Secretory pattern of leptin and LH during lactational amenorrhoea in breastfeeding normal and polycystic ovarian syndrome women

T. Sir-Petermann1,4, S. E. Recabarren2, A. Lobos2, M. Maliqueo1 and L. Wildt3

1Division of Endocrinology, Department of Internal Medicine, School of Medicine, University of Chile, Santiago, Chile, 2Laboratory of Animal Physiology and Endocrinology, School of Veterinary Medicine, University of Concepción, Chillán, Chile and 3Division of Gynecological Endocrinology and Reproductive Medicine, Department of Obstetrics and Gynecology, University of Erlangen, Germany

4To whom correspondence should be addressed at: Las Palmeras 299, Interior Quinta Normal, Casilla 33052, Correo 33, Santiago, Chile. E-mail: tsir@entelchile.net

Several studies have suggested that leptin modulates hypothalamic–pituitary–gonadal axis function. A synchronicity of LH and leptin pulses has been described in healthy women and in patients with polycystic ovarian syndrome (PCOS), suggesting that leptin may modulate the episodic secretion of LH. The aim of the present investigation was to assess the episodic fluctuations of circulating LH and leptin during lactational amenorrhoea in fully breastfeeding normal and PCOS women at 4 and 8 weeks postpartum, in order to establish LH–leptin interactions in the reactivation of the gonadal axis during this period. Six lactating PCOS patients and six normal lactating women of similar age and body mass index were studied. During a 12 h period on the 4th and 8th weeks postpartum, blood samples were collected at 10 min intervals for 12 h (22:00–10:00). Serum LH and leptin concentrations were measured in all samples. For pulse analysis, the cluster algorithm was used. To detect an interaction between LH and leptin pulses, an analysis of co-pulsatility was employed. LH concentrations tended to increase in both groups between the 4th and 8th weeks postpartum; however, serum leptin concentrations were not modified. Leptin pulse frequencies were similar at the 4th and 8th weeks postpartum, and did not differ between groups. Moreover, leptin pulse frequency was higher than LH pulse frequency in both groups, and in the two study periods. There was no synchronicity between LH and leptin pulses, and there were no increments in leptin concentration during the night. The fact that leptin concentrations were not modified and no synchronicity between LH and leptin pulses was observed suggests that, during lactational amenorrhoea, circulating leptin is probably not involved as a primary signal in promoting the reactivation of pulsatile LH secretion.

Key words: lactation/leptin/LH/polycystic ovarian syndrome

Introduction

Leptin, the protein hormone coded by the ob gene which is implicated in the control of body weight and thermogenesis (Zhang et al., 1994), also appears to act as a metabolic signal influencing the reproductive axis (Barash et al., 1996). In ob/ob mice, leptin deficiency results in hypogonadotrophic hypogonadism, impaired sexual maturation and infertility, which are corrected by leptin administration (Ahima et al., 1996; Chehab et al., 1996). In humans, a role for circulating leptin in the regulation of reproduction has also been suggested by many studies (Magoffin and Huang, 1998; Rosenbaum and Leibel, 1998; Mantzoros, 2000). Leptin appears to play a role in promoting hypothalamic function and maintaining adequate levels of gonadotrophin secretion (Laughlin et al., 1998). According to earlier studies (Yu et al., 1997a,b), leptin may stimulate gonadotrophin-releasing hormone (GnRH) release from the hypothalamus, and LH and FSH release from the pituitary, probably by acting on its own receptor and promoting nitric oxide release.

In addition, a synchronicity of LH and leptin pulses in the follicular phase of the menstrual cycle of healthy women (Licinio et al., 1998) and in patients with polycystic ovarian syndrome (PCOS) has been demonstrated (Sir-Petermann et al., 1999a), suggesting that leptin may regulate the episodic oscillations in plasma concentrations of LH.

It is apparent that puberty and the postpartum period share some common neuroendocrine features in the initiation and reactivation of menstrual cyclicity. Increments in LH secretion during the night are a feature of the postpartum period, analogous to that observed during early puberty (Liu and Park,
LH and leptin in lactating PCOS and normal women

1988); thus, the postpartum period resembles a ‘miniature puberty’ (Yen, 1998). This ‘miniature puberty’ could serve as a neuroendocrine model in exploring the link between central and peripheral influences in the reactivation of the gonadal axis in normal conditions, and in reproductive dysfunctions such as PCOS. Therefore, it is possible that, during the postpartum period (and similar to what has been suggested for the prepubertal period; Clayton et al., 1997; Garcia-Mayor et al., 1997; Mantzoros et al., 1997), rising leptin concentrations could be implicated in triggering the reactivation of pulsatile gonadotrophin secretion.

PCOS is a heterogeneous endocrine-metabolic disorder of unknown cause in women, characterized by hyperandrogenerinaemia, chronic anovulation, increased LH concentrations and high incidence of obesity and insulin resistance (Dunaif et al., 1989; Poretsky and Piper, 1994; Franks, 1995). Thus, PCOS may serve as a useful model to study LH–leptin interactions in pathological conditions in humans.

The aim of the present investigation was to assess the episodic fluctuations of circulating LH and leptin during lactational amenorrhoea in fully breastfeeding normal and PCOS women at 4 and 8 weeks postpartum, in order to establish LH–leptin interactions in the reactivation of pulsatile LH secretion during this period.

Materials and methods

Subjects
Six women with PCOS with normal-term pregnancies were selected for the study from patients attending the Unit of Reproductive Medicine, University of Chile. The patients’ mean age was 24.2 (range 21–33) years, and their mean body mass index (BMI) was 28.3 (range 25.2–37) kg/m². A preconceptional diagnosis of PCOS was made according to the following clinical and endocrine criteria: chronic oligo- or amenorrhoea; hirsutism; plasma testosterone concentration >0.6 ng/ml or free androgen index >5.0; and a characteristic ovarian morphology on ultrasound based on previously described criteria (Adams et al., 1986). A normal LH/FSH ratio was not considered an exclusion criterion. All women were amenorrhoeic and anovulatory according to progesterone measurements and ultrasound examinations. Hyperprolactinaemia, androgen-secreting neoplasm, Cushing’s syndrome and attenuated 21-hydroxylase deficiency as well as thyroid disease were excluded by appropriate tests.

Before the study, informed consent was obtained from all subjects. This study was approved by the local ethical committee.

Patients included in the study were selected from a group of 20 women with PCOS attending the Unit of Reproductive Medicine who desired fertility, and were placed on a 6-month diet and exercise programme which consisted of a 1000 kcal, low-fat diet and a daily walk of 30 min. During this programme, six patients ovulated and became pregnant and were included in the present study. With this intervention, the pregnancy rate was 30%; these were values similar to those reported previously (Hollmann et al., 1996; Huber-Buchholz et al., 1999).

By design, six normal lactating (NL) women of similar age [mean 23.8 (19–30) years] and BMI [mean 26.7 (20.8–30.0) kg/m²] participated in the study. Each woman had a history of regular 28- to 32-day menstrual cycles, absence of hirsutism and other manifestations of hyperandrogenism, absence of galactorrhoea, thyroid dysfunction and family history of diabetes. All were healthy, were not receiving any drug therapy, and had a normal-term pregnancy and vaginal delivery of a healthy child. These women were recruited from the maternity unit of the same hospital.

Both groups of lactating women maintained exclusive breastfeeding, and all were amenorrhoeic when the study was performed.

In addition, two reference groups for LH and leptin concentrations and pulse characteristics, comprising six healthy normally cycling women (HW) and six PCOS non-pregnant, non-lactating women, comparable in age [HW 28.6 (21.0–35.0) years; PCOS 26.0 (21.0–33.0) years] and BMI [HW 25.5 (21.2–31.2); PCOS 27.2 (22.0–32.0) kg/m²] with the lactating groups, were included. All PCOS women were amenorrhoeic and anovulatory according to progesterone measurements and ultrasound examinations. Normally cycling women were studied in the early follicular phase of the menstrual cycle (days 3–7). In the amenorrhoeic patients, the study began whenever feasible.

Study protocol
The women were admitted to the Clinical Research Center on the evening before the study on the 4th and 8th weeks postpartum. Infants accompanied their mothers, and breastfeeding was continued ad libitum. The study was initiated at the 4th week postpartum to avoid the effect of placentary steroids and peptide hormones. During a 12 h period (22:00–10:00), blood samples were collected every 10 min, using a sampling device that allowed the continuous withdrawal of blood through a heparinized catheter (Sir-Petermann et al., 1995). For the reference group, samples were collected at 10 min intervals for 6 h beginning at 09:00. Serum LH and leptin concentrations were determined in all samples. Serum total testosterone and oestradiol were determined in the first fasting sample. Prolactin (PRL) was measured in samples taken before and 30 min after each suckling episode.

Follicular development was monitored by ultrasound examination of the ovaries from the 4th week postpartum every 10 days. Scans were made using a 5.0 MHz vaginal probe (Aloka, Tokyo, Japan).

Hormone assays
Serum LH and oestradiol concentrations were determined by electrochemiluminescence (Roche, Basel, Switzerland; range: 0.1–200 IU/l for LH and 10–4000 pg/ml for oestradiol). The intra- and inter-assay coefficients of variation were 1.1 and 2.1% for LH, and 2.7 and 5% for oestradiol respectively. Serum leptin was measured by radioimmunoassay (Linco-Research Inc., St Louis, MO, USA); the intra- and inter-assay coefficients of variation were 2.5 and 3.6% respectively.

Testosterone, insulin and prolactin were measured by radioimmunoassay using commercial kits (DPC, Los Angeles, CA, USA). The intra- and inter-assay coefficients of variation were 7.0 and 11% for testosterone and 1.1 and 1.6% for PRL respectively.

Pulse analysis and statistical evaluation
For pulse analysis, the computerized version of the cluster pulse algorithm was used (Veldhuis and Johnson, 1986). A cluster configuration of 1×2 (one sample for the test peak and two for the test nadir), and a t-value of 2.1/2.1 to constrain the likelihood of false positive pulse determination to <5% were selected. The following mean properties of LH and leptin pulsatile concentrations were analysed: pulse frequency (number of significant peaks/h), pulse height, and pulse amplitude.

For the analysis of co-pulsatility, the ANCOPULS program (Albers et al., 1993) was used, as described previously (Sir-Petermann et al., 1999a). Statistical analyses were performed using the Wilcoxon test for comparisons within groups, and the Mann–Whitney test for comparisons between groups. Results are expressed as means and
Pulse frequencies were similar at the 4th and 8th weeks in women with similar characteristics to those observed in non-pregnant, non-lactating normal and PCOS women (Table I). The clinical and endocrine characteristics for lactating PCOS (LPCOS) women compared with normal lactating (NL) women at the 4th and 8th weeks postpartum.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4th week</th>
<th></th>
<th>8th week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NL</td>
<td>LPCOS</td>
<td>NL</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.8 (19–30)</td>
<td>24.2 (21–33)</td>
<td>24.0 (20–30)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7 (20.8–30.0)</td>
<td>28.3 (25.2–30.7)</td>
<td>26.73 (20.5–30.8)</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.13 (0.1–0.2)</td>
<td>0.21 (0.10–0.31)</td>
<td>0.11 (0.10–0.16)</td>
</tr>
<tr>
<td>Oestradiol (pg/ml)</td>
<td>12.9 (10.0–19.53)</td>
<td>19.8 (12.1–36.1)</td>
<td>16.3 (10.0–37.7)</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>200.1 (112.8–320.8)</td>
<td>148.7 (52.2–248.3)</td>
<td>212.6 (99.3–338.7)</td>
</tr>
</tbody>
</table>

Values are mean (range). *P < 0.05, NL versus LPCOS.

Ranges in the tables as means in the figures. Differences were considered significant when P < 0.05.

**Results**

**Clinical and endocrine characteristics**

The clinical and endocrine characteristics for lactating PCOS (LPCOS) women compared with NL women in the two study periods are shown in Table I.

Postpartum period resembles a miniature puberty, with increments in LH secretion during night-time as the first signal of reactivation of the ovarian axis. The BMI was similar in both groups, and did not change between the 4th and 8th weeks postpartum. In both groups, and in the two study periods, serum steroid concentrations remained low and follicular development assessed by ultrasound was not detected from the 4th to the 8th week postpartum.

**Mean serum concentrations**

Serum LH concentrations tended to increase in both groups between the 4th and 8th weeks postpartum, but remained lower than those observed in non-pregnant, non-lactating women (Table II). In contrast, serum leptin concentrations were not modified between these two periods and were comparable with values found in non-pregnant, non-lactating women (Table II). Moreover, in both groups and in the two study periods, a nocturnal leptin surge was not observed (Figure 1).

Mean serum prolactin concentrations were slightly lower in LPCOS patients compared with NL women at the 4th week postpartum, but at 8 weeks the mean serum prolactin concentrations were significantly lower in LPCOS women than in NL women (Table I).

**Pulse analysis**

The LH and leptin pulse characteristics in NL and LPCOS women at the 4th and 8th week postpartum are shown in Table II. In both groups of lactating women, LH and leptin pulse activities were observed at the 4th and 8th weeks postpartum. At the 8th week postpartum, in PCOS women, LH pulse frequency tended to increase as compared with that observed in NL women in the same sampling period. However, leptin pulse frequencies were similar at the 4th and 8th weeks postpartum and did not differ between the groups. Moreover, leptin pulse frequency was higher than LH pulse frequency in both groups, and in the two study periods.

Compared with non-pregnant non-lactating women, leptin pulse characteristics in both groups and in the two study periods were similar.

There was no synchronicity between LH and leptin pulses according to the Ancopuls analysis (Figure 2).

**Discussion**

In this study, the episodic fluctuations of circulating LH and leptin in normal and PCOS lactating women at 4 and 8 weeks postpartum were evaluated. In a state of partial inhibition of GnRH–LH pulse activity, the pulsatile pattern of leptin was present in both groups of women with similar characteristics to those observed in non-pregnant, non-lactating women. In both groups of lactating women, there were no changes in leptin concentrations between the two study periods, there were no increments in night-time leptin concentrations, and there was no synchronicity between LH and leptin pulses during the two study periods.

The stimulatory effect of leptin on the neuroendocrine–reproductive axis has been established in some species, including rat (Cagampang et al., 1990), monkey (Finn et al., 1998; Nagatani et al., 1998) and human (Laughlin and Yen, 1997; Laughlin et al., 1998). Rising leptin concentrations have been associated with the initiation of puberty in animals and humans, and normal leptin concentrations are needed for the maintenance of menstrual cycles and normal reproductive function. On the other hand, it has been suggested that the postpartum period resembles a ‘miniature puberty’, with increments in LH secretion during night-time as the first signal of reactivation of the ovarian axis. Due to this apparent similarity between puberty and the postpartum period, rising leptin concentrations during different states of this period and an increase in leptin concentrations at night would be expected. However, this was not observed in the present study. On the contrary, the pulsatile pattern of leptin was present in both groups of women with similar characteristics to those observed in non-pregnant, non-lactating normal and PCOS women.
TABLE II. LH and leptin concentrations and pulse characteristics in normal lactating (NL) women and lactating PCOS (LPCOS) women at the 4th and 8th weeks postpartum

<table>
<thead>
<tr>
<th></th>
<th>4th week</th>
<th>8th week</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NL</strong></td>
<td></td>
<td></td>
<td><strong>HW</strong></td>
</tr>
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</table>
| LH (IU/l)      | 1.0 (0.32–1.86)   | 2.73 (1.58–3.54)  | 4.76 (2.57–6.41)^
| LH pulse (number/h) | 0.65 (0.25–0.92) | 0.58 (0.33–0.75) | 1.0 (0.83–1.16)^
| LH pulse height (IU/l) | 1.44 (0.35–2.68) | 3.03 (1.68–7.05) | 5.75 (3.57–9.46)^
| LH amplitude (IU/l) | 0.62 (0.04–1.72) | 1.09 (0.22–1.14) | 1.86 (0.7–2.68)^
| **LPCOS**      |                   |                   | **PCOS**        |
| LH (IU/l)      | 2.11 (0.9–4.59)   | 3.26 (0.89–6.42)  | 9.92 (6.6–13.0)^
| LH pulse (number/h) | 0.63 (0.42–0.75) | 0.76 (0.66–0.92) | 1.02 (0.83–1.16)^
| LH pulse height (IU/l) | 2.64 (0.80–6.39) | 3.9 (0.79–9.04)  | 11.25 (7.29–15.03)^
| LH amplitude (IU/l) | 1.25 (0.06–3.83) | 1.21 (0.11–3.24) | 2.37 (1.3–3.8)^
| **NL**         |                   |                   | **HW**          |
| Leptin (ng/ml) | 20.6 (4.54–44.24) | 19.86 (8.51–33.28)| 21.69 (19.16–27.33) |
| Leptin pulse (number/h) | 1.06 (0.83–1.33) | 1.05 (0.83–1.25) | 1.03 (1.0–1.16)^
| Leptin pulse height (ng/ml) | 25.37 (5.81–58.0) | 26.18 (12.32–43.9) | 26.79 (22.77–33.61) |
| Leptin amplitude (ng/ml) | 6.95 (1.64–18.19) | 9.45 (4.48–14.2) | 7.58 (3.78–14.33) |
| **LPCOS**      |                   |                   | **PCOS**        |
| Leptin (ng/ml) | 17.29 (11.16–29.94) | 17.24 (7.97–25.41) | 16.98 (9.85–23.28) |
| Leptin pulse (number/h) | 1.03 (0.83–1.25) | 1.01 (0.83–1.25) | 1.06 (1.0–1.16)^
| Leptin amplitude (ng/ml) | 3.04 (1.77–5.40) | 5.18 (1.84–8.44) | 4.05 (2.01–6.23)^

Values are mean and range.

^P < 0.05 between reference values and 4th week postpartum.

^bP < 0.05 between reference values and 8th week postpartum.

^cP < 0.05 between normal women and PCOS women.

^dP < 0.05 between LH and leptin pulses.

HW = healthy, normally cycling women; PCOS = non-pregnant, non-lactating PCOS women.

According to our previous observations (Sir-Petermann et al., 1999a,b) and the data of the present study, leptin pulsatility persists despite a partial inhibition of GnRH–LH activity, suggesting again that circulating leptin might regulate GnRH–LH secretion, though this is apparently not involved in modulating episodic leptin release.

The absence of a nocturnal surge of leptin is concordant with results obtained in lactating rats (Pickavance et al., 1998), in healthy normally cycling women (Marsh et al., 2000) and in ewe lambs (Recabarren et al., 2000), where no differences between daytime and night-time leptin concentrations were observed. This allowed us to compare leptin concentrations and pulse characteristics of these women with those observed in normal cycling women and PCOS non-pregnant, non-lactating women during the daytime.

In healthy women (Licinio et al., 1998) and in patients with PCOS (Sir-Petermann et al., 1999a), a synchronicity of LH and leptin pulses has been observed, suggesting that leptin may regulate the oscillations in serum LH concentrations. However, the present results did not demonstrate that circulating leptin and LH are synchronized during lactation in normal and PCOS women. This phenomenon could be due to the fact that during lactation, GnRH–LH secretions are partially inhibited, which probably prevents the coupling of LH and leptin release.

During the postpartum period, breastfeeding imposes a negative energy balance and an elevation of plasma prolactin concentrations. When leptin concentrations during lactational amenorrhoea are compared with daytime concentrations found in healthy, normally cycling women during the early follicular phase and in non-pregnant, non-lactating PCOS women, it is...
observed that leptin concentrations and leptin pulse characteristics are similar. These findings are in agreement with those reported earlier (Butte et al., 1997), which showed that leptin concentrations did not differ between lactating and non-lactating women. This suggests that during lactation in humans, leptin concentrations are not modified—contrary to what is described in rodents, where leptin concentrations are decreased during lactation (Pickavance et al., 1998; Brogan et al., 1999).

This decrease in leptin concentrations has been attributed to the metabolic drain of milk production and to the inhibitory effect of PRL on leptin secretion (Brogan et al., 1999). However, in humans, the metabolic drain of milk production is not comparable with that seen in rodents (Neville et al., 1994; Stanley et al., 1998), suggesting that this factor is probably not involved as a modulatory factor in leptin secretion during lactation in humans.

According to the results of the present study, lactating PCOS patients exhibited lower mean plasma PRL concentrations than NL women, although leptin concentrations were comparable. This observation is in accordance with the assumption that, in humans, prolactin probably also plays a secondary role in modulating leptin concentrations during lactation.

In summary, from the 4th week postpartum, both leptin and LH exhibit a pulsatile pattern of secretion in normal and PCOS lactating women, in the absence of follicular activity. Leptin concentrations were comparable in normal and PCOS lactating women, probably because the BMI of both groups was similar and did not change between the 4th and 8th week postpartum. Leptin secretion is not related to LH secretion, or to the PCOS condition during lactational amenorrhea. This suggests that leptin should not be considered as a primary promoting metabolic signal for triggering reactivation of the gonadal axis in the ‘miniature puberty’ of the postpartum period, as has been proposed by some authors for the onset of puberty. However, this does not exclude that, during the postpartum period, leptin may exert a modulatory effect on the neuroendocrine–reproductive axis.

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