Abstract. Plasma LRH-immunoreactive substance in puerperal women was very low on days 5 and 10 post-partum and significantly increased between days 10 and 20 post-partum, but remained at a similar level on day 30 post-partum, which was significantly lower than that in eumenorrhoeic women on day 8 or 9 of the normal cycle. Although LRH administration did not significantly increase serum FSH on day 11 post-partum, daily injections of LRH from days 5 through 10 post-partum caused a significantly increased secretion of FSH on day 11 post-partum, both basally and in response to LRH. No significant difference was observed in serum oestradiol and Prl levels on day 11 post-partum between the two groups of women with and without LRH pre-treatment. It is concluded that the amount of hypothalamic LRH secretion may be small during the first month post-partum, and low basal FSH and unresponsiveness of the pituitary to LRH observed in the early puerperium may result from hypothalamic hypofunction.

Many studies have been made on the pituitary function in puerperal women; most investigators (Canales et al. 1974; Jeppsson et al. 1974; Friedman et al. 1976; Keye & Jaffe 1976; Miyake et al. 1978) demonstrated the pituitary refractoriness to 25–100 µg of LRH administration as well as the low level of serum FSH prior to 2 weeks post-partum, though Marrs et al. (1981) found a small but significant increase in serum FSH after 150 µg of LRH administration on day 10 post-partum. However, the hypothalamic function which regulates gonadotrophin secretion in puerperal women still remains obscure. The present study was designed to investigate the hypothalamic function in puerperal women during the first month post-partum and to elucidate the cause of the pituitary unresponsiveness to LRH stimulation in the early puerperium.

Materials and Methods

The study was performed in volunteer women on days 5–30 in the puerperium. All women had had normal menstrual cycles before the pregnancy and experienced normal puerperal breast engorgement and lactation. None of the women received hormonal therapy during the puerperium. For comparison, regularly menstruating women volunteered for this study. Blood samples for assay of LRH-immunoreactive substance (LRHIS) were taken around 10.30 a.m. in a resting position, after at least a 3 h fast and after a 2 h non-lactation. Blood samples for LRHIS assay were obtained longitudinally from 5 puerperal women on days 5, 10, 20 and 30 post-partum and from 1 puerperal woman on days 5, 20 and 30 post-partum. Blood samples were also obtained around 10.30 a.m. from 6 eumenorrhoeic women on day 8 or 9 of the normal cycle. The samples were collected in heparinized tubes treated with aprotinin (Bayer, Leverkusen, Germany), chilled on ice, and plasma was separated. All samples for hormone assays were stored at −20°C until assayed.

On day 11 post-partum, 7 of 12 puerperal women were given an iv infusion of 100 µg of synthetic LRH (Daiichi-seiyaku, Tokyo, Japan) and 5 were given 200 µg, since it was suggested that the difference between the results of
Marrs et al. (1981) and the other investigators might depend on the dose of LRH injected. Baseline blood samples for assays of FSH, Prl and oestradiol were started around 10:30 a.m., after at least a 3 h fast and after a 2 h non-lactation, and obtained immediately prior to the injection of LRH, which was called zero time. Subsequent samples for FSH assay were obtained at 15, 30, 60 and 120 min after the LRH injection. Blood samples for FSH, Prl and oestradiol assays were also obtained around 10:30 a.m. from 10 eumenorrhoeic women on day 8 or 9 of the normal cycle. The samples were chilled at 4°C, and serum was separated. Five puerperal women received a single daily injection of 100 µg of synthetic LRH from days 5 through 10 post-partum. On day 11 post-partum, they were given an iv infusion of 100 µg of synthetic LRH and blood samples were drawn as stated above. The maximum increase in serum FSH levels (max. FSH) was used as the index of gonadotrophin response to LRH (Canales et al. 1974).

Plasma LRHIS was measured by the RIA reported previously (Saito et al. 1975). LRH antiserum cross-reacted with C-terminal fragments and the cross-reactions with the 2–10 nonapeptide and 3–10 octapeptide of LRH were 83.3 and 2.4%, respectively. The recovery of exogenous synthetic LRH from plasma after extraction was 89.6%. The lowest limit of assay sensitivity was 1.5 pg/assay tube and the intra-assay coefficient of variation was 6.4%. Serum FSH, Prl and oestradiol were determined using RIA kits commercially supplied (Daichi Radioisotope Laboratories, Tokyo, Japan). The cross-reactivity of FSH antiserum was less than 0.2% with LH, hCG and GH, and less than 1% with TSH. The cross-reactivity of Prl antiserum was 0.16% with GH, 0.03% with human placental lactogen, and 0% with FSH, LH and TSH. The cross-reactivity of oestradiol antiserum was 4.5% with oestrone and 1% with oestradiol. The concentration of FSH was expressed as mIU/ml of the second International Reference Preparation of Human Menopausal Gonadotrophin. The lowest limits of assay sensitivities were 0.98 mIU/ml for FSH, 0.78 ng/ml for Prl and 6.2 pg/ml for oestradiol. The intra-assay coefficients of variation for FSH, Prl and oestradiol were 6.6, 5.0 and 6.8%, respectively. All relevant comparisons were performed in the same RIA. Statistical analyses of data were performed by Student's t-test, but when variances were unequal, Welch's test was employed.

Results

Plasma LRHIS in puerperal women was very low on days 5 and 10 post-partum and significantly increased between days 10 and 20 post-partum, but remained at a similar level on day 30 post-partum, which was significantly lower than that in eumenorrhoeic women on day 8 or 9 of the normal cycle (Fig. 1). Serum FSH and oestradiol levels were significantly lower in puerperal women on day 11 post-partum than in eumenorrhoeic women on day 8 or 9 of the normal cycle, but serum Prl level was significantly higher in puerperal women than in eumenorrhoeic women (Table 1).

On day 11 post-partum, 100 or 200 µg of LRH administration did not significantly increase serum FSH. Daily injections of 100 µg of LRH from days 5 through 10 post-partum not only caused a significant increase in basal level of FSH on day 11 post-partum, but also augmented significantly the FSH response to LRH (Fig. 2 and Table 1). However, LRH pre-treatment caused no significant change in serum Prl and oestradiol levels on day 11 post-partum (Table 1).
Table 1.
Basal levels of serum FSH, Prl and oestradiol in 11 day post-partum women with or without daily injections of 100 µg of LRH from days 5 through 10 post-partum and in eumenorrhoeic women on day 8 or 9 of the normal cycle.

<table>
<thead>
<tr>
<th></th>
<th>Day 11 post-partum</th>
<th>Day 8 or 9 of normal cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LRH pre-treatment</td>
<td>No pre-treatment</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(12)</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.8 ± 0.6*</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Prl (ng/ml)</td>
<td>138.0 ± 59.4</td>
<td>106.9 ± 15.4</td>
</tr>
<tr>
<td>Oestradiol (pg/ml)</td>
<td>39.2 ± 8.5</td>
<td>37.5 ± 3.0</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM of the number of observations in parentheses.

* P < 0.01 compared to no pre-treatment group.

** P < 0.001 compared to 11 day post-partum women.

Discussion
The present study demonstrates that plasma LRHIS level was low during the first month post-partum, especially for the first 10 days. As in our assay system LRH antiserum cross-reacts with the 2–10 nonapeptide and 3–10 octapeptide of LRH, LRHIS may contain these metabolic breakdown products of LRH as well as LRH. Plasma LRHIS level, however, may be a reflection of hypothalamic LRH secretion. The present result suggests that the amount of hypothalamic LRH secretion may be

![Fig. 2.](#)

Mean (± SEM) serum FSH responses to LRH administration in puerperal women on day 11 post-partum. ■—■ 100 µg LRH test in seven 11 day post-partum women; ●—● 200 µg LRH test in five 11 day post-partum women; ▲—▲ 100 µg LRH test in five 11 day post-partum women receiving daily injections of 100 µg of LRH from days 5 through 10 post-partum. * P < 0.02 compared to basal level.
small throughout the first month post-partum, especially for the first 10 days. The cause of small release of LRH during the first month post-partum remains unknown, but the following mechanism may be relevant. If the high concentrations of steroid hormones during pregnancy suppress only the release of LRH from the hypothalamus, plasma LRHIS on day 20 post-partum should recover to a level which is comparable to that in eumenorrheic women on day 8 or 9 of the normal cycle, because Reyes et al. (1972) and Said et al. (1973) showed that the concentrations of serum steroid hormones decreased to mid-follicular levels or less than those by 2 weeks after delivery. However, plasma LRHIS level on day 20 post-partum was significantly lower than that in eumenorrheic women. Therefore, it is likely that during pregnancy the high levels of steroid hormones produced by the placenta may suppress not only the release of LRH from the hypothalamus but also the synthesis of LRH in the hypothalamus, which may result in a reduction in plasma LRHIS level during the first month post-partum. In support of this speculation, puerperal rats were reported to have significantly less LRH activity in the hypothalamus and significantly less LH in serum than cycling female rats (Minaguchi & Meites 1967; Lu et al. 1976).

The small amount of LRH secretion from the hypothalamus in the early puerperium may be followed by the hypofunction of the pituitary not only in secretion but also in synthesis of gonadotropin, leading to the unresponsiveness of the pituitary to LRH stimulation in the early puerperium, since daily injections of LRH from days 5 through 10 post-partum caused the significantly increased secretion of FSH on day 11 post-partum, both basally and in response to LRH, and no significant difference was observed in serum Prl and oestradiol levels on day 11 post-partum between the two groups of women with and without daily LRH pre-treatment.

Acknowledgments

The authors would like to thank Daiichiseiyaku Co., Ltd. for the gift of synthetic LRH, and Miss Mariko Itsuki and Miss Yoshie Kondo for technical assistance.

References