# Leptin levels as function of age, gender, auxological and hormonal parameters in 202 healthy neonates at birth and during the first month of life

S. Bellone[1], A. Rapa[1], A. Petri[1], A. Zavallone[1], L. Strigini[1], E. Chiorboli[1], L. Ciardi[2], A. Aguzzi[1], and G. Bona[1]

[1]Unit of Pediatrics, Department of Medical Sciences, University of Piemonte Orientale; [2]Laboratory analysis, Maggiore Hospital, Novara, Italy

**ABSTRACT. Leptin signals to the brain energy stores and balance while integrating neuroendocrine functions. Leptin levels in adults are higher in females than in males, while a gender-related difference in newborns is controversial. To clarify this point, in 202 healthy neonates we measured dynamic changes in leptin levels over the first month of life and looked for correlation between leptin levels and auxological and hormonal parameters. Cord leptin concentration in females was higher ($p< 0.001$) than in males. IGF-I, IGF-II, insulin, testosterone and 17β-estradiol levels were similar in both sexes while insulin-like growth factor binding protein 3 (IGF-BP3) levels in females were slightly higher than in males. Leptin levels were positively associated to body weight, gestational age, IGF-BP3 levels, insulin levels and maternal body mass index (BMI) at time of delivery. In a subset of subjects (no.= 65), in comparison with cord levels, serum leptin levels were decreased on the 5th day of life ($p<0.0001$) and then increased at 1 month ($p<0.0001$). Positive association between leptin and weight was lost on the 5th day of life but present again at 1 month. In conclusion, our findings in a large population of neonates definitely show that leptin levels at birth are functions of gender, body weight and gestational age but not of length, cranial circumference, IGF-I and IGF-II levels. These findings, coupled with weight-independent prompt decrease after birth followed by weight-dependent increase at one month of life, suggest that leptin secretion in neonates as well as in adults mainly signals the nutritional state to the brain.**

**(J. Endocrinol. Invest. 27: 18-23, 2004)**



## INTRODUCTION

Leptin, the adipose-derived hormone, is an important signal to the brain reflecting both energy stores and energy balance (1-3) and integrating neuroendocrine functions (4-5). Leptin synthesis and secretion are closely correlated with the degree of adiposity (2, 6) and are markedly inhibited by caloric restriction and fasting (4, 7). It has been shown that leptin centrally inhibits food intake and stimulates energy expenditure (2, 3) and reduction in its circulating levels, probably triggers orexigenic behavior (2, 3, 8). Insulin stimulates ob-gene expression and leptin release *in vitro* (3, 9) while, at least after prolonged administration of supraphysiological dose, it is able to increase leptin levels in humans independently from changes in fat mass (10, 11). Glucocorticoids enhance while β-adrenoreceptor activation inhibits leptin (12, 13) which, in turn, plays an important role in the neuroendocrine control of gonadotroph, corticotroph, thyrotroph and also somatotroph function (3). Influence of GH and IGF-I levels on leptin secretion has, in turn, been suggested (14).

Leptin levels are always higher in women than in men (3, 15, 16) and even after adjustment for age, waist-to-hip ratio, fat mass and insulin levels, estradiol and testosterone are directly and inversely correlated, respectively, with leptin levels (17, 18). The gender-related difference in leptin levels is present in prepubertal children as well as in adults (15, 19). On the other hand, in newborns higher

*Key-words:* Leptin, gender, newborns.

*Correspondence:* G. Bona, MD, Unità di Pediatria, Dipartimento di Scienze Mediche, Università del Piemonte Orientale "A.Avogadro", Corso Mazzini 18, 28100 Novara, Italy.

*E-mail:* gianni.bona@maggioreosp.novara.it

Accepted August 1, 2003.

leptin concentration in females than in males has been reported by some (20-23) but not by other authors (24-26).
The mechanisms by which fetal/neonatal growth and metabolism are regulated are poorly understood. It is widely accepted that, besides genetic, nutritional and environmental factors, hormones, particularly insulin and IGFs, play a major role in regulating fetal and postnatal growth (27, 28). The role of leptin in this context is still unclear.
In order to further clarify the pattern of leptin secretion as a function of age, gender, body composition and hormonal parameters in newborns, in a large population of healthy neonates of both sexes we measured dynamic changes in leptin levels over the first month of life and looked for correlations between leptin levels and auxological parameters as well as IGF-I, IGF-II and IGF binding protein 3 (IGF-BP3) levels.

## SUBJECTS AND METHODS

Two hundred and two neonates (105 boys and 97 girls) were studied. All the infants were categorized as appropriate for gestational age (gestational age: 39.1±0.1 weeks, weight 3282.8±20.6 g, length 49.7±0.1 cm, cranial circumference 43.5±2.5 cm), born in our hospital, from consecutively enrolled vaginal and cesarean full-term deliveries. Venous cord blood was drawn immediately after birth. For each newborn we evaluated gestational age, birth weight, length and placental weight and collected a cord blood sample for determination of leptin, IGF-I, IGF-II, IGF-BP3, insulin, 17β-estradiol and testosterone. Leptin levels were also evaluated on the 5$^{th}$ and the 30$^{th}$ day of life.
In all children, chromosomal disorders, congenital malformations, dysmorfic features, intrauterine infections or organic disorders had been ruled out.
The study protocol was approved by the local Ethical Committee and informed consent was obtained from all the infants' parents.
Cord blood samples for leptin, IGF-I, IGF-II and IGFBP-3, insulin, 17β-estradiol and testosterone determination were centrifuged to separate serum, which was kept at –20 C for subsequent analysis.
Leptin was measured in duplicate by radioimmunometric assay using a commercially available kit (Linco Research Inc., St Charles, MO). The sensitivity was 0.5 ng/ml. The intra- and inter-assay coefficients of variation were 3.4-8.3% and 3.0-6.2%, respectively.
IGF-I, IGF-II and IGFBP-3 were measured in duplicate by a two-site radioimmunometric assay (Diagnostic System Laboratories Inc., Webster, TX). IGFs were assayed after an acid-ethanol extraction. Sensitivity was 0.8 ng/ml for IGF-I, 12 ng/ml for IGF-II and 0.5 ng/ml for IGFBP-3. The intra-assay coefficients of variation (CV) were 1.5-3.4% for IGF-I, 4.3-7.2% for IGF-II and 1.8-3.9% for IGF-BP-3. The inter-assay coefficients of variation were 1.5-8.2% for IGF-I, 6.3-10.4% for IGF-II and 0.5-1.9% for IGFBP-3.
Insulin was measured by chemiluminescent enzyme-labelled immunometric assay (Diagnostic Products Corporation, Los Angeles, CA). Sensitivity: 2 μUI/ml. Intra- and inter-assay CV ranges: 2.5-8.3 and 4.4-8.6%.
Total testosterone and estradiol were evaluated by a chemiluminescent competitive immunoassay (Immulite 2000 - Diagnostic Products Corporation, Los Angeles, CA). Analytical sensitivity was 10 ng/dl for total testosterone and 15 pg/ml for estradiol. The intra-assay CV were 4.9-16% for total testosterone and 6.7-16% for estradiol. The inter-assay CV were 7.2-22% for total testosterone and 4.3-9.8% for estradiol.
Student t-test was used to explore differences in birth weight, length, gestational age and placental weight between sexes. Anthropometric data were expressed as mean±SEM. Since hormonal variables were skewed toward low values, analysis of their levels were performed by non-parametric statistical tests. The analysis of the difference in IGF-I, IGF-II, testosterone, insulin, leptin, (ng/ml) and IGFBP-3 (μg/ml) concentrations between sexes was performed with Mann Whitney U test. Hormonal data were expressed as median (25$^{th}$ -75$^{th}$ centile).
The possible association between hormonal levels and anthropometric parameters was examined using Spearman correlation. Sex, birth weight, gestational age, IGFBP-3 levels and maternal weight at the time of delivery were tested in multivariate regression models to determine their independent contribution on leptin concentrations at birth.
Leptin levels at birth, on the 3$^{rd}$ and the 30$^{th}$ day of life were first compared by Friedman two-way analysis of variance and then by the Wilcoxon rank sum test with posthoc Bonferroni adjustment. Two-tailed tests were used and $p<0.05$ was considered significant. The statistical analysis was performed using STATISTICA version 5.1 (Stat Soft, Inc., Tulsa, OK).

## RESULTS

Weight and length in males (3326.7±30.2 g and 49.9±0.1 cm) were significantly higher ($p<0.009$ and 0.03) than in females (3236.0±27.2 g and 49.4±0.2 cm). Gestational age (males 39.1±0.1, females 39.1±0.1 weeks) and placental weight (males 584.2±12.7, females 567.9±20.8 g) were similar in both sexes.
Total IGF-I (males: 39.0, 23.4-52.9; females: 37.2, 22.3-58.3 ng/ml), IGF-II (males: 390.0, 343.0-452.0; females, 402.0, 333.0-497.0 ng/ml) and insulin levels (males: 3.1, 2.1-5.8; females: 4.1, 2.9-8.0 ng/ml) were similar in both sexes, while IGF-BP3 levels in females (1455.0, 1119.5-1741.5 ng/ml) were slightly higher ($p<0.05$) than in males (1299.0, 1070.0-1530.5 μg/ml) (Fig. 1). Testosterone (male: 3.5, 2.4-9.6; females: 2.4, 1.7-8.2 ng/ml) and 17β-estradiol (male: 10846.0; 6360.0-20000; female: 9525.0; 4776.0-20000 pg/ml) levels were similar in both sexes.
Cord plasma leptin concentration in females was higher than in males (8.5, 5.4-12.8 vs 6.6, 4.1-9.5 ng/ml, $p<0.001$) (Fig. 1).
Cord leptin levels were positively associated to body weight (r=0.28, $p<0.0002$), gestational age (r=0.24, $p<0.0001$), IGF-BP3 levels (r=0.22, $p<0.01$) and insulin levels (r=0.3, $p<0.006$) as well as to maternal body mass index (BMI) at time of delivery (r=0.22, $p<0.01$) (Fig. 2).

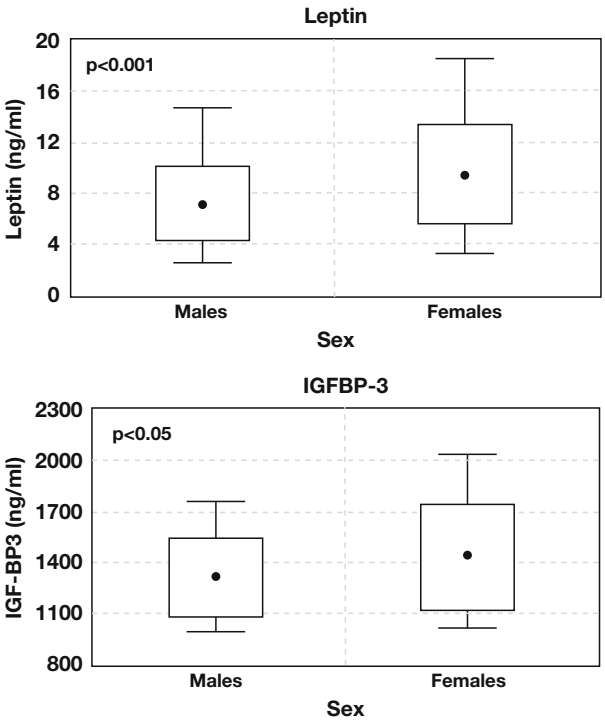


Fig. 1 - *Gender-related differences in leptin and IGFBP-3 levels: points are median values, box are interquartiles, and whiskers are 10th-90th centile.*

Leptin levels were not associated to placental weight, length and cranial circumferences of the infants as well as to IGF-I and IGF-II levels which, in turn, were positively correlated to each other ($p<0.02$, r=0.2) or with IGFBP-3 ($p<0.0001$, r=0.4 and $p<0.0001$, r=0.6 respectively). Leptin levels were also independent of testosterone and estradiol levels.

In a multiple regression model including sex, birth weight, IGFBP-3 levels and maternal BMI at delivery, sex (β: 0.325, $p=0.004$) and birth weight (β: 0.274, $p=0.02$) resulted as the independent predictors of cord leptin concentrations (multiple r=0.46, $p=0.0008$). However, when gestational age was introduced into the model, birth weight lost prediction power ($p=0.07$) for leptin levels. In this model (multiple r=0.47, $p=0.002$) sex resulted as being the only independent predictor of leptin levels at birth (β: 0.314, $p=0.007$).

In a subset of subjects (no.= 65, 36 M and 29 F), in comparison with cord levels (7.2 ng/ml), serum leptin levels were decreased on the 5th day of life (1.8 ng/ml, $p<0.0001$) and then increased at 1 month (3.2 ng/ml, $p<0.0001$) (Fig. 3).

Positive association between leptin and weight was lost on the 5th day of life but was present once again at 1 month when leptin levels positively correlated also with the 1-month weight variation ($p<0.0005$, r=0.6).

## DISCUSSION

The results of the present study in a large population of healthy neonates definitely show that leptin levels at birth are functions of gender, body weight and gestational age but not of other auxological parameters, such as length and cranial circumfer-

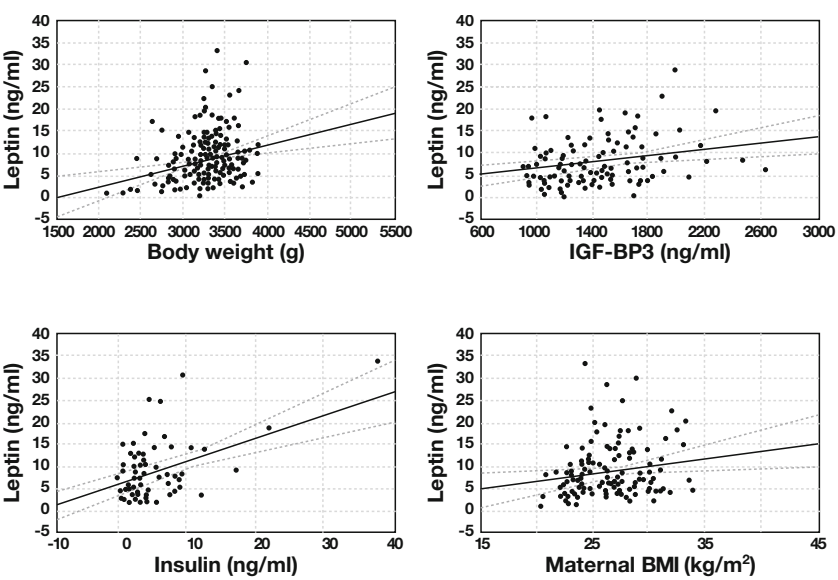


Fig. 2 - *Correlations between leptin levels of newborns and neonatal body weight, IGF-BP3, insulin and maternal BMI.*

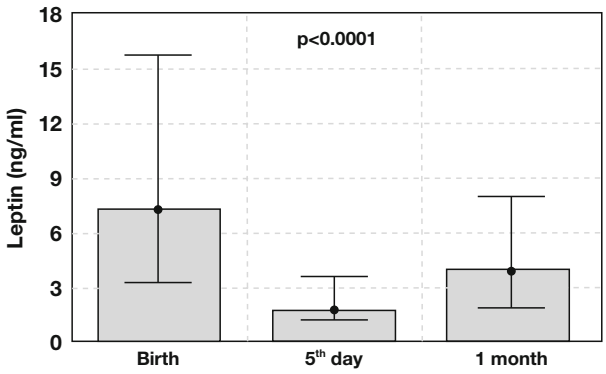


Fig. 3 - *Leptin levels in cord blood and on the 5th and the 30th day of life: points are median values, whiskers are 10th-90th centile.*

ence, as well as with IGF-I and IGF-II levels. These findings are coupled with weight-independent prompt decrease on the 5th day after birth followed by weight-dependent increase at 1 month of life.

Gender-related difference in leptin levels had been definitely demonstrated in adults and children, females having hormone levels always higher in women than in men even after adjustment for age, waist-to-hip ratio and fat mass (3, 15, 25). Gonadal steroids have been suggested as playing a major role in this gender-related difference, estradiol stimulating while testosterone inhibiting leptin synthesis and secretion (29-32).

The presence of gender-related difference in leptin levels in newborns was still uncertain. In newborns, higher leptin concentration in females than in males had been reported by some (20-23) but not by other authors (25-27). Our present data obtained in a large population of healthy newborns studied so far definitely demonstrate that at birth too leptin levels in females are higher than in males. Healthy female and male neonates in our population had similar gestational age and placental weight while, as expected, weight and length in males were higher than in females. In agreement with previous findings (26, 33, 27) leptin levels were positively associated to weight and gestational age while they were independent of other auxological parameters including length and cranial circumference. The positive association between leptin and weight did not prevent the gender-related difference despite higher mean weight in males.

As there was no significant difference between sexes in terms of estradiol and testosterone levels, it is clear that the gender-related difference at birth is independent of the influence of gonadal steroids. Potential explanations of the gender-related differences of leptin levels include a peculiar sensitivity of female adipose tissue to hormones, such as insulin and glucocorticoids, or other substances stimulating leptin production (34) and the evidence that sc gynoid fat produces more leptin mRNA than visceral android fat (35). Meanwhile, it is clear that the influence of gender is even stronger than that of weight, body composition and fat mass, in agreement with what has been described in adulthood (16, 36, 37). Thus, an as yet undetermined factor operating during the fetal period is responsible for the gender-related difference in the rate of secretion of leptin by the adipose tissue of neonates or, alternatively, by the placenta (22, 33, 38). It is very unlikely that this influence is played by growth factors such as IGF-I and IGF-II, which were not associated to leptin levels in agreement with previous studies (20, 39, 40). Though a functional link between leptin and GH/IGFs axis has been hypothesised as influencing fetal growth (20), in agreement with other studies (40) our findings do not favour this possibility. In fact, while leptin levels were not associated to length and cranial circumference, these parameters were positively correlated with IGFs. Thus, more probably leptin secretion in neonates as well as in adults mainly signals the nutritional state to the brain (2, 3 5). This assumption agrees with the early decrease in leptin levels on the 5th day of life and might be important as a signal stimulating feeding behavior and the acquisition of energy homeostasis in the neonate (21).

The prompt decrease in leptin levels after birth had been already reported (33, 39) and could reflect hormonal changes including variations in insulin, cortisol and thyroid hormone variations (39). The rapid decline of leptin levels could also be due to the removal of placenta at birth (33, 38, 41). That early leptin decrease is more likely to be an orexigenic signaling is also suggested by the lack of association between leptin and weight on the 5th day. This positive association was restored after 30 days when leptin levels showed significant increase. It is likely that at 1 month of life the feeding behavior of the infant is well-established and leptin synthesis and secretion is adapted to reflect a more stable association with body composition and fat stores.

In conclusion, our findings in a large population of healthy neonates definitely show that leptin levels at birth are a function of gender, body weight and gestational age but not of other auxological parameters as well as with IGF-I and IGF-II levels. These findings suggest that leptin secretion in neonates as well as in adults mainly signals the nutritional state to the brain.

## REFERENCES


1. Lonnqvist F, Arner P, Nordfors L, Schalling M. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nat Med 1995, 1: 950-3.
2. Mantzoros CS, Moschos SJ. Leptin: in search of role(s) in human physiology and pathophysiology. Clin Endocrinol (Oxf) 1998, 49: 551-67.
3. Wauters M, Considine RV, Van Gaal LF. Human leptin: from an adipocyte hormone to an endocrine mediator. Eur J Endocrinol 2000, 143: 293-311.
4. Ahima RS, Prabakaran D, Mantzoros C, et al. Role of leptin in the neuroendocrine response to fasting. Nature 1996, 382: 250-2.
5. Yu WH, Kimura M, Walczewska A, Karanth S, McCann SM. Role of leptin in hypothalamic-pituitary function. Proc Nat Acad Sci USA 1997, 94: 1023-8.
6. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996, 334: 292-5.
7. Maccario M, Aimaretti G, Corneli G, et al. Short-term fasting abolishes the sex-related difference in GH and leptin secretion in humans. Am J Physiol Endocrinol Metab 2000, 279: 411-6.
8. Mantzoros CS. Obesity, eating disorders and restrained eating: is leptin the missing link? Mol Psychiatry 1997, 2: 377-80.
9. Patel BK, Koenig JI, Kaplan LM, Hooi SC. Increase in plasma leptin and Lep mRNA concentrations by food intake is dependent on insulin. Metabolism 1998, 47: 603-7.
10. Kolaczynski JW, Nyce MR, Considine RV, et al. Acute and chronic effects of insulin on leptin production in humans: Studies *in vivo* and *in vitro*. Diabetes 1996, 45: 699-701.
11. Boden G, Chen X, Kolaczynski JW, Polansky M. Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. J Clin Invest 1997, 100: 1107-13.
12. Miell JP, Englaro P, Blum WF. Dexamethasone induces an acute and sustained rise in circulating leptin levels in normal human subjects. Horm Metab Res 1996, 28: 704-7.
13. Giacobino JP. Role of the beta3-adrenoceptor in the control of leptin expression. Horm Metab Res 1996, 28: 633-7.
14. Carro E, Senaris R, Considine RV, Casanueva FF, Dieguez C. Regulation of *in vivo* growth hormone secretion by leptin. Endocrinology 1997, 138: 2203-6.
15. Blum WF, Englaro P, Hanitsch S, et al. Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. J Clin Endocrinol Metab 1997, 82: 2904-10.
16. Saad MF, Damani S, Gingerich RL, et al. Sexual dimorphism in plasma leptin concentration. J Clin Endocrinol Metab 1997, 82: 579-84.
17. Mannucci E, Ognibene A, Becorpi A, et al. Relationship between leptin and oestrogens in healthy women. Eur J Endocrinol 1998, 139: 198-201.
18. Behre HM, Simoni M, Nieschlag E. Strong association between serum levels of leptin and testosterone in men. Clin Endocrinol (Oxf) 1997, 47: 237-40.
19. Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF. Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. J Clin Endocrinol Metab 1997, 82: 2849-55.
20. Maffeis C, Moghetti P, Vettor R, Lombardi AM, Vecchini S, Tato L. Leptin concentration in newborns' cord blood: relationship to gender and growth-regulating hormones. Int J Obes Relat Metab Disord 1999, 23: 943-7.
21. Kiess W, Siebler T, Englaro P, et al. Leptin as a metabolic regulator during fetal and neonatal life and in childhood and adolescence. J Pediatr Endocrinol Metab 1998, 11: 483-96.
22. Tome MA, Lage M, Camina JP, Garcia-Mayor RV, Dieguez C, Casanueva FF. Sex-based differences in serum leptin concentrations from umbilical cord blood at delivery. Eur J Endocrinol 1997, 137: 655-8.
23. Shekhawat PS, Garland JS, Shivpuri C, et al. Neonatal cord blood leptin: its relationship to birth weight, body mass index, maternal diabetes, and steroids. Pediatr Res 1998, 43: 338-43.
24. Vatten LJ, Nilsen ST, Odegard RA, Romundstad PR, Austgulen R. Insulin-like growth factor I and leptin in umbilical cord plasma and infant birth size at term. Pediatrics 2002, 109: 1131-35.
25. Koistinen HA, Koivisto VA, Andersson S, et al. Leptin concentration in cord blood correlates with intrauterine growth. J Clin Endocrinol Metab 1997, 82: 3328-30.
26. Schubring C, Kiess W, Englaro P, et al. Levels of leptin in maternal serum, amniotic fluid, and arterial and venous cord blood: relation to neonatal and placental weight. J Clin Endocrinol Metab 1997, 82: 1480-3.
27. Kirel B, Tekin N, Tekin B, Kilic FS, Dogruel N, Aydogdu SD. Cord blood leptin levels: relationship to body weight, body mass index, sex and insulin and cortisol levels of maternal-newborn pairs at delivery. J Pediatr Endocrinol Metab 2000, 13: 71-7.
28. Gluckman PD, Grumbach MM, Kaplan SL. The neuroendocrine regulation and function of growth hormone and prolactin in the mammalian fetus. Endocr Rev 1981, 2: 363–95.
29. Gluckman PD. The endocrine regulation of fetal growth in late gestation. The role of insulin-like growth factors. J Clin Endocrinol Metab 1995, 80: 1047–50.
30. Kristensen K, Pedersen SB, Richelsen B. Regulation of leptin by steroid hormones in rat adipose tissue. Biochem Biophys Res Commun 1999, 259: 624-30.
31. Shimizu H, Shimomura Y, Nakanishi Y, et al. Estrogen increases *in vivo* leptin production in rats and human subjects. J Endocrinol 1997, 154: 285-92.
32. Wabitsch M, Blum WF, Muche R, et al. Contribution of androgens to the gender difference in leptin production in obese children and adolescents. J Clin Invest 1997, 100: 808-13.
33. 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.

34. Tritos N, Mantzoros CS. Leptin: its role in obesity and beyond. Diabetologia 1997, 40: 1371-9.
35. Saad MF, Riad Gabriel MG, Khan A, et al. Diurnal and ultradiurnal rhytmicity of plasma leptin: effects of gender and adiposity. J Clin Endocrinol Metab 1998, 83: 453-9.
36. Havel PJ, Kasim-Karakas S, Dubuc GR, Mueller W, Phinney SD. Gender differences in plasma leptin concentrations. Nat Med 1996, 2: 949-50.
37. Wauters M, Mertens I, Considine R, Leeuw ID, Van Gaal. Are leptin levels dependent on body fat distribution in obese men and women? Eat Weight Disord 1998, 3: 124-30.
38. Hassink SG, de Lancey E, Sheslow DV, et al. Placental leptin: an important new growth factor in intrauterine and neonatal development? Pediatrics 1997, 100, E1.
39. Schubring C, Siebler T, Kratzsch J, et al. Leptin serum concentrations in healthy neonates within the first week of life: relation to insulin and growth hormone levels, skinfold thickness, body mass index and weight. Clin Endocrinol (Oxf) 1999, 51: 199-204.
40. Christou H, Connors JM, Ziotopoulou M, et al. Cord blood leptin and insulin-like growth factor levels are independent predictors of fetal growth. J Clin Endocrinol Metab 2001, 86: 935-8.
41. Senaris R, Garcia-Caballero T, Casabiell X, et al. Synthesis of leptin in human placenta. Endocrinology 1997, 138: 4501-4.