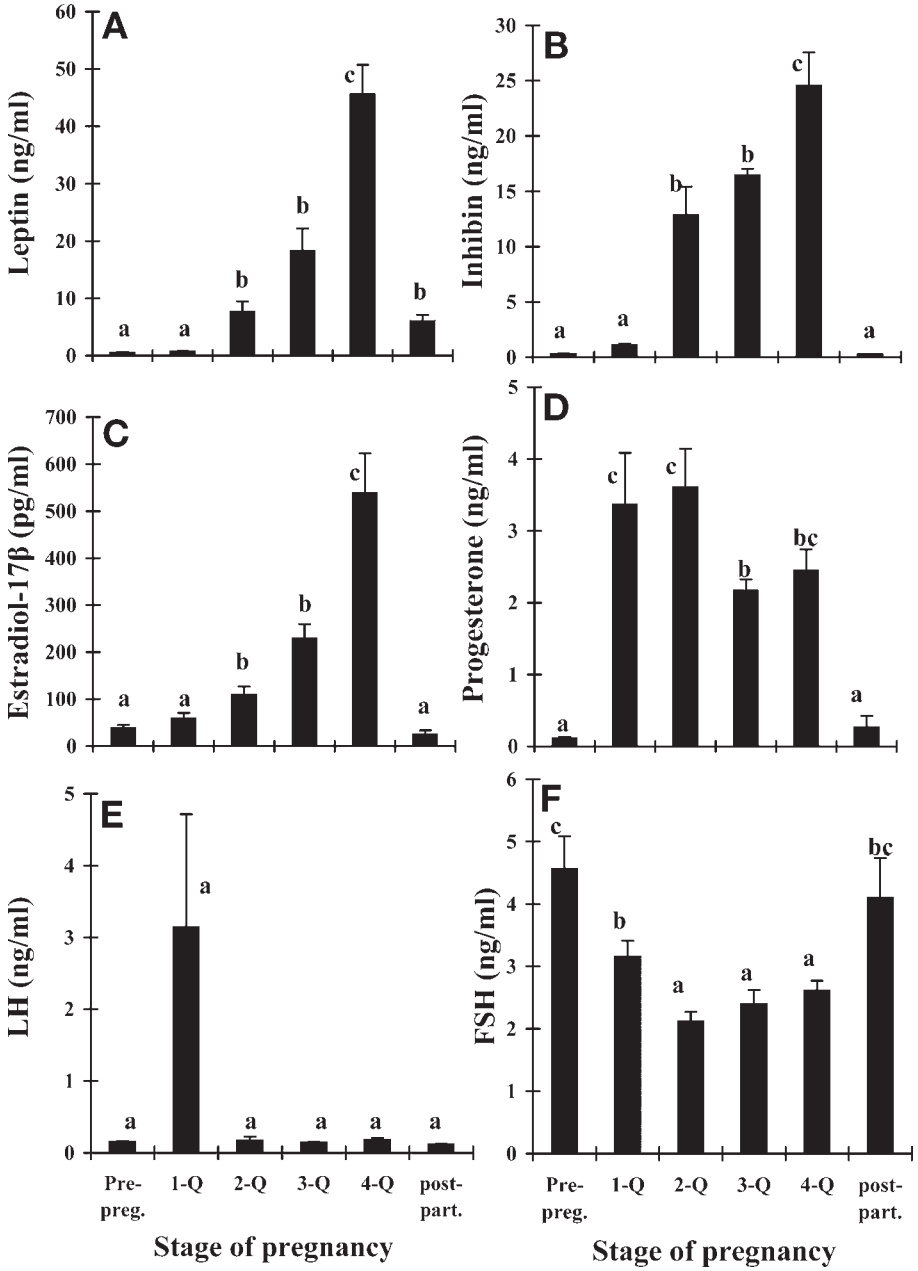

Fig. 2. Changes in the plasma concentrations of leptin (**A**), ir-inhibin (**B**) estradiol-17β (**C**), progesterone (**D**), LH (**E**), and FSH (**F**) during pre-pregnancy (pre-preg.), first quarter (1-Q), second quarter (2-Q), third quarter (3-Q), fourth quarter (4-Q) of pregnancy, and post-partum (post-part.) periods in Japanese monkeys. Each value represents the mean ± SEM of six monkeys. Means without a common character differ significantly ($p < 0.05$).

lactation, the average level was equal to that of the second quarter of pregnancy (Fig. 2A).

During the first quarter of pregnancy, no significant change was observed in the plasma concentration of ir-inhibin. A significant increase was observed since the second quarter of pregnancy ($p < 0.001$), and this increase continued through the third quarter of pregnancy and reached its peak during the fourth quarter of pregnancy. After parturition, plasma ir-inhibin sharply decreased to the levels of the pre-pregnancy (Fig. 2B).

In all of the experimental animals, the plasma concentration of estradiol-17β during the first quarter of pregnancy was not significantly changed compared with that of the pre-pregnant period. During the second quarter of pregnancy, circulating levels of estradiol-17β significantly increased compared with pre-pregnancy ($p<0.05$). It increased through the third quarter of pregnancy reaching its peak at the fourth quarter of pregnancy. Compared with the pre-pregnancy, circulating levels of estradiol-17β during the fourth quarter of pregnancy are more than ten times higher ($p < 0.0001$; Fig. 2C). In addition, plasma concentrations of progesterone significantly increased during the first quarter of pregnancy and remained at a high level until parturition (Fig. 2D). There was a nonsignificant elevation of lutein-

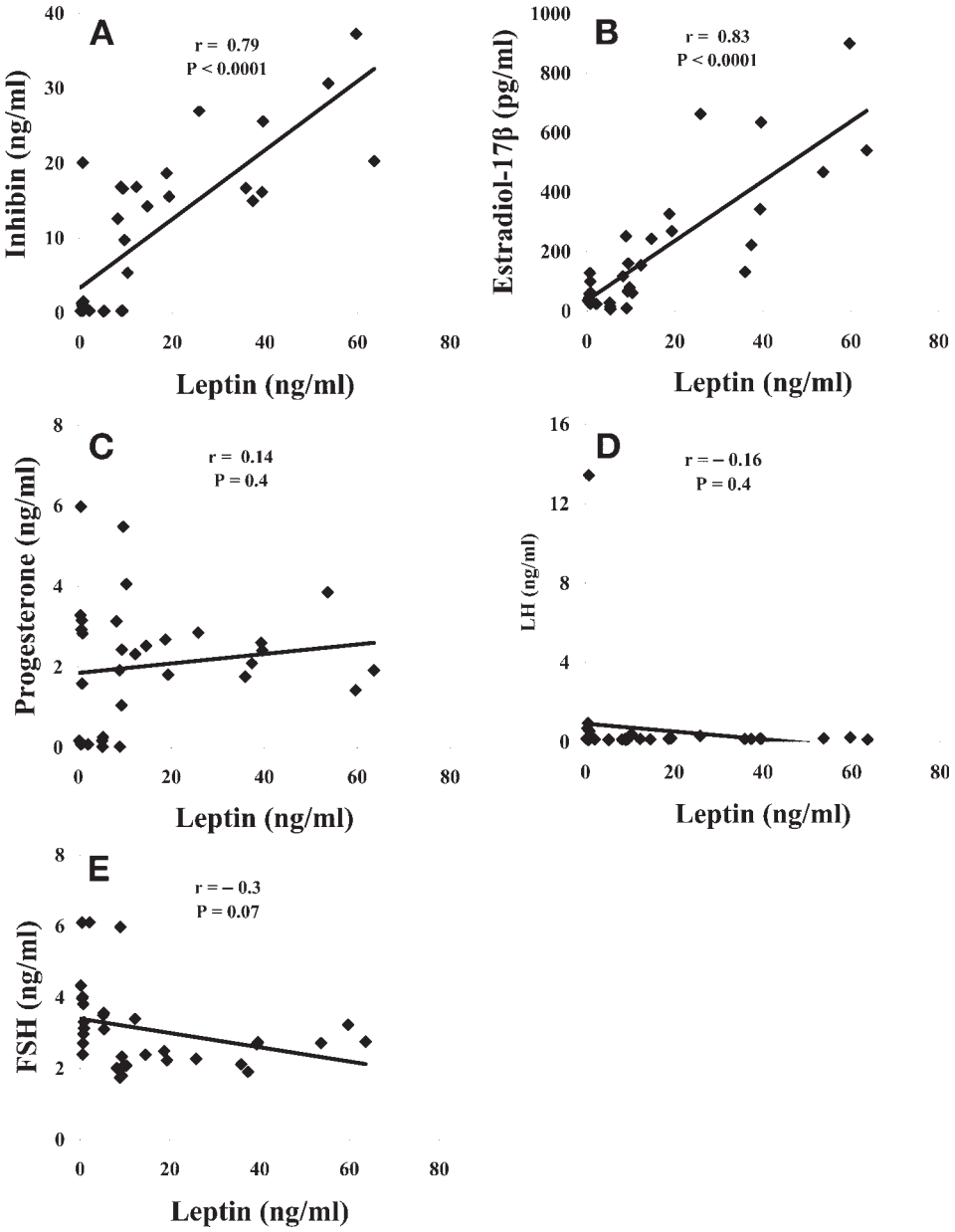

Fig. 3. Correlation between leptin and ir-inhibin (**A**), estradiol-17β (**B**), progesterone (**C**), LH (**D**), and FSH (**E**) of Japanese monkeys. Coefficients (*r*) and *p* values are indicated.

izing hormone (LH) during the first quarter of pregnancy (Fig. 2E) and significant low levels of follicle-stimulating hormone (FSH) in the pregnant females from first to fourth quarter compared with the pre-pregnancy (Fig. 2F).

Correlation of Leptin with Ir-Inhibin, Estradiol-17β, Progesterone, LH, FSH

There was a strong positive correlation between plasma concentration of leptin and ir-inhibin ($r = 0.79$, $p < 0.0001$) (Fig. 3A). Furthermore, a strong positive correlation also existed between the plasma concentration of leptin and estradiol-17β ($r = 0.83$, $p < 0.0001$) (Fig. 3B). No significant correlation existed among leptin, progesterone, LH and FSH (Figs. 3C,D,E).

Placental Leptin

As shown in the dose–response curve (Fig. 1), placental homogenate dose–response curve was well paralleled with the human leptin standard. It contained a high level of leptin (7.25 ± 0.226 ng/g placental tissue). This showed that placenta could secrete relatively large amount of leptin and it may be another potential source of leptin.

Discussion

Japanese monkey shows a clear seasonal breeding under natural conditions and is one of the nonhuman primates used as a model for the human physiology investigation. Although leptin is known as an important factor linking

metabolism and endocrinology and a factor with a strong effect on the regulation of reproduction, its secretion during pregnancy and lactation in Japanese monkey is still unknown. Whether placenta is another source of leptin during pregnancy in this species is still unclear. The present study clearly showed that, in the Japanese monkey, circulating leptin levels significantly increased beginning from the second quarter of the pregnancy and peaked during the fourth quarter of pregnancy. After parturition, circulating leptin levels sharply decreased to the level of pre-pregnancy. Supporting the present findings, several studies have demonstrated that maternal peripheral leptin levels are enhanced during pregnancy *(5–10,12)*. In human pregnancy, maternal serum leptin concentrations are greater than those of nonpregnant women *(5)*. By analyzing large number of samples around the term, Henson and Castracane *(29)* suggested that maternal serum leptin concentrations increased throughout pregnancy. However, following midterm, leptin levels appeared to decline prior to parturition. In nonhuman primate pregnancy, peripheral serum leptin concentrations in cycling versus postpartum and nonpregnant baboons were similar, but in pregnant animals, leptin concentrations in circulation were dramatically higher than in either cycling or postpartum baboons *(30,31)*. In rodents, such as pregnant mice, serum leptin levels peaked on d 17 and were many folds higher than the nonpregnant levels. Leptin mRNA in adipose tissue increased several folds over that in nonpregnant *(21,32)*. In the pregnant rats, serum leptin increases during pregnancy *(33)*, and declined before parturition *(34)*.

The placenta is a peculiar organ that, in addition to the regulation of respiratory and nutrients exchange between mother and fetus, intervenes in a variety of endocrine functions. Some of these mimic the function of the body endocrine glands, but many other unsolved functions are far from being understood *(35)*. The present study clearly demonstrated that large amounts of leptin were present in placental homogenate of Japanese monkey obtained just before term. Thus, the placenta-derived leptin could at least partially contribute to the hyperleptinemia during middle and late stages of pregnancy reported here, which were approx 50- to 100-fold higher than those during pre-pregnancy and the first quarter of pregnancy. Supporting the present hypothesis, several studies have demonstrated that placenta can produce leptin *(13–15)*. The placenta may be both a source of leptin and a target for its action, as leptin *(13)* and leptin-receptor mRNA *(16,36)* have been detected in human placental trophoblasts. In rodents, leptin mRNA transcripts are expressed in various tissues *(22,33,34)*, including the uterus, placenta, and maternal adipose tissue. However, there are species differences in the production of leptin in the placenta. Primate placenta produces more leptin than that of rodents. The regulating mechanism and the function of leptin profile observed in the late pregnancy are still unclear. During pregnancy, the maternal metabolism adapted to support a rapidly growing conceptus and prepare for lactation. Therefore, based on the principal function of leptin as a regulator of energy homeostasis, a decline in maternal leptin levels might be expected during pregnancy to facilitate optimal nutritional intake. Conversely, as shown in the present study, circulating leptin levels are enhanced throughout gestation. The substantial increases in early pregnancy, before the occurrence of any notable increase in body weight due to progressive gestation, imply that factors other than increased adiposity mediate maternal leptin levels *(10)*. As shown in the present study, leptin was highly positively correlated with estradiol-17β and ir-inhibin throughout pregnancy, suggesting a dynamic relationship between the changing levels of leptin and reproductive hormones that are released during pregnancy. Previous work showed that circulating estrogen concentrations rise with advancing primate gestation and play key roles in placental progesterone biosynthesis and fetal adrenal maturation (*17,25,37,38)*. Other studies suggested that, in humans, leptin production is regulated by estrogen *(12,26)*, which had receptors in the adipose tissues *(39)* and was elevated during pregnancy. Estrogen has also been reported to enhance leptin mRNA expression and leptin secretion in rat adipocytes in a dose-dependent manner. Both were inhibited by a specific estrogen receptor antagonist *(40)*. Ovariectomy reduced serum leptin levels in both rodents *(41)* and humans *(42)*. Although inhibin has a strong positive correlation with plasma leptin, whether it has receptor on the adipocytes is still unknown. During pregnancy in Japanese monkeys, inhibin A, inhibin B, and activin A were elevated with gestational age and decreased after parturition and the placenta trophoblast expressed inhibin βA and βB subunit *(43,44)*. The co-expression of leptin and inhibin/activin in the placenta further suggest that there may be relationships between the regulation of leptin secretion and the production of inhibin/activin in the placenta. Further studies are needed to determine the pregnancy-specific role(s) of leptin and the regulating mechanism between circulating leptin and other factors, especially the factors derived from the ovary and placenta.

In conclusion, the present findings demonstrate that circulating leptin concentration increased from the second quarter of the pregnancy and remained elevated throughout pregnancy and sharply decreased after parturition. Placenta could secrete large amounts of leptin and may be another potential source of leptin production during pregnancy in the Japanese monkey.

Materials and Methods

Animals, Blood Sampling, and Placenta Collection

Six sexually mature female Japanese monkeys (*Macaca fuscata fuscata*) 8 yr old and weighing 7–9 kg were used in this study. Animals were kept at the Primate Research Institute of Kyoto University, Inuyama, Japan and housed indi-

vidually in an air-conditioned room with controlled temperature (20–25°C) and lighting (light-on: 06:00–18:00 h). The animals were fed a standard monkey pellet food supplemented with sweet potatoes or fruit daily, and had free access to water. Females were mated from d 11 to 15 of their menstrual cycles by introducing a male into each female's cage.

The day of LH surge was designated as d 0 of pregnancy. The pregnancy was divided into four stages as follow: the first quarter (0–39 d), the second quarter (40–79 d), the third quarter (80–119 d), and the fourth quarter (120 d until parturition). Blood samples were collected into heparinized tubes from cubital vein without anesthesia once in each quarter of pregnancy. Additional blood samples were collected before pregnancy and within 10 d after parturition. Monkeys were well acclimated to blood collection. The plasma was collected after centrifugation at 1700*g* for 10 min at 4°C and stored at −30°C until assayed for leptin, FSH, LH, estradiol-17β, ir-inhibin and progesterone concentrations. Placenta was collected at the late stage of pregnancy (d 120 of pregnancy) by caesarean section under initial anesthesia induced by 10 mg/kg ketamine hydrochloride followed by deep anesthesia with nitrous oxide gas and halothane. The fetus was used for another study. Animal care and the experimental protocol were approved by "Guidelines for the care and use of laboratory primates" prepared by the Primate Research Institute, Kyoto University, Japan (2002).

Tissues Homogenization

Placenta was homogenized with normal saline (1 g placenta/mL) using a homogenizer (Phiscotoron, Nichion, Tokyo, Japan), centrifuged at 25,000*g* for 30 min at 4°C, and the supernatant was collected and kept at −20°C for leptin RIA.

Hormone Assays

Leptin

Immunoreactive leptin concentrations in plasma and placental homogenates were measured using a human double-antibody radioimmunoassay (RIA). This employed recombinant human leptin for radioiodination (# AFP496C iodinated by the chloramine T procedure) and reference standard. Both standard and samples were then incubated with multi-species leptin antibody (458LxL LINCO Research Inc., USA) diluted in buffer (0.05 *M* EDTA, 0.05 *M* PBS) containing 0.4% normal rabbit serum. After the initial incubation, ^{125}I-labeled human leptin (counting 5000 cpm) were added to each tube and the incubation was continued for an additional 24 h at 4°C. The bound and free ligands were separated by adding of a specific anti-rabbit gamma globulin and incubated for 24 h at 4°C. The unbound 125I-human leptin was separated by centrifugation at 1700*g* for 30 min at 4°C and decanting of the supernatant. The remaining radioactivity in the precipitate was counted with a gamma counter. The results are expressed in terms of human leptin. The intra- and interassay coefficients of variation were 7.4% and 10.5%, respectively.

LH and FSH

Plasma concentrations of LH and FSH were measured by a heterologous double-antibody RIA using methods described previously (*28*). Anti-ovine LH serum (YM #18; kindly provided by Dr. Y. Mori, Tokyo University, Tokyo, Japan) was used as the first antibody and rat NIDDK-rat LH-I-5 was used for radioiodination. In the FSH assay, anti-ovine FSH (NIDDK-H-31) was used as the first antibody and rat-NIDDK-rat FSH-I-5 was used for radioiodination. Results are expressed in terms of NIDDK rat LH-RP-2 and rat FSH-RP-2. Because a large amount of monkey chorionic gonadotropin (mCG) existed in the plasma of pregnant monkeys and it gave displacement curves parallel to that of rat LH RP-2 standard in the LH RIA system, LH levels are referred as LH/mCG equivalents. The intra- and interassay coefficients of variation were 7.8% and 10.3% for the LH assay and 7.8% and 8.5% for the FSH assay.

Estradiol-17β and Progesterone

Plasma concentrations of progesterone and estradiol-17β were determined by the double antibody RIA system using ^{125}I-labeled radioligands as described previously (*45*). Antisera against progesterone (GDN 337) and estradiol-17β (GDN 244) were kindly provided by Dr. G. D. Niswender (Animal Production and Biotechnology, Colorado State University, Fort Collins, CO, USA). The intra- and interassay coefficients of variation were 2.8% and 5.5% for progesterone and 2.7% and 7.9% for estradiol-17β, respectively.

Ir-Inhibin

Concentrations of ir-inhibin in the plasma were measured by the double-antibody RIA based on a bovine inhibin RIA (*46*). Iodinated preparations were bovine 32 kDa (bFF 32 kDa) inhibin. The antiserum used was anti-bFF 32 kDa inhibin (TNDH-1) and the results are expressed in terms of bFF 32 kDa inhibin. The intra- and interassay coefficients of variation were 5.4 and 6.4%, respectively.

Statistics

Data are presented as means ± SEM. When there was heterogeneity of variance and the standard deviations were proportional to the means, logarithmic transformation was carried out before ANOVA. The significant changes in the concentrations of each hormone during the pre-pregnancy, pregnancy, and early lactation were analyzed by a two-way ANOVA, with animal and day of pregnancy as the two factors. Significance of difference between means was compared by Duncan's multiple range test. The linear coefficients of correlation (r) were calculated among the plasma concentrations of leptin and ir-inhibin, estradiol-17β, progesterone, LH, and FSH. All differences with values of $p < 0.05$ were considered significant.

Acknowledgments

We are grateful to Dr. A. F. Parlow and the National Institute of Diabetes and Digestives and Kidney Diseases (NIDDK), CA, USA for providing RIA materials for leptin, LH, and FSH; Dr. G. D. Niswender, Animal Reproduction and Biotechnology, Colorado State University, Fort Collins, CO, USA for providing antisera to progesterone (GDN #337) and estradiol-17β (GDN#244); Dr. Y. Mori, University of Tokyo, Tokyo, Japan, for providing antisera to ovine LH (YM#18). This work was supported in part by a Cooperation Research Program of Primate Research Institute, Kyoto University; a Grant-in-Aid for the Biodiversity Research of the 21st Century Center-of-Excellence Program, A14 to Kyoto University, and a Grant-in-Aid for Scientific Research (The 21st Century Center-of-Excellence Program, E-1 to Gifu University) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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. Lonqvist, F., Arner, P., Nordfors, L., and Schalling, M. (1995). *Nat. Med.* **1,** 950–953.
4. Considine, R. V., Sinha, M. K., and Heiman, M. L. (1996). *N. Engl. J. Med.* **334,** 292–295.
5. Hardie, L., Trayhurn, P., Abramovich, D., and Fowler, P. (1997). *Clin. Endocrinol.* **47,** 101–106.
6. Butte, N. F., Hopkinson, J. M., and Nicolson, M. A. (1997). *J. Clin. Endocrinol. Metab.* **82,** 585–589.
7. Schubring, C., Kiess, W., Englaro, P., et al. (1997). *J. Clin. Endocrinol. Metab.* **82,** 1480–1483.
8. Schubring, C., Englaro, P., Siebler, T., et al. (1998). *Horm. Res.* **50,** 276–283.
9. Helland, I. B., Reseland, J. E., Saugstad, O. D., and Drevon, C. A. (1998). *Pediatrics* **101,** E12.
10. Highman, T. J., Friedman, J. E., Huston, L. P., Wong, W. W., and Catalano, P. M. (1998). *Am. J. Obstet. Gynecol.* **178,** 1010–1015.
11. Jaquet, D., Leger, J., Levy-Marchal, C., Oury, J. F., and Czernichow, P. (1998). *J. Clin. Endocrinol. Metab.* **83,** 1243–1246.
12. Sivan, E., Whittaker, P. G., Sinha, D., et al. (1998). *Am. J. Obstet. Gynecol.* **179,** 1128–1132.
13. Hassink, S. G., de Lancey, E., Sheslow, D. V., et al. (1997). *Pediatrics* **100,** E1.
14. Senaris, R., Garcia-Caballaro, T., Casabiell, X., et al. (1997). *Endocrinology* **138,** 4501–4504.
15. Masuzaki, H., Ogawa, Y., Sagawa, N., et al. (1997). *Nat. Med.* **3,** 1029–1033.
16. Luoh, S. M., Di Marco, F., Levin, N., et al. (1997). *J. Mol. Endocrinol.* **18,** 77–85.
17. Henson, M. C. (1998). *Hum. Reprod. Update* **4,** 389–405.
18. Ashworth, C. J., Hoggard, N., Thomas, L., Mercer, J. G., Wallace, J. M., and Lea, R. G. (2000). *Rev. Reprod.* **5,** 18–24.
19. Kawai, M., Yamaguchi, M., Murakami, T., Shima, K., Murata, Y., and Kishi, K. (1997). *Biochem. Biophys. Res. Commun.* **240,** 798–802.
20. Gavrilova, O., Barr, V., Marcus-Samuels, B., and Reitman, M. (1997). *J. Biol. Chem.* **272,** 30546–30551.
21. Tomimatsu, T., Yamaguchi, M., Murakami, T., et al. (1997). *Biochem. Biophys. Res. Commun.* **240,** 213–245.
22. Amico, J. A., Thomas, A., Crowley, R. S., and Burmeister, L. A. (1998). *Life Sci.* **63,** 1387–1395.
23. Malik, N. M., Carter, N. D., Wilson, C. A., Scaramuzzi, R. J., Stock, M. J., and Murray, J. F. (2005). *Placenta* **26,** 47–52.
24. Kronfeld-Schor, N., Zhao, J., Silvia, B. A., et al. (2000). *Biol. Reprod.* **63,** 274–280.
25. Albrecht, E. D. and Pepe, G. J. (1999). *Placenta* **20,** 129–139.
26. Stock, S. M., Sande, E. M., and Bremme, K. A. (1999). *Fertil. Steril.* **72,** 657–662.
27. Nigi, H. (1975). *Primates* **16,** 207–216.
28. Watanabe, G., Nozaki, M., Taya, K., Katakai, Y., and Sasamoto, S. (1990). *Biol. Reprod.* **43,** 196–201.
29. Henson, M. C. and Castracane, V. D. (2000). *Biol. Reprod.* **63,** 1219–1228.
30. Henson, M. C., Castracane, V. D., O'Neil, J. S., et al. (1999). *J. Clin. Endocrinol. Metab.* **84,** 2543–2549.
31. Castracane, V. D., Hendrickx, A. G., and Henson, M. C. (2005). *Exp. Biol. Med.* **230,** 251–254.
32. Hoggard, N., Hunter, L., Lea, R. G., Trayhurn, P., and Mercer, J. G. (2000). *Br. J. Nutr.* **83,** 317–326.
33. Garcia, M. D. C., Casanueva, F. F., Diequez, C., and Senaris, R. M. (2000). *Biol. Reprod.* **62,** 698–703.
34. Chien, E. K., Hara, M., Rouard, M., et al. (1997). *Biochem. Biophys. Res. Commun.* **237,** 476–480.
35. Petrglia, F., Florio, P., Nappi, C., and Genazzani, A. (1996). *Endocr. Rev.* **17,** 156–186.
36. Henson, M. C., Swan, K. F., and O'Neil, J. S. (1998). *Obstet. Gynecol.* **92,** 1020–1028.
37. Nozaki, M., Watanabe, G., Taya, K., et al. (1990). *Biol. Reprod.* **43,** 444–449.
38. Kondo, M., Udono, T., Jin, W.Z., et al. (2001). *J. Endocrinol.* **168,** 257–262.
39. Pedersen, S. B., Fuglsig, S., Sjogren, P., and Richelsen, B. (1996). *Eur. J. Clin. Invest.* **26,** 1051–1056.
40. Machinal, F., Dieudonne, M. N., Leneveu, M. C., Pecquery, R., and Giudicelli, Y. (1999). *Endocrinology* **140,** 1567–1574.
41. Yoneda, N., Saito, S., Kimura, M., et al. (1998). *Horm. Metab. Res.* **30,** 263–265.
42. Messinis, I. E., Milingos, S. D., Alexandris, E., Kariotis, I., Kollios, G., and Seferiadis, K. (1999). *Hum. Reprod.* **14,** 913–918.
43. Kojima, C., Kondo, M., Jin, W., et al. (2002). *Endocrine* **18,** 21–25.
44. Kondo, M., Kojima, C., Watanabe, G., et al. (2003). *Endocrine* **22,** 239–243.
45. Taya, K., Watanabe, G., and Sasamoto, S. (1985). *Japan. J. Anim. Reprod.* **31,** 186–197.
46. Hamada, T., Watanabe, G., Kokuho, T., et al. (1989). *J. Endocrinol.* **122,** 697–704.