Short- and long-term fluctuations in plasma prolactin concentration in normal subjects

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Abstract. The physiological changes in plasma prolactin concentration were studied in 447 normal subjects, including 65 men, 75 pre-menopausal women and 307 post-menopausal women. The within-day and day-to-day variation as well as the circadian and circannual rhythm of plasma prolactin levels were determined. Furthermore, the relationship between changes in prolactin and oestradiol-17ß levels during the normal menstrual cycle and in the climacteric was studied.

Pre-menopausal women had significantly (P < 0.01) higher basal plasma prolactin concentration than men and post-menopausal women. Furthermore, they had significantly (P < 0.01) higher day-to-day variation than men. This suggests that prolactin in women is secreted in a pulsatile fashion. Only small seasonal variations in both sexes were seen.

The levels of plasma prolactin during the ovulatory and the luteal phase in the cycle were significantly (P < 0.02) higher than that of the follicular phase, and a positive correlation between changes in plasma concentration of oestradiol-17ß and prolactin was found. Also in post-menopausal women a relationship between plasma concentration of prolactin and oestradiol-17ß was seen.

It is concluded that the assessment of prolactin concentration in blood is dependent on the physiological variation recorded during sleep in both sexes. However, only in women day-to-day changes and the changes related to the menstrual cycle and the climacteric are of importance.

Specific and sensitive radioimmunoassays for determinations of human prolactin (Prl) in plasma have been available since 1971 (Hwang et al. 1971). A large amount of information has been collected about the control of Prl secretion and its clinical significance. However, data on physiological changes of serum Prl concentration are limited. In this study we have determined the within-day and day-to-day variation as well as diurnal and circannual rhythm in serum Prl levels in normal subjects. We have in addition studied the relationship between plasma concentrations of Prl and oestradiol-17ß during the normal menstrual cycle and in post-menopausal women.

Materials and Methods

Sixtyfive normal adult male subjects between the age of 21 and 64 years, 75 pre-menopausal women between the age of 19 and 46 years and 307 post-menopausal women between the age of 45 and 54 years of age were studied. The men and pre-menopausal women were members of hospital personal and none had any known disease. All pre-menopausal women had regular menstrual cycles and were except for the women studied during the menstrual cycle, in the follicular phase of their cycle. The post-menopausal women were selected as follows: of 11809 questionnaires sent to all women aged 45–54
years in certain districts of Copenhagen, 9,411 were returned. From information on the data of last vaginal bleeding, earlier gynaecological operations and drug intake 307 women participated and fulfilled the criteria: menstrual bleeding had stopped spontaneously within the last ½ to 3 years; medical examination including history, gynaecological examination and breast palpation was normal; systolic and/or diastolic blood pressure was below 170 and 105 mm of mercury and no biochemical evidence of kidney or liver disease was found. All had post-menopausal plasma concentration of follicle stimulating hormone (FSH). None were taking any medication known to influence plasma Prl levels. After at least 15 min rest basal blood samples were obtained between 9 a.m. and 12 a.m. in all subjects.

Within-day variation was determined in 9 of the normal male subjects and 6 of the pre-menopausal women. In all subjects blood samples were obtained after 30 min rest from an iv catheter with 15 min interval for 120 min.

Day-to-day variation was determined in 6 of the normal men and 7 of the normal pre-menopausal women. Blood samples were obtained after 30 min rest by venous puncture on five consecutive days.

Diurnal rhythm was determined in 8 of the normal men. All subjects were at sleep from about 11 p.m. to 7 a.m. The subjects were admitted to the ward the day before the study and carefully briefed about the procedure. One hour before the first blood sample a polyethylene catheter was placed in an antecubital vein and connected to a 3 meter catheter. Thirteen blood samples were collected with hourly to 4-hourly intervals. During night sampling was done in an adjoining room.

Circannual rhythm was determined in 9 normal men and 9 normal pre-menopausal women. In all subjects blood samples were obtained after 30 min rest by venous puncture each month except for June and November. The mean serum Prl levels for each month was compared to the previous and following month.

Variation during the menstrual cycle was studied in 17 normal women aged 22–32 years who had 10 blood samples taken throughout an ovulatory cycle of between 26 and 32 days of duration for the measurement of prolactin, luteinizing hormone (LH), FSH, progesterone and oestriadiol-17β. In all women the cycles were characterized by a peak of LH in relation to a rise in progesterone to above 15 nmol/l and by a biphasic behaviour of oestriadiol. For each woman the mean levels of the hormones were calculated for the follicular, ovulatory and luteal phases (defined by Judd & Yen 1973), the ovulatory period corresponding to the two days prior to and the two days following the LH peak. Hormone concentrations from at least two separate days were used as the mean value for each period.

The relationship between plasma oestradiol-17β and plasma Prl concentration in post-menopausal women were studied in 60 of the 307 post-menopausal women. The 60 women were at random selected and plasma oestradiol-17β concentration was measured in the 10 women with the highest and the 10 with the lowest basal plasma Prl concentration.

Blood samples were centrifuged, plasma separated and stored at −20°C until assayed. Samples from each subject were analysed in the same assay.

Plasma Prl was measured by a double antibody radioimmunoassay (McNeilly & Hagen 1974) employing highly purified human prolactin (kindly supplied by Prof. H. G. Friesen, University of Manitoba, Winnipeg) and an anti-serum raised against human prolactin in the rabbit (AR 1-7, kindly supplied by Prof. H. G. Friesen). In this assay 1 ng of H. G. Friesen prolactin equals 22.3 μg of the MRC prolactin standard (75/504, assuming 650 μg per ampoule). The between- and within-day variation was determined on 9 replicate samples and assays respectively on three dose levels.

LH and FSH were measured by double antibody radioimmunoassays (McNeilly & Hagen 1974). In the LH and FSH assay LH MRC 68/40 (assuming 77 μU/ampoule) and FSH MRC 69/104 (assuming 10 IU/ampoule) were used as standards, respectively. Plasma concentrations of oestradiol-17β and progesterone were measured by radioimmunoassays after extraction with n-hexane/ ethylacetate for oestradiol-17β and petroluemether for progesterone using tritiated steroids (New England Nuclear) and specific antibodies raised in rabbits against 6-keto-17β-oestradiol 6-CMO: Bovine Serum Albumin and progesterone 3-CMO: Bovine Serum Albumin (Steraloids Inc).

For statistical analysis the Wilcoxon and Mann-Whitney rank sum test for paired and unpaired comparisons respectively, and Spearmans rank correlation were used. Analysis of variance was performed calculating the within- and between-day variation of plasma Prl concentrations in men and pre-menopausal women.

<p>| Table 1. The analytical variation expressed as the standard deviation (SD, μg/l) and the coefficients of variation (CV %) found at three different plasma prolactin concentrations (Prl μg/l). |
|-----------------------------------|----|----|----|----|</p>
<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>SD</th>
<th>CV</th>
<th>Prl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>9</td>
<td>1.0</td>
<td>17.9</td>
<td>5.8</td>
</tr>
<tr>
<td>Medium</td>
<td>9</td>
<td>1.1</td>
<td>11.9</td>
<td>11.9</td>
</tr>
<tr>
<td>High</td>
<td>9</td>
<td>2.1</td>
<td>10.0</td>
<td>21.1</td>
</tr>
</tbody>
</table>
Results

The analytical variations of Prl at the three dose levels are shown in Table 1. The within-day component of variance was not significant at any dose level ($P > 0.05$, analysis of variance, F-test), thus the between-day variation was found to be the dominant component of analytical variance.

The basal plasma Prl concentration in 65 men, 75 pre-menopausal and 307 post-menopausal women is shown in Fig. 1. Pre-menopausal women had significantly ($P < 0.01$) higher plasma Prl concentrations than men and post-menopausal women.

The within-day variation in plasma Prl was not significantly different in 6 pre-menopausal women and 9 men ($P > 0.05$, analysis of variance, F-test). The standard deviations of within-day observations were 2.2 μg/l and 1.9 μg/l for pre-menopausal women and men respectively. The individual coefficients of variation were 16.6% (7.0%–32.6%) and 17.0% (11.9%–87.5%) (median and range) for pre-menopausal women and men, respectively (Fig. 2A).

The between-day variation in 7 pre-menopausal women was significantly higher ($P < 0.01$, analysis of variance, F-test) than the corresponding variation in 6 men. The standard deviation between-day was 4.0 μg/l and 1.9 μg/l for pre-menopausal women and men respectively. The individual co-
Table 2.
Prl, LH, FSH, oestradiol-17β (Oe2) and progesterone (P) concentration in the three phases of the normal menstrual cycle from 17 women. The ovulatory period corresponding to the 2 days prior to and the 2 days following the LH peak. Mean ± SEM.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Prl μg/l</th>
<th>LH IU/l</th>
<th>FSH IU/l</th>
<th>Oe2 pmol/l</th>
<th>P nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular phase</td>
<td>13.5 ± 1.8</td>
<td>5.3 ± 0.3</td>
<td>4.2 ± 0.4</td>
<td>353 ± 44</td>
<td>0.5</td>
</tr>
<tr>
<td>Ovulatory phase</td>
<td>17.4 ± 2.4</td>
<td>12.4 ± 1.8</td>
<td>6.7 ± 1.2</td>
<td>970 ± 95</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td>Luteal phase</td>
<td>19.8 ± 3.2</td>
<td>5.4 ± 0.6</td>
<td>2.5 ± 0.3</td>
<td>649 ± 68</td>
<td>35.2 ± 4.2</td>
</tr>
</tbody>
</table>

The mean plasma Prl levels for males and females as a function of time during one year are given in Fig. 2D. Only small seasonal variations were observed in both sexes. In males the highest mean levels were found in January and March, and the lowest mean value in February (P < 0.05). In females the mean plasma prolactin levels were virtually constant except for significantly higher values in September and October (P < 0.05).

The mean plasma concentrations of Prl, LH, FSH, oestradiol-17β and progesterone during the three phases of the normal menstrual cycle are shown in Table 2. Four of the 17 women had the highest plasma Prl concentration recorded on the day of the midcycle peak, 6 in the ovulatory phase and 11 in the luteal phase of the cycle. The mean levels of Prl during the ovulatory and luteal phase of the cycle were higher than that of the follicular phase in 14 and 15, respectively of the 17 women which were highly significant (P < 0.02). However, no significant (P > 0.05) difference between Prl levels in the ovulatory and luteal phase could be shown. There was a significant positive correlation between changes in plasma concentrations of oestradiol and Prl from the follicular to the ovulatory and luteal phase of the cycle (R = 0.54, P < 0.05).

Among the 60 post-menopausal women the 10 women with the highest plasma Prl concentration had significantly (P < 0.01) higher plasma oestradiol-17β levels than the 10 women with the lowest plasma prolactin concentration (Fig. 3). No significant (P > 0.05) differences between FSH levels between the two groups were found.

Fig. 3.
Plasma concentrations of FSH and oestradiol-17β in 10 post-menopausal women with low (6.2 ± 0.6 μg/l, mean ± SEM) ■ and 10 with high (20.4 ± 1.7 μg/l, mean ± SEM) □ prolactin levels.

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Discussion

Our data document a number of physiological variations in serum Prl concentration in human beings. The study of Prl levels in normal subjects when blood samples are drawn at intervals of minutes demonstrates considerable fluctuations suggesting that Prl is secreted in a pulsatile fashion (Sassin et al. 1972; McNeilly et al. 1974). Cowden et al. (1979) reported that the day-to-day variability of Prl levels was more pronounced in women than in men. In the present study this difference was significant and might be due to an effect of oestrogens. But the observation of similar within-day changes of Prl levels in male and female subjects is against this possibility. However, the women included were in the early follicular phase of their cycle, during which period oestrogen levels are at their lowest. Furthermore, oestrogen administration in normal subjects has been shown to increase the fluctuating pattern in Prl levels (Vekemans & Robyn 1975) as well as the reactivity of the pituitary lactotrophs to the influence of thyrotrophin releasing hormone (TRH) (Reymond & Lemarchand-Béraud 1976).

While this study confirms the findings of Reinberg et al. (1978) showing no annual plasma Prl variation in male human beings, it shows seasonal plasma Prl variations in women very similar to the observations done by Beck et al. (1978) in rhesus monkeys. They found that the rhesus monkey had significantly higher plasma Prl levels during September, October and November, compared to the rest of the year. Seasonal variations in plasma Prl have also been demonstrated in other animals (Schams & Reinhardt 1974; Hart et al. 1978). In ruminants plasma Prl raise in spring, are on highest level during summer and decline in autumn. However, observations in rats (Relkin 1972) have opposed theories about the influence of light or temperature being the cause of this seasonal variation. Therefore, the aetiology of these variations remains to be explained. In man, the small annual changes are of little importance for the determination of basal Prl concentration.

The present study confirms that plasma Prl concentration in normal men is raised during sleep and does not reach basal levels before one to two hours after the subjects are awake (Sassin et al. 1972). To avoid falsely elevated Prl values in patients and in normal subjects blood samples should be drawn at least 2 h after waking.

Physiological and pharmacological changes in oestrogen concentration influence plasma prolactin levels. Thus, the present study confirms that premenopausal women have higher prolactin levels than men and post-menopausal women (Tyson & Friesen 1973). Furthermore, the group of postmenopausal women with the highest plasma Prl concentration had significantly higher plasma oestradiol-17β levels than those with the lowest Prl levels. During the normal menstrual cycle reports on changes in basal plasma Prl levels have been conflicting. Three studies (Ehara et al. 1973; Guyd & Friesen 1973; McNeilly & Chard 1974) found no significant variation in Prl throughout the menstrual cycle. In contrast, others found higher Prl levels at times with raised oestrogen concentration at midcycle and in the luteal phase (Vekemans et al. 1977; Franchimont et al. 1976).

Our data confirm these last reports and the influence of oestrogens on Prl levels was substantiated by the relationship between changes in the hormones throughout the cycle.

In conclusion, the only significant variation in basal Prl levels in men is related to sleep. Whereas, when assessing the results of dynamic procedures or of single values obtained on different occasions in women, the day-to-day changes and the changes during the menstrual cycle as well as changes related to the climacteric, must be born in mind.

Acknowledgments

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References


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