Original Paper

Biology of the Neonate

Biol Neonate 2000;78:315–320

Plasma Leptin Levels in Rat Mother and Offspring during Pregnancy and Lactation

E. Herrera[a] M.A. Lasunción[b] L. Huerta[b] A. Martín-Hidalgo[b]

[a]Universidad San Pablo-CEU, Madrid, and [b]Servicio de Bioquímica-Investigación, Hospital Ramón y Cajal, Madrid, Spain

Key Words

Leptin · Pregnant rat · Fetus · Newborn · Adipose tissue

Abstract

The profiles of plasma leptin levels in pregnant and lactating rats and their offspring were determined. The plasma leptin levels increased on days 12 and 20 of gestation and declined on day 21 of gestation, remaining at this level during lactation. These changes were similar for lumbar adipose tissue weight, and a significant correlation was found when both variables were plotted with individual values. During the last 2 days of intrauterine life, the plasma leptin levels in the fetuses were in the same range as in their mothers, declining from day 20 to day 21. On the 1st day of life, the leptin levels increased to decline in suckling newborns after 4 days, remaining stable until day 20 of life. The enhancement in maternal white adipose tissue mass that takes place during pregnancy and its decline around parturition and lactation are proposed to contribute actively to the changes in the plasma leptin profile detected at these stages. Besides the contribution of placental leptin for the fetus and milk leptin for the suckling newborn, it is proposed that brown adipose tissue, which is the first form of adipose tissue that appears during development in the rat, is responsible for most of the changes in plasma leptin levels seen around birth, whereas its later decline could be mediated by the hormonal changes occurring after birth.

Introduction

The body fat accumulation increases progressively during pregnancy, although it stops or even declines during the last trimester, both in women [1, 2] and in rats [3–5], and this change seems to account for a substantial proportion of the net (conceptus not included) maternal body weight increase during gestation. From studies in pregnant rats, body fat accumulation during gestation may be associated with hyperphagia [6], increased adipose tissue lipogenesis and glycerolgenesis [7] and hyperinsulinemia prior to the development of insulin resistance which normally takes place during late gestation [8].

During pregnancy, the plasma levels of leptin, the product of the adipose tissue ob gene, has been shown to be high in women [9–11], mice [12], and rats [13, 14]. The placenta seems to contribute to the hyperleptinemia of pregnancy, since it has been shown to express leptin mRNA both in women [15–17] and in rats [18], although in the latter this finding has not been confirmed by some authors [13].

According to studies in nonpregnant animals [19, 20], leptin is thought to be a satiety factor which acts in the hypothalamus to reduce food intake and increase energy expenditure. The increase in leptin in pregnancy, during which hyperphagia is a common feature both in women [21, 22] and in rats [6, 23], would, therefore, indicate a leptin-resistant condition similar to that reported in obesity [24]. Since the physiological role of an increased plas-

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0006–3126/00/0784–0315$17.50/0

Accessible online at:
www.karger.com/journals/bon

Dr. E. Herrera
Facultad de Ciencias Experimentales
Universidad San Pablo-CEU, Ctra. Boadilla del Monte, km 5,300
E-28668 Madrid (Spain)
Tel. +34 91 352 01 44, Fax +34 91 351 0496

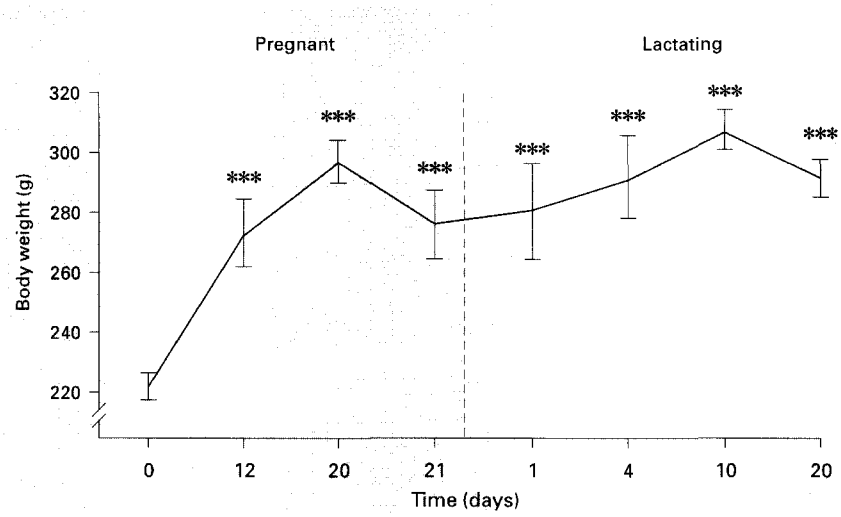

Fig. 1. Effect of pregnancy and lactation on maternal body weight in the rat. Values are mean ± SE of 5–9 animals for each time point. Significant difference versus values of virgin rats: *** $p < 0.001$.

ma leptin level during pregnancy is yet to be determined, we have examined plasma leptin levels during pregnancy and lactation in rat dams and their offspring, and the values have been related to both body and adipose tissue weights.

Materials and Methods

Animals and Sample Collection

Female Sprague-Dawley rats from our colony, weighing 180–200 g, were kept at 22 ± 1 °C under standard conditions of light (on from 08.00 to 20.00 h) and food (Purina chow; Panlab, Barcelona, Spain). The experimental protocol was approved by the Animal Research Committee of the Hospital Ramón y Cajal and University San Pablo-CEU.

The experimental groups were virgin controls (day 0; n = 7), pregnant rats (days 12, 20, and 21; n = 5, 9, and 6, respectively), and lactating rats (days 1, 4, 10, and 20; n = 6, 5, 5, and 6, respectively). The rats were mated with normal males, and positive pregnancy was determined by the appearance of spermatozoids in vaginal smears. The litter sizes were adjusted to 9–11 pups at birth. The animals were fed ad libitum and had free access to tap water. They were killed between 10.00 and 11.00 h by decapitation on days listed above, after normal night access to food, and trunk blood was collected in ice-chilled heparinized tubes for immediate separation of plasma at 4 °C that was kept at −20 °C until analysis.

Lumbar fat pads of all adult rats were rapidly dissected and weighed. In pregnant rats, the two uterine horns were excised and weighed with their content to obtain the whole conceptus weight. This value was subtracted from the body weight before death to obtain the net maternal body weight. Fetuses and neonates were weighed and decapitated, and their blood was collected as indicated above. Blood from all fetuses and neonates coming from the same dam was pooled and processed in parallel to that of the adults.

Plasma leptin levels were assayed using a commercially available RIA kit from Linco Research Laboratories (St. Charles, Mo., USA). The components of the kit include recombinant rat leptin standards, radioiodinated recombinant rat leptin, guinea pig antirat leptin, and quality control pools representing low and high concentration.

Statistical Analysis

Values are mean ± SE. The statistical significance of changes was assessed at the 5% level using Anova followed by pairwise multiple comparisons using the Student-Newman-Keuls method. Pearson correlation coefficients were calculated to quantify the association between intrapair differences in leptin concentrations and corresponding differences in other variables.

Results

As shown in figure 1 the maternal net body weight (conceptus not included) in the pregnant rat progressively increases until day 20 of gestation, slightly decreases on day 21 of pregnancy, and remains augmented during lactation. As shown in figure 2, the maternal lumbar adipose tissue weight increases until the 20th day of pregnancy, when it reaches a value significantly higher than in virgin rats (day 0). The maternal lumbar adipose tissue weight declines on day 21 of gestation to values that do not differ from those in virgin rats, and this variable remains stable throughout lactation.

As shown in figure 3, the plasma leptin levels in rats progressively increase on days 12 and 20 of gestation to decline on day 21 of gestation to levels found in virgin rats, remaining around these values during lactation.

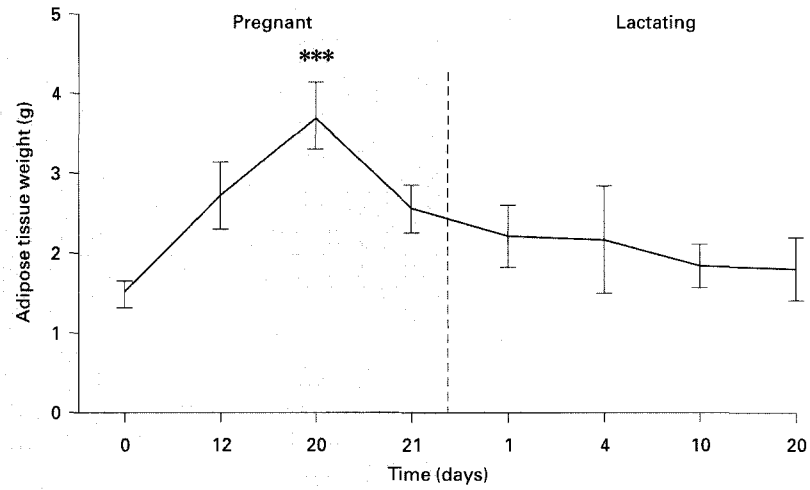

Fig. 2. Effect of pregnancy and lactation on maternal lumbar adipose tissue weight. Values are mean ± SE of 5–9 animals for each time point. Significant difference versus values of virgin rats: *** $p < 0.001$.

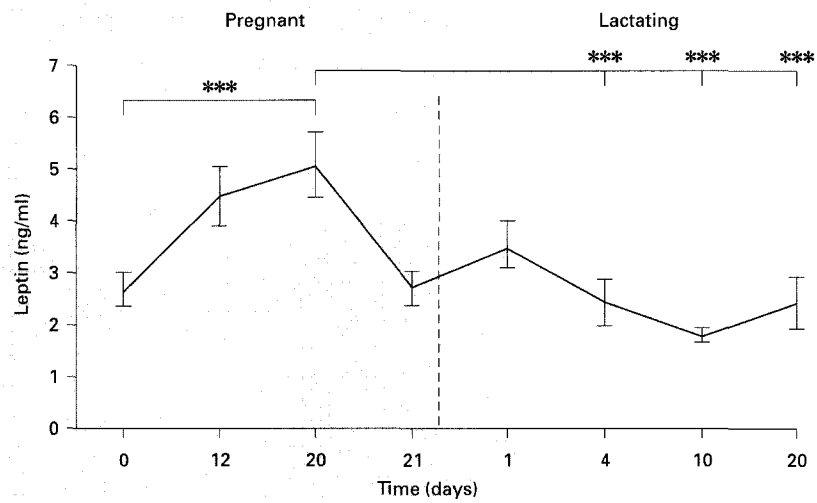

Fig. 3. Changes in the plasma leptin concentration during pregnancy and lactation in the rat. Values are mean ± SE of 5–9 animals for each time point. Significant differences of values in pregnant rats are versus those in virgin rats, whereas values in lactating rats are versus those in 20-day pregnant rats: *** $p < 0.001$.

Similarities in the changes of plasma leptin levels and body weight and lumbar adipose tissue during pregnancy prompted us to calculate the linear correlation for individual values between these variables. Whereas net body weight did not correlate significantly with plasma leptin levels when values of virgin, pregnant, and lactating rats were calculated together ($r = 0.300$, not significant, $n = 49$), the correlation reached a significant level ($r = 0.543$, $p < 0.05$, $n = 20$), when the values of pregnant rats were considered separately. However, as shown in figure 4, a highly significant correlation was found when plasma leptin levels in all studied animals were plotted against their lumbar adipose tissue weights. Significant correlations were also found between plasma leptin levels and lumbar adipose tissue weights for virgin and pregnant rats ($r = 0.656$, $p < 0.001$, $n = 27$) or for lactating rats ($r = 0.485$, $p < 0.05$, $n = 22$) separately.

The plasma leptin levels in fetuses on days 20 and 21 of pregnancy, neonates (day 1 after birth), and suckling newborns are shown in figure 5. The plasma leptin level in fetuses during the last days of intrauterine life was in the same range as in their mothers and changed similarly, declining from day 20 to day 21. The plasma leptin level increased on the 1st day of life to decline in 4-day old suckling newborns and remaining stable until day 20 of life. During lactation, the maternal plasma leptin levels correlated significantly with those in their respective pups ($r = 0.533$, $p < 0.05$, $n = 22$).

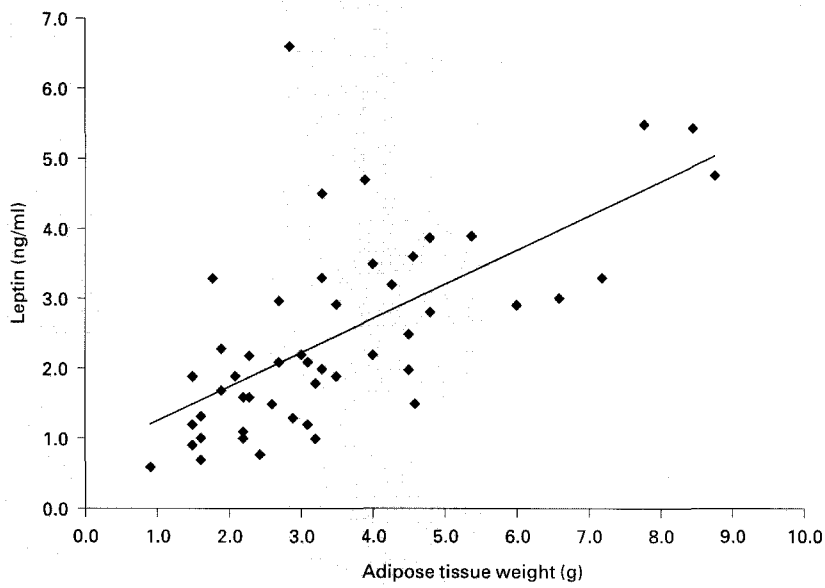

Fig. 4. Linear correlation between maternal plasma leptin concentration and lumbar adipose tissue weight ($y = 0.4864x + 0.7661$, $r = 0.6570$, $n = 49$, $p < 0.01$).

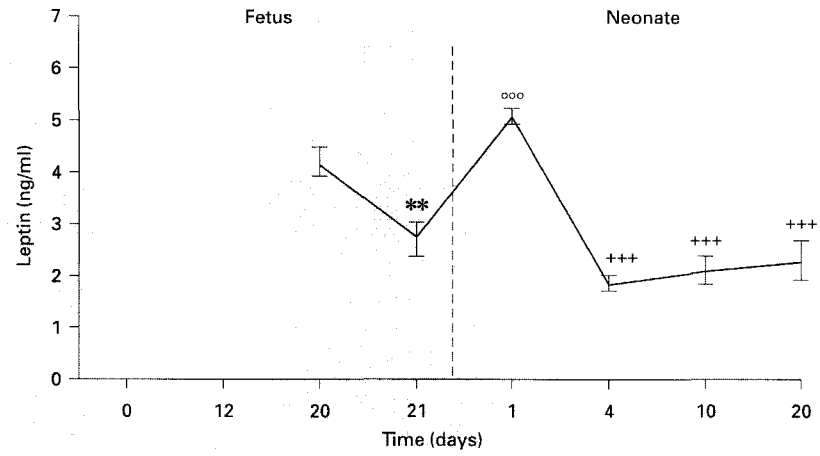

Fig. 5. Changes in plasma leptin concentration in fetuses and newborn rats. Values are mean ± SE of a pool of samples from 5–9 mothers for each time point. Statistical comparisons versus 20-day fetuses: ** $p < 0.01$; versus 21-day fetuses: ^{ooo} $p < 0.001$, and versus 1-day-old neonates: $^{+++}$ $p < 0.001$.

Discussion

The present study shows that in rats, the plasma leptin levels increase up to day 20 of gestation and decline just before parturition to remain low throughout lactation, agreeing with previously reported findings [13, 18]. This study demonstrates for the first time that these changes in plasma leptin levels during pregnancy linearly correlate with the maternal lumbar adipose tissue weight. Since changes in net maternal body weight during gestation mainly correspond to maternal fat accumulation [4, 5], the present findings, therefore, indicate that maternal fat depots contribute to the changes in plasma leptin levels. This fat accumulation occurs during the two first parts of gestation [3, 5, 25], is caused by enhanced adipose tissue lipogenesis [7, 26], and is related to maternal hyperphagia, since it does not occur in food-restricted or food-deprived rats [6]. On day 21 of gestation, adipose tissue lipogenesis sharply declines [7] which together with an enhanced lipolytic activity [27, 28] and a decreased hydrolysis and uptake of circulating triglycerides resulting from a decreased lipoprotein lipase activity [28–30] reflects the decline in adipose tissue mass seen in the rat around parturition. This change does not cause a signifi-

cant decline in maternal body weight during lactation, probably as a result of the enhanced mammary gland weight [5, 31] as well as the high body water content [32] known to occur during this phase. Late pregnancy is characterized by high plasma insulin levels and insulin resistance [8, 33, 34]. Therefore, the high plasma leptin levels found in pregnant rats occur in the presence of hyperphagia which indicates leptin resistance, increased lumbar adipose tissue weight, high plasma insulin levels, and insulin resistance, all of which are similar changes to those normally seen in human obesity [35–37].

Enhanced plasma leptin levels during pregnancy could be partially attributed to its production by the placenta, since it has been previously shown that the placenta expresses leptin mRNA both in women [10, 16, 17] and in rats [18]. In the case of the rat placenta, however, the matter remains controversial, since other authors reported absence of leptin mRNA expression [13]. Besides, the presence of a leptin-binding activity during pregnancy causing a reduced renal clearance of bound leptin cannot be claimed in the rat, as it has been, however, shown in mice [12], since such leptin-binding activity has not been found in pregnant rats [12, 36]. An enhanced production of leptin by adipose tissue, proportional to its augmented mass, may, therefore, be proposed as the main factor responsible for the hyperleptinemia found in pregnant rats, and this hypothesis is supported by the significant correlation reported in this study between maternal plasma leptin levels and lumbar adipose tissue mass. This conclusion agrees with the high leptin content and mRNA expression found in adipose tissue of pregnant rats [13, 18], suggesting that both the increase in fat mass and the enhanced gene expression and protein production of leptin in adipose tissue are the main responsible factors for the development of maternal hyperleptinemia during pregnancy. This situation changes around parturition and during lactation, when both adipose tissue mass and plasma leptin levels decline. During lactation food intake is even greater than during pregnancy, and although the reduction in plasma leptin levels reaches the same values as in nonpregnant rats, the rapid decline around parturition could contribute to the massive food intake required to sustain the enhanced energy demand that takes place during this period.

Although the presence of leptin in fetal plasma may be influenced by placental synthesis, it could also come from the own fetus. In the rat, brown adipose tissue is the first form of adipose tissue that appears during development, and in Wistar rats, interscapular brown adipose tissue expresses leptin mRNA at birth, although not in fetuses 1 day before birth [38]. The presence of leptin in 20- and 21-day fetuses of Sprague-Dawley rats found here agrees with data from Kawai et al. [13], and given the differences in the metabolic maturation sequence that takes place around birth in rats of this strain as compared with Wistar rats [39], it may be suggested that brown adipose tissue could contribute to the plasma leptin levels found in the former. In fact, the rapid increase in plasma leptin level found in our rats just on the 1st day after birth could be the consequence of the abrupt expansion of brown adipose tissue known to occur at this stage [40]. The contribution of milk leptin to the changes in plasma leptin levels found in the newborn rats cannot be ruled out on the background of the significant correlation found between mothers' and pups' plasma leptin levels and the recent finding of leptin production by the mammary gland in lactating mice [41]. The later decline in plasma leptin levels found in our newborn rats could, however, be mediated by hormonal changes occurring after birth, like the fall in plasma insulin levels [42]. A decline in plasma leptin levels has been reported to occur in healthy human neonates [43] and might be important to regulate body weight gain and feeding during the early neonatal period and to acquire progressively the adult plasma leptin pattern. In fact, in adults, the close correlation between adiposity and circulating leptin levels supports the generally held concept that leptin is a signal from the body fat mass to the brain where it affects satiety. This concept is, however, difficult to apply to the fetal situation, where the mother controls the energy supply to the fetus which has no need for feelings of hunger and satiety.

Acknowledgments

We thank Dr. Beatriz Ramos for her editorial help. This work was supported by a grant (No. PB92/0918) from the Dirección General de Investigación Científica y Técnica of the Ministry of Education and Culture to A. Martín-Hidalgo.

References

1 Hytten FE, Leitch I: The gross composition of the components of weight gain; in Hytten FE, Leitch I (eds): The Physiology of Human Pregnancy, ed 2. Oxford, Blackwell, 1971, pp 370–387.

2 Villar J, Cogswell M, Kestler E, Castillo P, Menendez R, Repke JT: Effect of fat and fat-free mass deposition during pregnancy on birth weight. Am J Obstet Gynecol 1992;167:1344–1352.

3 López-Luna P, Muñoz T, Herrera E: Body fat in pregnant rats at mid- and late-gestation. Life Sci 1986;39:1389–1393.

4 Herrera E, Lasunción MA, Gómez-Coronado D, Aranda P, López-Luna P, Maier I: Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. Am J Obstet Gynecol 1988; 158:1575–1583.

5 López-Luna P, Maier I, Herrera E: Carcass and tissue fat content in the pregnant rat. Biol Neonate 1991;60:29–38.

6 Moore BJ, Brassel JA: One cycle of reproduction consisting of pregnancy, lactation, and recovery: Effects on carcass composition in ad-libitum-fed and food-restricted rats. J Nutr 1984;114:1548–1559.

7 Palacín M, Lasunción MA, Asunción M, Herrera E: Circulating metabolite utilization by periuterine adipose tissue in situ in the pregnant rat. Metabolism 1991;40:534–539.

8 Muñoz C, López-Luna P, Herrera E: Glucose and insulin tolerance tests in the rat on different days of gestation. Biol Neonate 1995;68: 282–291.

9 Hartmann BW, Wagenbichler P, Söregi G: Maternal and umbilical-cord serum leptin concentrations in normal, full-term pregnancies. N Engl J Med 1997;337:863.

10 Helland IB, Reseland JE, Saugstad OD, Drevon CA: Leptin levels in pregnant women and newborn infants: Gender differences and reduction during the neonatal period. Pediatrics 1998;101:E12–E15.

11 Tamura T, Goldenberg RL, Johnston KE, Cliver SP: Serum leptin concentrations during pregnancy and their relationship to fetal growth. Obstet Gynecol 1998;91:389–395.

12 Gavrilova O, Barr V, Marcus-Samuels B, Reitman M: Hyperleptinemia of pregnancy associated with the appearance of a circulating form of the leptin receptor. J Biol Chem 1997;272: 30546–30551.

13 Kawai M, Yamaguchi M, Murakami T, Shima K, Murata Y, Kishi K: The placenta is not the main source of leptin production in pregnant rat: Gestational profile of leptin in plasma and adipose tissues. Biochem Biophys Res Commun 1997;240:798–802.

14 Chien EK, Hara M, Rouard M, Yano H, Phillippe M, Polonsky KS, Bell GI: Increase in serum leptin and uterine leptin receptor messenger RNA levels during pregnancy in rats. Biochem Biophys Res Commun 1997;237: 476–480.

15 Hassink SG, de Lancey E, Sheslow DV, Smith-Kirwin SM, O'Connor DM, Considine RV, Opentanova I, Dostal K, Spear ML, Leef K, Ash M, Spitzer AR, Funanage VL: Placental leptin: An important new growth factor in intrauterine and neonatal development? Pediatrics 1997;100:E1–E6.

16 Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, Nishimura H, Yoshimasa Y, Tanaka I, Mori T, Nakao K: Nonadipose tissue production of leptin: Leptin as a novel placenta-derived hormone in humans. Nat Med 1997;3:1029–1033.

17 Señarís R, García-Caballero T, Casabiell X, Gallego R, Castro R, Considine RV, Dieguez C, Casanueva FF: Synthesis of leptin in human placenta. Endocrinology 1997;138:4501–4504.

18 Amico JA, Thomas A, Crowley RS, Burmeister LA: Concentrations of leptin in the serum of pregnant, lactating, and cycling rats and of leptin messenger ribonucleic acid in rat placental tissue. Life Sci 1998;63:1387–1395.

19 Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P: Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science 1995;269: 546–549.

20 Pelleymounter MA, Cullen MJ, Baker MO, Hecht R, Winters D, Boone T, Collins F: Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995;269: 540–543.

21 Piers LS, Diggavi SN, Thangam S, Van Raaij JMA, Shetty PS, Hautvast JGAJ: Changes in energy expenditure, anthropometry, and energy intake during the course of pregnancy and lactation in well-nourished Indian women. Am J Clin Nutr 1995;61:501–513.

22 Murphy SP, Abrams BF: Changes in energy intakes during pregnancy and lactation in a national sample of US women. Am J Public Health 1993;83:1161–1163.

23 Weijs PJM, Schreurs VVAM, Grooten HNA: Meal feeding and leucine utilization in pregnant rats. Br J Nutr 1995;73:253–258.

24 Cusin I, Rohner-Jeanrenaud F, Stricker-Krongrad A, Jeanrenaud B: The weight-reducing effect of an intracerebroventricular bolus injection of leptin in genetically obese fa/fa rats. Diabetes 1996;45:1446–1450.

25 Knopp RH, Boroush MA, O'Sullivan JB: Lipid metabolism in pregnancy. II. Postheparin lipolytic activity and hypertriglyceridemia in the pregnant rat. Metabolism 1975;24:481–493.

26 Fain JM, Scow RO: Fatty acid synthesis in vivo in maternal and fetal tissues in the rat. Am J Physiol 1966;210:19–25.

27 Knopp RH, Herrera E, Freinkel N: Carbohydrate metabolism in pregnancy. VIII. Metabolism of adipose tissue isolated from fed and fasted pregnant rats during late gestation. J Clin Invest 1970;49:1438–1446.

28 Martín-Hidalgo A, Holm C, Belfrage P, Schotz MC, Herrera E: Lipoprotein lipase and hormone-sensitive lipase activity and mRNA in rat adipose tissue during pregnancy. Am J Physiol 1994;266:E930–E935.

29 Otway S, Robinson DS: The significance of changes in tissue clearing-factor lipase activity in relation to the lipaemia of pregnancy. Biochem J 1968;106:677–682.

30 Hamosh M, Clary TR, Chernick SS, Scow RO: Lipoprotein lipase activity of adipose and mammary tissue and plasma triglyceride in pregnant and lactating rats. Biochim Biophys Acta 1970;210:473–482.

31 Vilaró S, Viñas O, Remesar X, Herrera E: Effects of chronic ethanol consumption on lactational performance in rat: Mammary gland and milk composition and pups' growth and metabolism. Pharmacol Biochem Behav 1987; 27:333–339.

32 Spray CM: A study of some aspects of reproduction by means of chemical analysis. Br J Nutr 1949;3:354–360.

33 Martín A, Zorzano A, Caruncho I, Herrera E: Glucose tolerance tests and 'in vivo' response to intravenous insulin in the unanaesthesized late pregnant rat and their consequences to the fetus. Diabète Métab 1986;12:302–307.

34 Knopp RH, Ruder HJ, Herrera E, Freinkel N: Carbohydrate metabolism in pregnancy. VII. Insulin tolerance during late pregnancy in the fed and fasted rat. Acta Endocrinol (Copenh) 1970;65:352–360.

35 Collins S, Kuhn CM, Petro AE, Swick AG, Surwit RS: Role of leptin in fat regulation. Nature 1996;380:677.

36 Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fey H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM: Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1995;1: 1155–1161.

37 Suzuki M, Shimomura Y, Satoh Y: Diurnal changes in lipolytic activity of isolated fat cells and their increased responsiveness to epinephrine and theophylline with meal feeding in rats. J Nutr Sci Vitaminol (Tokyo) 1983;29:399–411.

38 Dessolin S, Schalling M, Champigny O, Lönnqvist F, Ailhaud G, Dani C, Ricquier D: Leptin gene is expressed in rat brown adipose tissue at birth. FASEB J 1997;11:382–387.

39 Galan X, Llobera M, Ramírez I: Lipoprotein lipase in developing rat tissues: Differences between Wistar and Sprague-Dawley rats. Biol Neonate 1993;64:295–303.

40 Nedergaard J, Cannon B: Brown adipose tissue: Development and function; in Polin RA, Fox WW (eds): Fetal and Neonatal Physiology, ed 2. Philadelphia, Saunders, 1998, vol 1, pp 478–489.

41 Aoki N, Kawamura M, Matsuda T: Lactation-dependent down-regulation of leptin production in mouse mammary gland. Biochim Biophys Acta 1999;1427:298–306.

42 Blázquez E, Sugase T, Blázquez M, Foà PF: Neonatal changes in the concentration of rat liver cyclic AMP and of serum glucose, free fatty acids, insulin, pancreatic, and total glucagon in man and in the rat. J Lab Clin Med 1974;83: 957–967.

43 Schubring C, Siebler T, Englaro P, Blum WF, Kratzsch J, Triep K, Kiess W: Rapid decline of serum leptin levels in healthy neonates after birth. Eur J Pediatr 1998;157:263–264.