High repeatability of circadian prolactin rhythm assessment results in children

Renata Stawerska [1,2], Joanna Smyczynska [1,2], Maciej Hilczer [1,2], Andrzej Lewinski [2,3], Michal Karasek [3,4]

1. Department of Pediatric Endocrinology, Chair of Endocrinology and Metabolic Diseases, Medical University of Lodz, Poland.
2. Department of Endocrinology and Metabolic Diseases, Chair of Endocrinology and Metabolic Diseases, Medical University of Lodz, Polish Mother's Memorial Hospital – Research Institute, Lodz, Poland.
3. Department of Endocrinology and Metabolic Diseases, Chair of Endocrinology and Metabolic Diseases, Polish Mother's Memorial Hospital – Research Institute, Lodz, Poland.
4. Department of Neuroendocrinology, Chair of Endocrinology, Medical University of Lodz, Poland.

Correspondence to: Prof. Michal Karasek M.D., Ph.D.
Department of Neuroendocrinology
Chair of Endocrinology, Medical University of Lodz
92-216 Lodz, Czechoslowacka 8/10, Poland
tel./fax: +48 42 675 7613; e-mail: karasek@csk.umed.lodz.pl

Submitted: 2008-11-08 Accepted: 2008-11-21 Published online: 2008-12-29

Key words: **prolactin; circadian rhythm; repeatability; intrasubject variability**

Neuroendocrinol Lett 2008; **29**(6):889–894 **PMID:** 19125151 NEL290608A23

Abstract

OBJECTIVES: In normal conditions, prolactin (Prl) secretion manifests a circadian pattern. So far, there have only been but few studies, concerning intrasubject variability and repeatability of the circadian Prl secretion pattern, based on pulse analysis. It seems, that macroscopic analysis based on measurement of Prl concentration at nine time points every 3 hours during 24 hours is an appropriate method to assess Prl profile for clinical purposes. The aim of the study was to assess the repeatability of that circadian Prl secretion pattern in a group of short children without hormonal disorders.

MATERIALS AND METHODS: The analysis comprised the results of two circadian Prl profiles, performed from 2 to 14 months in 23 prepubertal children (16 boys) with idiopathic short stature, aged: 10.3±2.4 yrs.

RESULTS: There were no statistical differences between Prl concentrations at the same time points in the two consecutive profiles, but the comparison of Prl concentrations at 8:00 gave results which were close to the border of statistical significance (p=0.055), what indicated low repeatability of measurement results at that particular time point. There were no statistical differences between the values of particular parameters in macroscopic profile analysis in the first and in the second test.

CONCLUSION: Circadian Prl profile, based on nine Prl concentration measurements, taken every 3 hours during one day, is characterized by high repeatability of the results and low intrasubject variability in children, despite the results of Prl concentration at 08:00 o'clock.

ORIGINAL ARTICLE

INTRODUCTION

Prolactin (Prl) is not only a lactotrophic hormone; it has over 300 separate biological activities and, besides its roles in reproduction, it also plays multiple homeostatic functions (Freeman *et al*. 2000). In normal condition, both in adults and in children, Prl secretion manifests a circadian pattern, with lower serum concentrations during the day and with about twice as high at night, when asleep (Sassin *et al*. 1972; Parker *et al*. 1973; Waldstreicher *et al*. 1996). Thus, it is important that, not only should Prl concentration be within standard range at fasting state, but circadian Prl rhythm should also be maintained in that pattern. In our earlier work, we presented simple criteria for a macroscopic analysis of circadian Prl rhythm, based on nine Prl measurements, taken every three hours during 24 hours period (Stawerska *et al*. 2007). A possible weak point of that study could be associated with the fact that it perhaps provided only approximate evaluation of circadian Prl secretion. It is well known that Prl secretion is modified by a number of external factors (light, stress, exercise, food intake, hypoglycemia) (Freeman *et al*. 2000; Karasek *et al*. 2006). Nevertheless, a question may be raised as to what an extent Prl profile, evaluated on the basis of nine serum Prl measurements, taken every three hours during one day, is reliable and repeatable and whether the most informative circadian rhythm parameters, such as the amplitude, the Xn/Xd ratio and the regression index, are reliable and repeatable as well.

Thus, the aim of the present study was to assess the repeatability of the pattern of circadian Prl secretion, evaluated on the basis of nine serum Prl measurements, taken every three hours during one day.

MATERIAL AND METHODS

The study was approved by the Local Committee for Studies in Human Subjects. The experimental protocol was explained to patient's parents and an informed consent was obtained.

The analysis comprised the results of two circadian Prl profiles (profile 1 and profile 2) which were performed in 23 children, hospitalized twice, due to short stature (from 2 to 14 months) at our Department. Children with signs of infection during any of the examinations, with chronic diseases and those on Prl concentration affecting medicaments were excluded from the analysis. The girls with Turner syndrome were excluded from the study, too. Besides Prl circadian rhythm assessment, tests were performed, concerning the growth hormone (GH) secretion in two stimulation tests, the concentrations of insulin-like growth factor type I (IGF-I), TSH and of free thyroxine (FT4), together with evaluations of other pituitary hormones, if considered necessary.

The qualification criteria into the study allowed enrolment of children with idiopathic short stature (normal growth hormone secretion, normal IGF-I concentration). Thus, the analysed material comprised 23 prepubertal children (16 boys and 7 girls), aged (during the first hospitalization) from 7.2 to 13.5 yrs, the mean age ± SD: 10.3±2.4 yrs.

In 5 cases, Prl concentration at 8:00 was slightly elevated, but its further values were within the normal range and hiperprolactinaemia was thus excluded.

Estimation of circadian Prl rhythm

All the subjects were admitted to hospital for Prl assessment, at least, 24 hours before the test. In each child, Prl circadian secretion profile was determined on the basis of Prl concentrations in serum, measured in samples obtained every 3 hours during 24 hours. Blood samples were collected at 08:00, 11:00, 14:00, 17:00, 20:00, 23:00, 02:00, 05:00 and 08:00 h. All the collected blood samples were left to clot for 45 minutes; serum was removed after centrifugation and stored at –20°C until assay. Prl concentrations were measured by the electrochemiluminescence method (ELICA, Roche, Elecsys®Systems 2010, its sensitivity being 0.47 ng/mL, within the range up to 470 ng/mL, the inter assay CV was 1.8–3.4%). All the measurements were performed at the Laboratory of Immunochemical Research, Polish Mother's Memorial Hospital – Research Institute, Lodz, Poland

Based on the measured Prl concentrations during 24 hours, the following circadian rhythm parameters were calculated (macroscopic analysis) (Cugini 1993):

- the mesor (the overall mean level),
- the median,
- the area under curve (AUC),
- the peak level (the maximal Prl concentration),
- the trough level (the minimal Prl concentration),
- the amplitude (the peak level and the mesor ratio),
- the mean nocturnal (Xn) concentration (the mean Prl concentration from three night time points: 23:00, 2:00 and 5:00 h),
- the mean diurnal (Xd) concentration (the mean Prl concentration from three day time points: 11:00, 14:00 and 17:00 h),
- the Xn/Xd ratio,
- the regression index (it is the directional index, i.e., the index of the slope of the regression straight line in relation to the axis of ordinates).

Moreover, for each Prl profile, the time point of maximal Prl concentration (acrophase) was established.

Statistical analysis

Prolactin concentrations in the particular time points of both profiles were compared by the sign test for assessment of intrasubject variability and repeatability of that profiles. Moreover, comparisons of particular mac-

Table 1. The mean (± SD) concentrations of Prl at particular time points of circadian rhythm in the first and the second profile in the analysed group of children.

Time points	Profile 1 Prl (ng/mL); mean ±SD	Profile 2 Prl (ng/mL); mean ± SD	Sign test	Correlation index	Variability
8:00	11.9 ± 7.4	15.8 ± 12.3	p=0.055	r=0.64, p<0.05	36.3 %
11:00	8.2 ± 5.1	8.4 ± 4.5	p=0.82	r=0.62, p<0.05	25.4%
14:00	11.7 ± 6.2	10.6 ± 4.5	p=1.0	r=0.60, p<0.05	27.6%
17:00	8.7 ± 4.4	9.3 ± 5.1	p=1.0	r=0.67, p<0.05	22.9%
20:00	12.1 ± 7.4	10.9 ± 6.0	p=0.66	r=0.22, p>0.05	27.1%
23:00	12.0 ± 8.4	14.8 ± 8.1	p=0.19	r=-0.13, p>0.05	40.4%
2:00	24.9 ± 9.5	26.1 ± 11.8	p=0.66	r=0.72, p<0.05	20.6%
5:00	21.9 ± 10.6	23.6 ± 10.7	p=0.65	r=0.72, p<0.05	21.1%
8:00	14.6 ± 8.4	16.1 ± 8.6	p=0.38	r=0.56, p<0.05	35.6%

Table 2. Comparison of macroscopic analysis parameters in the first and the second Prl circadian profiles in the analysed group of children.

Parameters	Profile 1 (mean ± SD)	Profile 2 (mean ± SD)	Sign test	Correlation index	Variability
mesor (ng/mL)	14.00 ± 4.83	14.94 ± 5.96	p=0.52	r=0.75; p<0.05	17.3%
median (ng/mL)	11.79 ± 5.57	11.85 ± 4.90	p=0.83	r=0.55; p<0.05	24.6%
AUC (ng/mL/24 hours)	338.37 ± 115.13	340.12 ± 145.72	p=0.83	r=0.74; p<0.05	18.7%
peak level (ng/mL)	28.24 ± 9.50	28.83 ± 9.50	p=1.0	r=0.77; p<0.05	15.1%
trough level (ng/mL)	6.29 ± 3.30	7.10 ± 4.15	p=0.66	r=0.79; p<0.05	21.2%
amplitude	1.98 ± 0.52	1.87 ± 0.47	p=0.19	r=0.70; p<0.05	18.9%
X_d (ng/mL)	9.53 ± 4.48	9.48 ± 4.17	p=1.0	r=0.68; p<0.05	22.6%
X_n (ng/mL)	19.61 ± 6.94	21.52 ± 8.88	p=1.0	r=0.74; p<0.05	15.4%
X_n/X_d ratio	2.43 ± 1.34	2.39 ± 0.91	p=0.38	r=0.58; p<0.05	18.6%
regression index	–0.59 ± 0.57	–0.63 ± 0.49	p=0.83	r=0.34; p>0.05	85%

roscopic parameters of Prl rhythm in both profiles were also performed by the sign test. The level of statistical significance, higher than 0.05 (p>0.05) for the sign test, indicated the good repeatability of the data.

Then, Pearson's correlation index was calculated among Prl measurements in profile 1 and in profile 2 for each particular time point. In that evaluation the level of statistical significance p below 0.05 (p<0.05) indicated the good repeatability of data.

The coefficient of variation was calculated for all repeated data.

RESULTS

The mean Prl concentrations at particular time points of the profile 1 and profile 2 in analysed group of children are presented in Table 1. There were no statistical differences (p>0.05) between Prl concentrations at the same time points of the two Prl profiles. However, the least values of probability of statistical significance were observed for Prl concentrations at 14:00, 17:00 and 11:00, higher values for 20:00, 2:00 and 5:00, while the comparison of Prl concentration results at 8:00 were close to the border of statistical significance (p=0.055).

Moreover, we observed the positive significant correlations between the results of Prl concentration in the first and in the second profile at each time point, except 20:00 and 23:00. The least values of Prl concentration variability (about 20%) was observed at night (at 2:00 and 5:00 time point) and during the day at 11:00 and 17:00 time point, while the subject variability both at 8:00 and 8:00' as well as at 23:00 time points were higher than 30% (Figure 1). However, in 5 cases we noticed differences in the status of children at 23:00: during one of the two hospitalisations periods', a child was asleep at 23:00, while during the second one, the same child was awake at 23:00. After the elimination of those cases, we observed significant correlation between Prl concentration at that time point in the first and the second profile and low (20%) subject variability at that time point.

In turn, the results of macroscopic analysis parameters of the Prl profile 1 and Prl profile 2 in analysed group of children were presented in Table 2. The sign

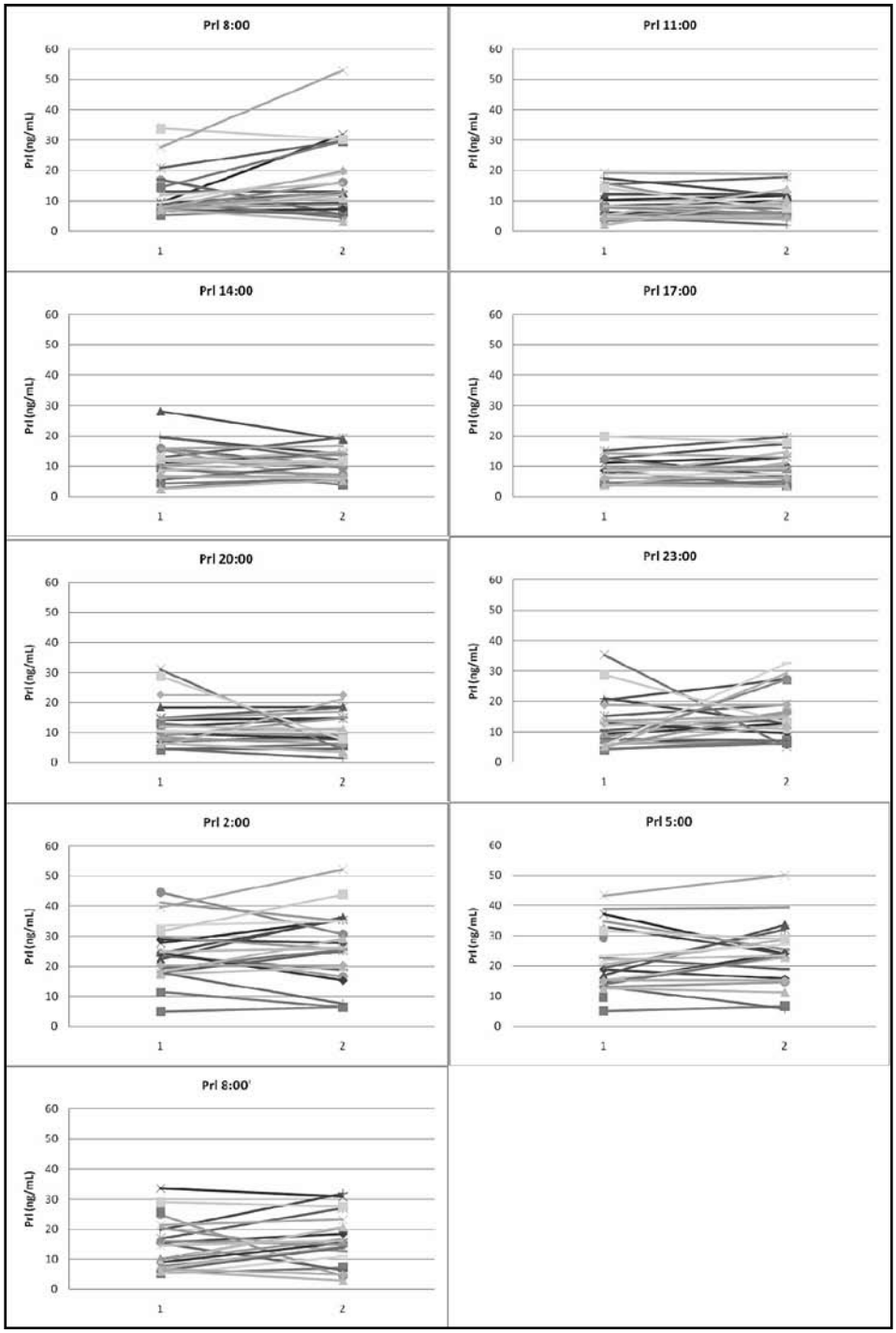

Figure 1. Variability of Prl concentration at each time point between the first and the second profile in the analysed group of children.

test has shown that there were no statistical differences between the values of particular parameters in the first and in the second profile with the least values of statistical significance probability for the mean nocturnal concentration, the mean diurnal concentration and peak level. Among the parameters describing Prl circadian rhythm, the Xn/Xd ratio and the amplitude were characterised by the least variability.

Next, a comparison of acrophase values was performed in the first and the second profile for each child. In 34.8% cases (8 children), acrophase took place in the same time point. In 43.5% cases (10 children), acrophase took place three hours earlier or later, however, in all those cases, it was observed during the night in both profiles. Only in 21.7% cases (5 remaining children), acrophase was shifted by more than one time point in the second profile. Moreover, in 4, out of 5 those cases, acrophase was observed at 8:00 (the most frequent time point for Prl measurement). The data, concerning the acrophase in the first and the second profile, are presented in Table 3. It is worth underlying that in most of cases (except only 5, out of all the 46 profiles) acrophase was observed at nocturnal hours or at 8:00 o'clock.

DISCUSSION

Assessment of Prl secretion is usually performed on the basis of serum Prl concentration in fasting state or after the stimulation with metoclopramide. An interpretation of these results should rather be careful, due to many falsely elevated results (Di Sarno *et al*. 2003; Karasek & Pawlikowski 2006). The most appropriate method for evaluation of lactotroph function is the assessment of circadian Prl secretion rhythm, especially pulse analysis. However, this method poses one set-back, namely, it is mainly appropriate for the purpose of experimental studies, demanding measurements of Prl concentrations in blood samples, collected every 10–25 minutes during 24 hours (Finkelstein *et al*. 1978; Shulman *et al*. 1989; Waterman *et al*. 1994) and the results must be submitted to computer for spectral resolution (Fourier analysis) (Cugini 1993; Lerchl & Partsch 1994, Merriam & Wachter 1982; Partsch *et al*. 1995; Van Cauter *et al*. 1981). However, so far, there have only been but few studies, concerning intrasubject variability and repeatability of the circadian Prl secretion pattern. Partsch *et al*. (1995) found good repeatability in Prl profile studies, while evaluating intra- and intersubject variability of Prl secretion rates in a group of 10 young, healthy men. The authors determined the profile of circadian Prl secretion on the basis of measurements, obtained every 20 minutes for 24 hours, followed by control studies in the same subjects after 2 weeks and three months. They demonstrated on the same basis that intrasubject variability of the parameters of pulsatile Prl release (mesor, nadir, minimal and maximal concentration, amplitude) was lower from the intersubject variability (i.e., variability among the patients).

The method of circadian rhythm analysis, which we have performed in our study, is the non-inferential chronobiometry (macroscopic analysis), which requires a few blood collections only, performed during a definite period of time. Thus, it is useful for clinical purposes and it can be performed at any hospital without special equipment or software support. Recently, we have presented simple, useful principles of Prl secretion rhythm interpretation, based on nine Prl serum measurements every 3 hours during 24 hours (Stawerska *et al*. 2007). But no studies have, so far, been performed, regarding Prl profile repeatability, evaluated in this way; therefore, our study seems to have been the first attempt at finding an appropriate answer to this question.

In the current study, we have shown a very high compatibility of Prl concentrations in particular time points in the same children, when Prl profile was repeated after not very long time period. It should be underlined that the children did not undergo any change of puberty stage, what could, otherwise, have affected the final results. Some investigators indicate a relationship between Prl concentration at fasting state and sex and the stage of puberty but the results have been controversial (Beck & Wuttke 1980; Minuto *et al*. 1984; Ehara *et al*. 1975; Yamai *et al*. 1976; Genazzani *et al*. 1994; Apter *et al*. 1978). It is interesting, that in 5 cases we observed elevated Prl concentrations at 8:00; however hiperprolactinaemia was not diagnosed in those children. It indicates that Prl concentration at that time point should not be treated as screening value. Moreover, the results of Prl concentration at 8:00 o'clock in two consecutive profiles were close to the border of statistic significance in sign test and characterised the highest variability, what indicated low repeatability of the Prl secretion at that time point. In about 20% of cases, acrophase was observed at that time point, although it should have occurred during night hours.

Another problem in the analysis of Prl circadian rhythm is connected with the time of falling asleep in children. When the child is not asleep at 23:00, the Prl concentration at this time point should not be accounted into the mean nocturnal Prl concentration. On the other hand, the Xn/Xd ratio and the amplitude ratio seem to be the best tools for the analysis of Prl circadian rhythm, irrespective of Prl measurement at 23:00 o'clock.

Table 3. The time point of peak Prl concentration (acrophase) in particular individuals in the first and the second Prl profile.

Nb of patient	Profile 1 acrophase (time point)	Profile 2 acrophase (time point)
1	2:00	2:00
2	8:00	8:00
3	14:00	2:00
4	5:00	2:00
5	8:00	5:00
6	8:00	2:00
7	5:00	2:00
8	5:00	2:00
9	2:00	2:00
10	2:00	5:00
11	8:00	8:00
12	2:00	5:00
13	23:00	2:00
14	2:00	23:00
15	2:00	23:00
16	14:00	14:00
17	2:00	5:00
18	2:00	2:00
19	20:00	20:00
20	8:00	2:00
21	2:00	2:00
22	5:00	2:00
23	8:00	2:00

Summing up, we confirm that circadian Prl profile, based on nine Prl measurements, taken every 3 hours during one day, is characterized by the high repeatability of the results and low intrasubject variability, despite the results of Prl concentration at 08:00 o'clock.

Acknowledgements

The study was supported by a grant from the Medical University of Lodz, No. 502-15-504.

REFERENCES

1 Apter D, Pakarinen A, Vihko R (1978). Serum prolactin, FSH and LH during puberty in girls and boys. Acta Paediatr Scand. **67:** 417–423.
2 Beck W, Wuttke W (1980). Diurnal variations of plasma luteinizing hormone, follicle-stimulating hormone, and prolactin in boys and girls from birth to puberty. J Clin Endocrinol Metab. **50:** 635–639.
3 Cugini P. Chronobiology: principles and methods (1993). Ann I[st] Super Sanita. **29:** 483–500.
4 Di Sarno A, Rota F, Auriemma R, De Martino MC, Lombardi G, Colao A (2003). An evaluation of patients with hyperprolactinaemia: have dynamics tests had their day? J Endocrinol Invest. **26 (supl. 7):** 39–47.
5 Ehara Y, Yen SS, Siler TM (1975). Serum prolactin levels during puberty. Am J Obstet Gynecol. **121:** 995–997.
6 Finkelstein JW, Kapen S, Weitzman ED, Hellman L, Boyar RM (1978). Twenty-four-hour plasma prolactin patterns in prepubertal and adolescent boys. J Clin Endocrinol Metab. **47:** 1123–1128.
7 Freeman ME, Kanyicska B, Lerant A, Nagy G (2000). Prolactin: structure, function, and regulation of secretion. Physiol Rev. **80:** 1523–1631.
8 Genazzani AD, Petraglia F, Gastaldi M, Massolo F, Cellini M, Iori G, *et al* (1994). Intrinsic secretory characteristics of luteinizing hormone and prolactin episodic release during pubertal development. Eur J Endocrinol. **131**: 80–85.
9 Karasek M, Pawlikowski M, Lewinski A (2006). Hiperprolaktynemia: przyczyny, diagnostyka i leczenie [(Hyperprolactinemia: causes, diagnosis, and treatment) [in Polish with English abstract)]. Endokr Pol - Pol J Endocrinol. **6:** 656–662.
10 Karasek M. Pawlikowski M (2006). Hyperprolactinaemia – the Essentials. Touch Briefings: European Endocrine Disease. 53–39.
11 Lerchl A, Partsch CJ (1994). Reliable analysis of individual human melatonin profiles by complex cosinor analysis. J Pineal Res. **16:** 85–90.
12 Merriam GR, Wachter KW (1982). Algorithms for the study of episodic hormone secretion. Am J Physiol. **243:** 310–318.
13 Minuto F, Barreca A, Ferrini S, Del Monte P, Bernasconi D, Giordano G (1984). Prolactin secretion in pubertal and adult male subjects. J Endocrinol Invest. **7:** 193–196.
14 Parker DC, Rossman LG, Vanderlaan EF (1973). Sleep-related, nyctohemeral and briefly episodic variation in human plasma prolactin concentrations. J Clin Endocrinol Metab. **36:** 1119–1124.
15 Partsch CJ, Lerchl A, Sippell WG (1995). Characteristics of pulsatile and circadian prolactin release and its variability in men. Exp Clin Endocrinol Diabetes. **103:** 33–43.
16 Sassin JF, Frantz AG, Weitzman ED, Kapen S (1972). Human prolactin: 24-hour pattern with increased release during sleep. Science. **177:** 1205–1207.
17 Shulman DI, Hu CS, Root AW, Bercu BB (1989). Pooled prolactin measurements in the evaluation of short children. J Clin Endocrinol Metab. **69:** 1261–1267.
18 Stawerska R, Smyczynska J, Hilczer M, Kowalska E. Lewinski A, Karasek M (2007). Assessment of prolactin secretion in children: profile of circadian prolactin secretion and principles of its interpretation. Endokrynol Pol - Polish J Endocrinol. **58:** 282–290.
19 Van Cauter E, L'Hermite M, Copinschi G, Refetoff S, Desir D, Robyn C (1981). Quantitative analysis of spontaneous variations of plasma prolactin in normal man. Am J Physiol. **241:** 355–363.
20 Waldstreicher J, Duffy JF, Brown EN, Rogacz S, Allan JS, Czeisler CA (1996). Gender differences in the temporal organization of prolactin (PRL) secretion: evidence for a sleep-independent circadian rhythm of circulating PRL levels – a clinical research center study. J Clin Endocrinol Metab. **81:** 1483–1487.
21 Waterman GS, Dahl RE, Birmaher B, Ambrosini P, Rabinovich H, Williamson D, *et al* (1994). The 24-hour pattern of prolactin secretion in depressed and normal adolescents. Biol Psychiatry. **35:** 440–445.
22 Yamaji T, Shimamoto K, Ishibashi M, Kosaka K, Orimo H (1976). Effect of age and sex on circulating and pituitary prolactin levels in human. Acta Endocrinol (Copenh). **83:** 711–719.