Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Nutritional Biochemistry 16 (2005) 138–143
Journal of Nutritional Biochemistry

Leptin treatment during lactation increases transfer of iodine through the milk☆

Márcia Clements Lins[a], Magna Cottini Fonseca Passos[b], Patrícia Cristina Lisboa[a], Isabela Teixeira Bonomo[a], Egberto Gaspar de Moura[a,*]

[a]Department of Physiological Sciences, State University of Rio de Janeiro, Rio de Janeiro 20550-030, Brazil
[b]Department of Applied Nutrition, Nutrition Institute, State University of Rio de Janeiro, Rio de Janeiro 20550-030, Brazil

Abstract

We have previously shown that protein restriction during lactation is associated with changes in iodine secretion into the milk and that a pup's serum leptin concentration was increased at the end of lactation. So, here we evaluate whether leptin treatment during lactation affects iodine transfer through the milk to the pups. Lactating rats were divided into two groups: the leptin (Lep) group, single injected with recombinant rat leptin (8 μg/100g of body weight, daily for 3 consecutive days), and the control (C) group that received the same volume of saline. We studied iodine transfer to the pups through the milk on Days 4, 12 and 21 of lactation. In those days, the dams were separated from their pups for 4 h. Then, the mothers received an injection of ^{131}I (2.22×10^4 Bq ip) and the pups were allowed to nurse for 2 h. The animals were sacrificed 2 h later. Leptin, total serum T3 and total serum T4 concentrations were higher ($P<.05$) in pups of Lep mothers only on Day 4, suggesting a higher transfer of leptin through the milk at this period, probably with a direct stimulatory effect on thyroid hormone secretion. In other periods, however, even without a detectable increase in a pup's serum leptin concentration, maternal leptin administration increased the pup's thyroid iodine uptake (Day 12, 39%; Day 21, 34%), probably caused by a higher transfer of iodine through the milk, since they had a higher gastric content of ^{131}I on Days 12 (31%) and 21 (128%).

Keywords: Iodine; Leptin; Milk; Rats; Lactation

1. Introduction

Iodine accumulated in the lactating mammary gland and secreted into milk is used by human newborns for thyroid hormone biosynthesis. An adequate supply of iodine for sufficient production of thyroid hormones is essential for the proper development of a newborn's nervous system [1]. Iodine deficiency at this early stage of life results in severe mental retardation [1].

Previously, we have shown that iodine metabolism is differently affected according to the kind of maternal malnutrition during lactation [2]. Protein restriction during lactation is associated with lower iodine secretion into the milk in the beginning of lactation. However, at the end of lactation, a higher transfer of iodine into the milk compensates the impairment of thyroid iodine uptake in these pups, observed during all lactations [2]. In this model, we observed a similar pattern of pups' serum leptin concentrations, with lower values in the beginning of lactation and higher values at the end [3]. On the contrary, the mothers' serum leptin concentrations were higher during all lactations (data not published). Here we hypothesized that these changes of iodine secretion in protein restriction could be mediated by maternal serum leptin concentration.

Leptin is a protein that regulates energy available in the peripheral adipose tissue by specific hypothalamic signals and affects many functions such as body weight, food intake, body temperature and metabolic rate [4,5]. Although leptin was initially reported to be secreted only by adipose tissue [6], recent studies have identified leptin production in

☆ This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo a Pesquisa do Rio de Janeiro (FAPERJ).

* Corresponding author. Departamento de Ciências Fisiológicas - 5° andar, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro 20550-030, Brazil. Tel.: +55 21 25876434; fax: +55 21 25876129.
E-mail address: egmoura@uerj.br (E.G. de Moura).

0955-2863/$ – see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.jnutbio.2004.12.004

a few other tissues including the pituitary, skeletal muscle, placenta, stomach and epithelial cells of the mammary gland [7–13].

Some authors demonstrated the longitudinal pattern of serum leptin during pregnancy and lactation in rats [14,15] and in humans [16,17], suggesting that leptin may play a role in this physiological condition. Leptin concentration increased as gestation progressed and reached the highest level on Days 18 and 21 of pregnancy and declined thereafter [14,15]. Although serum leptin concentration during lactation is lower than that in pregnancy, it is still higher than that in nonlactating women [17].

Increased serum leptin concentrations in late gestation may seem paradoxical since food consumption may decline. This could be deleterious for the mother and the fetus. However, Amico et al. [14] showed that food intake in fact did not diminish in late pregnancy until 24 h preceding delivery, suggesting a resistance to the anorectic effect of leptin.

During lactation, the decrease of serum leptin concentrations is associated to an increase in leptin clearance [18]. In addition, suckling stimulus is associated to maternal leptin suppression [19] and to hypothalamic NPY increase [20], increasing, in this way, food intake that is proper for energy accumulation necessary for milk production.

Most studies concerning leptin action on thyroid axis favor the concept that leptin acts primarily on the hypothalamus, stimulating directly or indirectly TRH production and release [21–25]. Recently, Ortiga-Carvalho et al. [25]

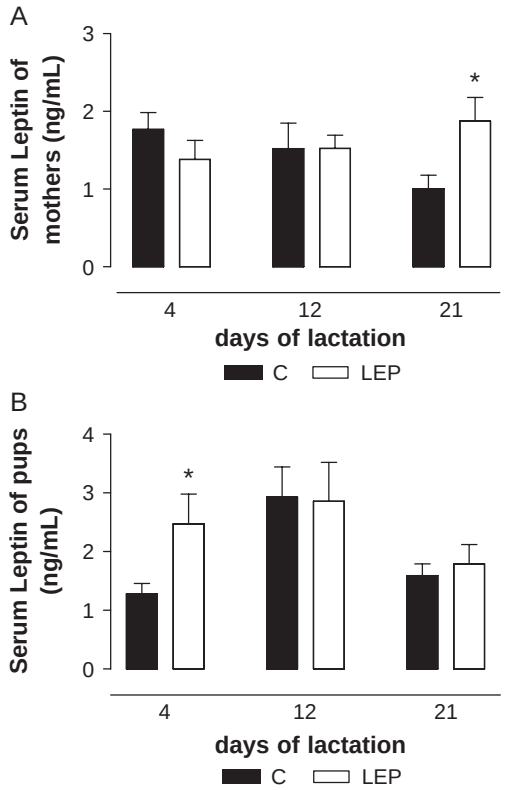

Fig. 1. A, Serum leptin in lactating rats of the C group (black bars) and the group injected with 8 μg of leptin/100 g of body weight (white bars) during lactation. B, Serum leptin in pups' nursing mothers that were treated with saline (C group; black bars) and pups' nursing mothers that were treated with leptin (white bars) during 3 days of lactation. Values are given as the mean±S.E.M. of 6 lactating rats and 12 pups. Asterisk indicates significant differences between the treated group and the C group, the level of significance set at $P<.05$.

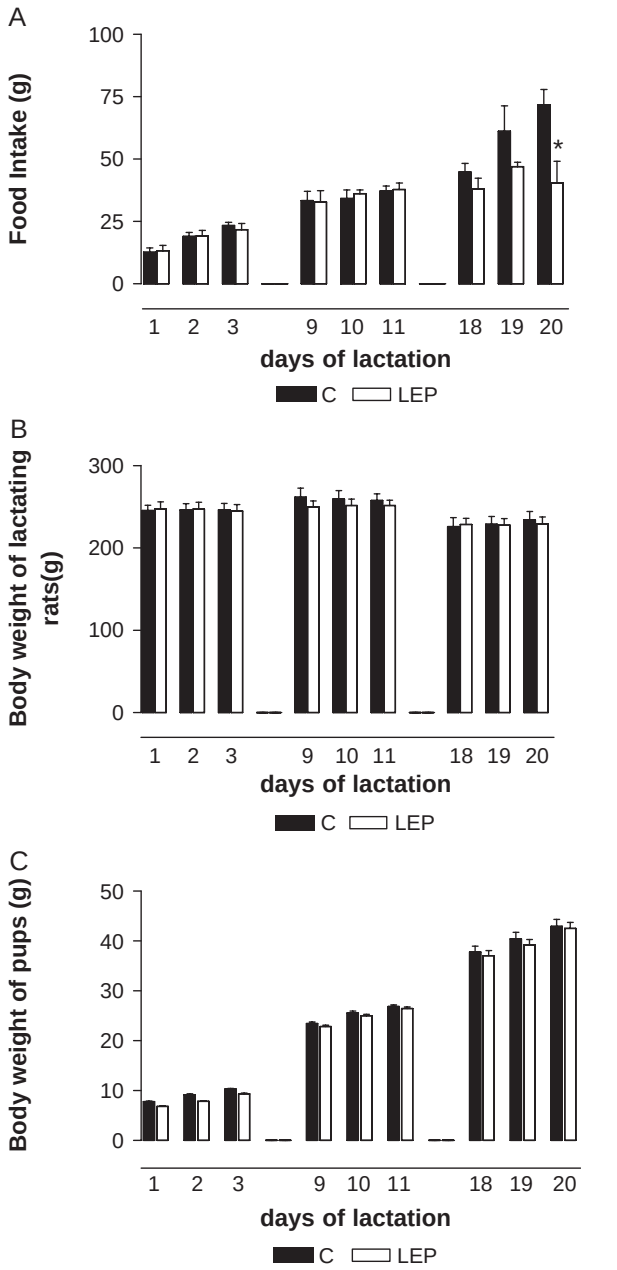

Fig. 2. Food intake (A) and body weight (B) in lactating rats of the C group (black bars) and the group injected with 8 μg of leptin/100 g of body weight (white bars). C, Body weight of pups' nursing mothers that were treated with saline (C group; black bars) and of pups' nursing mothers that were treated with leptin (white bars) during 3 days of lactation. Values are given as the mean±S.E.M. of 6 lactating rats and 12 pups. Asterisk indicates significant differences between the treated group and the C group, the level of significance set at $P<.05$.

showed a direct inhibitory effect of leptin on TSH release, by in vitro pituitary explants, suggesting that leptin may act as an autocrine/paracrine factor at the pituitary level.

Expression of the leptin receptor in the thyroid gland of control rats has been recently detected. Authors also showed, after subcutaneous leptin injection, a reduced serum TSH concentration, consequent to a normal feedback by higher thyroid hormone levels [26]. These findings provide the evidence that leptin stimulates the secretion of thyroid hormones through a direct mechanism involving the thyroid leptin receptor.

Hence, the present study was designed to evaluate whether maternal leptin treatment during lactation affects iodine transfer to the pups through the milk during three different periods of lactation and the consequence on thyroid hormone serum levels.

2. Material and methods

Wistar rats were kept in a room with controlled temperature (25±1°C) and with artificial dark–light cycles (lights on from 7:00 a.m. to 7:00 p.m.). Three-month-old nubile female rats were housed with a male rat, and, after mating, each female rat was placed in an individual cage with free access to water and food until parturition. The use of the animals was carried out according to the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro, which based its analysis on the principles described in the *Guide for the Care and Use of Laboratory Animals* [27]. Mothers were randomly assigned to one of the following groups: the leptin (Lep) group, single injected with 8 μg/100g of body weight of recombinant rat leptin (National Hormone and Pituitary Program, Harbor-UCLA, Medical Center, CA, USA), daily for 3 consecutive days, always at 4:00 p.m., and the control (C) group that received the same volume of saline (NaCl, 0.9%). Body weight and food intake of mothers as well as the body weight of pups were monitored daily during leptin treatment.

To study iodine transfer to the pups through the milk during the three different periods of lactation (Days 4, 12 and 21), we used six dams, which received the leptin injection during the last 3 days before the sacrifice, for each period.

On the day of sacrifice, the dams were separated from their pups for 4 h. After this period, the dams received a single intraperitoneal injection containing 2.22×10^4 Bq of ^{131}I (IPEN, São Paulo, Brazil) and the pups were allowed to nurse for 2 h [2]. Then, only two pups by litter were killed with a lethal dose of pentobarbital and blood was obtained by cardiac puncture. The thyroid gland and duodenum were excised and weighed. The stomach was excised and rinsed in normal saline. The stomach contents were obtained through an incision made in the fundal area of the stomach. The contents were carefully weighed. Gastric ^{131}I content was considered as ^{131}I milk content.

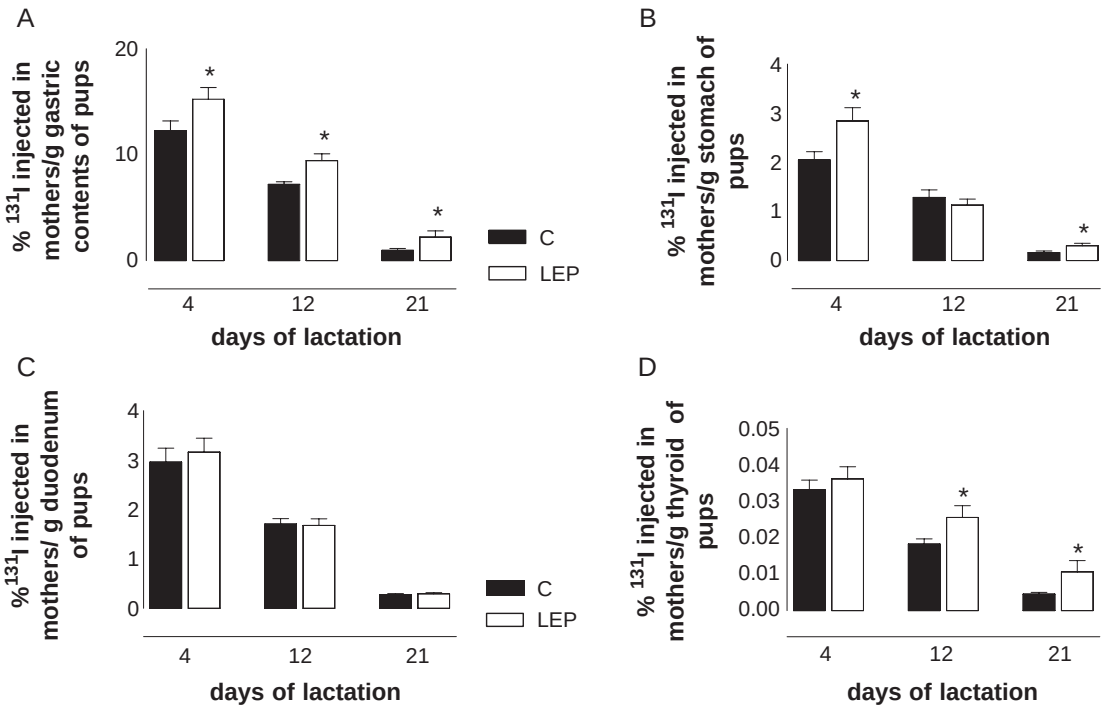

Fig. 3. ^{131}I gastric content (A), stomach (B), duodenum (C), and thyroid iodine uptake (D) of pups' nursing mothers that were treated with saline during 3 days of lactation and injected with ^{131}I 2 h before the sacrifice (black bars) and of pups' nursing mothers that were treated with leptin during 3 days of lactation and injected with ^{131}I 2 h before the sacrifice (white bars). Values are given as the mean±S.E.M. of 12 pups. Asterisk indicates significant differences between the treated group and the C group, the level of significance set at $P<.05$.

The ^{131}I uptake in all tissues was individually evaluated in a gamma counter (Cobra Auto-gamma, Packard Instrument Co., Downers Grove, IL, USA).

Total serum T3 (TT3) and total serum T4 (TT4) were measured by radioimmunoassay (RIA) using commercial kits (Coat-A-Coat, DPC, Los Angeles, CA, USA), in which we used control standard curves diluted in iodothyronine-free rat serum (charcoal treated). Serum TSH was determined by specific RIA using a kit for rat TSH supplied by the NIDDKD (Bethesda, MD, USA) and data were expressed in terms of the reference preparation provided (RP-3).

Leptin concentrations were measured using a commercial kit (Murine Leptin Elisa-DSL-10-24100, Diagnostic Systems Laboratories Inc., Webster, TX, USA). The inter- and intra-assay coefficients of variance were 3.1% and 4.2%, respectively, with a detection limit of 0.04 ng/100 ml.

The data are reported as mean±S.E.M. The statistical significance of experimental observations was determined by the Student's *t* test, with significance level set at $P<.05$.

3. Results

The mothers' and pups' serum leptin concentrations on the last day of treatment are shown in Fig. 1. The serum leptin concentration was significantly ($P<.05$) higher in the mothers only at the end of lactation (Fig. 1A). On the contrary, in the pups from Lep mothers, serum leptin concentration was significantly higher only on Day 4 (+92%; $P<.01$) (Fig. 1B).

The lactating rats that received leptin injection at the end of lactation had significantly lower (43%; $P<.05$) food intake on Day 20 (Fig. 2A), but there were no changes in their body weight (Fig. 2B). The pups from Lep mothers showed no significant changes in body weight compared with pups from the C group (Fig 2C).

Fig. 3 depicts the pups' ^{131}I gastric content, duodenum, stomach and thyroid iodine uptake. Pups of Lep mothers had a significant higher radioiodine gastric content from the beginning to the end of the lactation (24% on Day 4, 31% on Day 12 and 128% on Day 21; $P<.05$) compared with those from the C group (Fig. 3A). Pups from Lep mothers had a significant ($P<.05$) higher iodine uptake in the stomach on Days 4 (38%) and 21 (86%) (Fig. 3B), while, in the duodenum, no significant changes in radioiodine uptake were observed between the groups (Fig. 3C). The thyroid iodine uptake in pups from Lep mothers was significantly ($P<.01$) higher on Days 12 (41%) and 21 (134%) compared with that in pups from the C group (Fig. 3D).

TT3 and TT4 serum concentrations were significantly higher ($P<.05$) in pups from Lep mothers only on Day 4 (81% and 17%, respectively) compared with those in the controls, while the TSH serum concentrations were

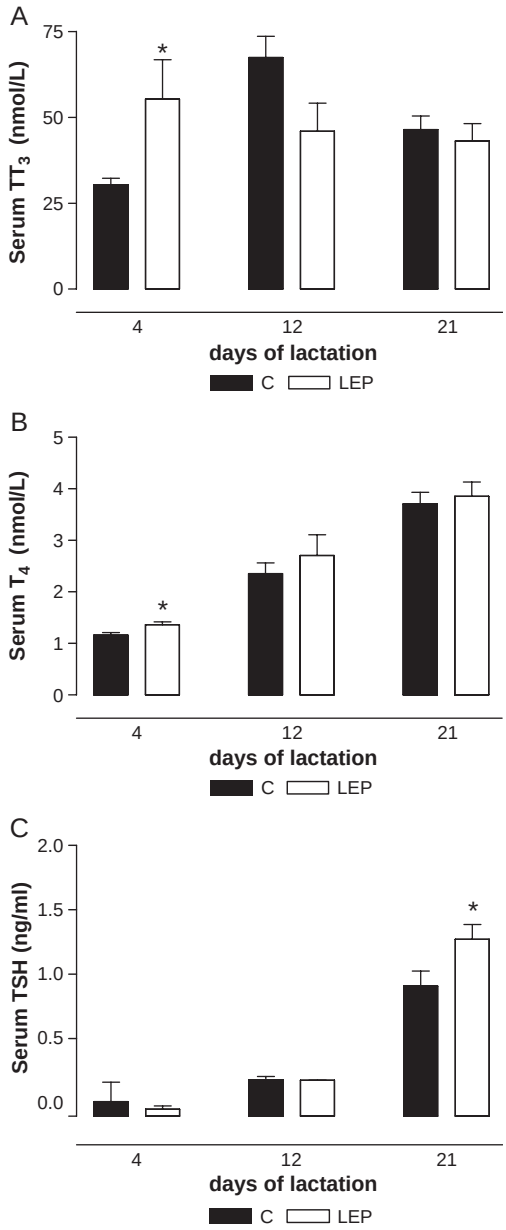

Fig. 4. Sera TT3 (A) and TT4 (B) and TSH (C) in pups' nursing mothers that were treated with saline (C; black bars) and in pups' nursing mothers that were treated with leptin (white bars) during 3 days of lactation. Values are given as the mean±S.E.M. of 12 pups. Asterisk indicates significant differences between the treated group and the C group, the level of significance set at $P<.05$.

significantly higher ($P<.05$) only at the end of lactation (Fig. 4).

4. Discussion

It was the first time that iodine metabolism and thyroid hormone serum concentration were evaluated in a model of maternal hyperleptinaemia during lactation.

Vernon et al. [18] demonstrated that lactation is associated with a decrease of serum leptin concentration

due to an increase of leptin clearance. This fact allows the mother to maintain food consumption in this period and nutritional support for the pups. In the present study, in almost all the periods studied, the maternal serum leptin did not increase, despite the exogenous supply of leptin. We believe that the higher leptin clearance can explain these findings. Besides, it is possible that those animals had a resistance to the anorectic effect of leptin, as suggested before in other models [14,28,29], which explain the maintenance of food consumption. Nevertheless, on the last day of lactation, the treated dams had a significant increase in serum leptin and decrease in food intake. This could be due to a reduction of leptin clearance that occurs at weaning. In addition, Vernon et al. [18] suggest that the nocturnal increase of leptin is reduced by suckling. This contributes to explain this last finding since the food intake decreases only at the end of lactation when the suckling stimulus declines.

In the present study, we corroborate findings of Casabiel et al. [30] that demonstrated in lactating rats injected with ^{125}I-leptin that it is transferred from a mother's circulation through the milk then to a 4-day-old pup's stomach and afterward to its blood. Our study suggests that leptin is transferred from the mothers' milk to the pups on Day 4 because leptin was increased in the pups' sera. An alternative explanation may be that the increase in maternal leptin could stimulate other stimulatory factors to pups' leptin production. One of these factors could be the milk's fat content since Mantzoros et al. [31] observed an association between this factor and pups' leptin concentrations. Besides this increase in serum leptin, the body weight of these animals did not change in our model. It is possible that these animals also present a resistance to the anorectic effect of leptin.

The body weight of pups from Lep mothers did not change in all periods studied, and on Days 12 and 21, coherent serum leptin concentration was normal. It is probable that for the anorectic and metabolic effects, both the low dose and short time of treatment were not sufficient to change these variables. The model used in our study ended to be a better model since this low dose was sufficient to change iodine metabolism and thyroid hormone concentration, as discussed in the Results section. This has made possible the separation of the direct effect of leptin from the indirect effect of malnutrition caused by leptin anorectic effect.

In the present work, the pups of Lep mothers had a significant increase in the radioiodine gastric content from the beginning to the end of the lactation and in the stomach on Days 4 and 21. We suggest that the mammary gland sodium iodine symporter (mg-NIS) transcriptional activity could be regulated by leptin. It has been demonstrated that leptin increases prolactin (PRL) secretion in isolated pituitaries of rats [32,33] and in *ob*/*ob* mice [34]. Rillema and Rowady [35] showed that PRL enhances iodide accumulation in the cultured mammary tissue of mice, suggesting a PRL stimulatory effect of mg-NIS. So, in our model, leptin could increase iodine transfer to the pups, directly or through PRL stimulation.

The data of higher thyroid ^{131}I uptake in pups from Lep mothers, on Days 12 and 21 of lactation, are, in part, in agreement with those shown by us in pups from protein-restricted mothers, which had higher transfers of iodine at the end of lactation [2]. We did not find studies about the effects of leptin on iodide thyroid uptake. However, Nowak et al. [26] showed that leptin chronic administration stimulates rat thyroid hormone secretion, probably through a direct mechanism involving the thyroid leptin receptor. So, it is possible that the higher ^{131}I thyroid uptake is due to a direct stimulatory effect of leptin. Probably, this increase is due both to the higher supply of iodine and to higher absorption since the radioiodine gastric content and iodine uptake in the stomach are higher during all lactations. Thus, as discussed in relation to the mammary gland and thyroid NIS, our data also suggest a stimulatory effect of leptin on gastric NIS expression.

On Day 4, when we observed an increase in the pups' serum leptin concentrations, T3 and T4 showed a significant increase, corroborating the finding of Nowak et al. [26], now in a neonatal model. At the end of lactation, the pups had an increase of TSH, which confirms findings of other authors showing a stimulatory role of leptin on TSH secretion in neonatal rats [21,25]. This higher TSH level could contribute to higher thyroid radioiodine uptake.

Therefore, our data suggest a stimulatory effect of leptin on NIS activity in different tissues that could contribute to a higher transfer of iodine from a mother to a pup and could help explain why malnourished mothers had higher iodine transfers at the end of lactation, associated with higher serum leptin concentrations both in pups and mothers.

References

[1] Stubbe P, Schulte FJ, Heidemann P. Iodine deficiency and brain development. Bibl Nutr Dieta 1986;38:206–8.
[2] Passos MCF, Ramos CF, Dutra SCP, Moura EG. Transfer of iodine through the milk in protein-restricted lactating rats. J Nutr Biochem 2001;12:300–3.
[3] Teixeira CV, Passos MCF, Ramos CF, Dutra SCP, Moura EG. Leptin serum concentration in rats whose mothers were submitted to malnutrition during lactation. J Nutr Biochem 2002;13:493–8.
[4] Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in *ob*/*ob* mice. Science 1995;269:540–3.
[5] Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–70.
[6] Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. J Clin Invest 1996;98:1101–6.
[7] Senaris R, Garcia-Caballero T, Casabiell X, Callero R, Castro R, Considine RV, et al. Synthesis of leptin in human placenta. Endocrinology 1997;138:4501–4.
[8] Bado A, Levasseur S, Attoub S, Kermongant S, Laigneau JP, Bortoluzzi MN, et al. The stomach is a source of leptin. Nature 1998;394:790–3.

[9] Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature 1998;393:684–8.
[10] Smith-Kirwin SM, O'Connor DM, De Johnston J, De Lancey ED, Hassink SG, Funanage VL. Leptin expression in human mammary epithelial cells and breast milk. J Clin Endocrinol Metab 1998; 83:1810–3.
[11] Morash B, Li A, Murphy PR, Wilkinson M, Ur E. Leptin gene expression in the brain and pituitary gland. Endocrinology 1999;1140(12):5995–8.
[12] Jin L, Burguera BG, Couce ME, Scheithauer BW, Lamson J, Eberhardt NL, et al. Leptin and leptin receptor expression in the normal and neoplastic human pituitary: evidence of a regulatory role of leptin on pituitary cell proliferation. J Clin Endocrinol Metab 1999; 84:2903–11.
[13] Jin L, Zhang S, Burguera BG, Couce ME, Osamura RY, Kulig E, et al. Leptin and leptin receptor expression in rat and mouse pituitary cells. Endocrinology 2000;141:333–9.
[14] 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; 63:1387–95.
[15] Herrera E, Lasuncion MA, Huerta L, Martin-Hidalgo A. Plasma leptin levels in rat mother and offspring during pregnancy and lactation. Biol Neonate 2000;78:315–20.
[16] Butte NF, Hopkinson JM, Nicholson MA. Leptin in human reproduction: serum levels in pregnant and lactating women. J Clin Endocrinol Metab 1997;82:585–9.
[17] Mukherjea R, Castonguay TW, Douglass LW, Moser-Veillon P. Elevated leptin concentrations in pregnancy and lactation: possible role as a modulator of substrate utilization. Life Sci 1999;65: 1183–93.
[18] Vernon RG, Denis RG, Sorensen A, Williams G. Leptin and the adaptations of lactation in rodents and ruminants. Horm Metab Res 2002;34:678–85.
[19] Brogan RS, Mitchell SE, Trayhurn P, Smith MS. Suppression of leptin during lactation: contribution of the suckling stimulus versus milk production. Endocrinology 1998;140:2621–7.
[20] Li C, Chen P, Smith MS. The acute suckling stimulus induces expression of neuropeptide Y (NPY) in cells in the dorsomedial hypothalamus and increases NPY expression in the arcuate nucleus. Endocrinology 1998;139:1645–52.
[21] Legradi G, Emerson CH, Ahima RS, Flier JS, Lechan RM. Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. Endocrinology 1997;138:2569–76.
[22] Harris M, Aschkenasi C, Elias CF, Chandrankunnel A, Nillni EA, Bjorbaek C, et al. Transcriptional regulation of the thyrotropin-releasing hormone gene by leptin and melanocortin signaling. J Clin Invest 2001;107:111–20.
[23] Nillni EA, Vaslet C, Harris M, Hollenberg A, Bjorbaek C, Flier JS. Leptin regulates prothyrotropin-releasing hormone biosynthesis. J Biol Chem 2000;275:36124–33.
[24] Kim MS, Small CJ, Stanley SA, Morgan DG, Seal LJ, Komg WM, et al. The central melanocortin system effects the hypothalamic-pituitary thyroid axis and may mediate the effect of leptin. J Clin Invest 2000;105:1005–11.
[25] Ortiga-Carvalho TM, Oliveira KJ, Soares BA, Pazos-Moura CC. Leptin role in the regulation of thyrotropin secretion in fed state — in vivo and in vitro studies. J Endocrinol 2002;174:121–5.
[26] Nowak KM, Kaczmarek P, Mackowiak P, Ziolkowska A, Albertin G, Ginda WJ, et al. Rat thyroid gland expresses the long form of leptin receptors, and leptin stimulates the function of the gland in euthyroid non-fasted animals. Int J Mol Med 2002;9:31–4.
[27] Bayne K. Revised guide for the care and use of laboratory animals available. Am Phys Soc Physiol 1996;39:208–11.
[28] Mounzih H, Qiu J, Ewart-Toland A, Chehab FF. Leptin is not necessary for gestation and parturition but regulates maternal nutrition via a leptin resistance state. Endocrinology 1998;139: 5259–62.
[29] Garcia MC, Casanueva FF, Diégues C, Senarís RM. Gestational profile of leptin messenger ribonucleic acid (mRNA) content in the placenta and adipose tissue in the rat, and regulation of the mRNA levels of the leptin receptor subtypes in the hypothalamus during pregnancy and lactation. Biol Reprod 2000;62:698–703.
[30] Casabiell X, Piñeiro V, Tomé MA, Peinó R, Diéguez C, Casanueva FF. Presence of leptin in colostrum and/or breast milk from lactating mothers: a potential role in the regulation of neonatal food intake. J Clin Endocrinol Metab 1997;82:4270–3.
[31] Mantzoros CS, Varvarigou A, Kaklamani VG, Beratis NG, Flier JS. Effect of birth weight and maternal smoking on cord blood leptin concentrations of full-term and preterm newborns. J Clin Endocrinol Metab 1997;9:2856–61.
[32] Watanobe H, Suda T, Wikberg JE, Schioth HB. Evidence that physiological levels of circulating leptin exert a stimulatory effect on luteinizing hormone and prolactin surges in rats. Biochem Biophys Res Commun 1999;263:162–5.
[33] Yu WH, Kimura M, Walczewska A, Karanth S, Mccann SM. Role of leptin in hypothalamicpituitary function. Proc Natl Acad Sci U S A 1997;94:1023–8.
[34] Watanobe H, Schioth HB, Suda T. Stimulation of prolactin secretion by chronic, but not acute, administration of leptin in the rat. Brain Res 2000;887:426–31.
[35] Rillema JA, Rowady DL. Characteristics of the prolactin stimulation of iodide uptake into mouse mammary gland explants. Proc Soc Exp Biol Med 1997;215:366–9.