Genetic and environmental influences on prolactin secretion during wake and during sleep

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Linkowski, Paul, Karine Spiegel, Myriam Kerkhofs, Mireille L’Hermité-Baléraux, Anne Van Onderbergen, Rachel Leproult, Julien Mendlewicz, and Eve Van Cauter. Genetic and environmental influences on prolactin secretion during wake and during sleep. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E909–E919, 1998.—To delineate the contributions of genetic and environmental factors in the regulation of human prolactin (PRL) secretion, the 24-h profile of plasma PRL was obtained at 15-min intervals in 10 pairs of monozygotic and 10 pairs of dizygotic twins. Sleep was monitored polygraphically. PRL secretory rates were derived from plasma concentrations by deconvolution. Diurnal (24-h) variations were quantified by a regression curve to define nadir, acrophase, and amplitude. Pulses of PRL secretion were identified using a computerized algorithm. A procedure specifically developed for twin studies was used to partition the variance into genetic and environmental contributions. Significant genetic effects were identified for daytime PRL concentrations, rhythm amplitude, and overall wave-shape of the daily PRL profile. In contrast, environmental effects were dominant for mean concentrations during sleep, total secretory output during sleep, overall 24-h concentrations, and total 24-h secretion. However, when interindividual variations in sleep fragmentation were taken into account, the estimates of genetic variance for PRL concentrations and secretion during sleep approached statistical significance. Significant genetic influences were identified for slow-wave sleep (SWS). Because SWS is associated with increased nocturnal PRL secretion, it is possible that genetic effects on PRL secretion during sleep reflect genetic influences on SWS. In conclusion, genetic factors determine partially both the basal daytime concentrations of PRL and the temporal organization of PRL secretion over the 24-h cycle in normal young men.

circadian rhythmicity; heredity; pulsatility; slow-wave sleep

IN CONTRAST TO OTHER pituitary hormones, the functions of prolactin (PRL) did not become specialized early during the course of evolution, and >100 biological actions of PRL have appeared progressively, many of which are species specific (6). In humans, the multiple functions of PRL are not completely delineated but seem to include, in addition to the control of the initiation and maintenance of lactation, a role in osmeregulation, reproduction, growth, and immunomodulation.

It is well established that the secretion of PRL is highly dependent on sleep. Plasma PRL concentrations are highest during sleep and lowest during the waking period (18, 24, 25, 33). Under normal conditions, ~50% of the total daily production of PRL occurs during the sleep period, i.e., a time interval representing less than one-third of the 24-h cycle. Studies of PRL concentrations during daytime naps or after shifts of the normal sleep period have demonstrated consistently increased PRL secretion associated with sleep onset (34). There is good evidence to indicate that the temporal organization of PRL secretion also is partially controlled by circadian rhythmicity, a sleep-independent endogenous oscillation with a near-24-h periodicity generated in the suprachiasmatic nuclei of the hypothalamus (8, 27). Studies performed under so-called “constant routine conditions,” i.e., at least 40 h of continuous wakefulness in a semirecumbent position with constant caloric intake and constant levels of ambient dim illumination, have shown that the sleep-independent circadian PRL rhythm is of considerably larger amplitude in women than in men (38).

Because of the marked stimulatory effect of sleep on PRL secretion, several studies have examined the possible relationship between internal sleep structure and pulsatile variations of plasma PRL concentrations. A relationship between the alternation of rapid eye movement (REM) and non-REM sleep stages and the occurrence of nadirs and peaks in plasma PRL pulses was described first by Parker et al. (19), but later studies failed to confirm this finding (23, 27, 32). A consistent temporal association between the onset of REM sleep and low PRL secretory activity has, however, been demonstrated (10, 27). Finally, a recent study, examining PRL secretory rates (rather than plasma concentrations) and spectral characteristics of the electroencephalogram (rather than visually scored sleep stages), demonstrated that PRL secretory rates and delta wave activity undergo simultaneous parallel variations during sleep (28).

Thus sleep and circadian rhythmicity interact in determining the temporal organization of PRL secretion during the 24-h period. The genetic control of various circadian rhythm characteristics has been well established for invertebrate (22, 41) and vertebrate (13, 20, 26, 29) species. In humans, despite the increased impact of social constraints on daily behavior, evidence for the genetic control of human circadian rhythmicity has also been obtained. Indeed, the timing of the nocturnal nadir of cortisol secretion, a robust marker of human circadian phase (31), has been shown to be under partial genetic control (15). Sleep quality also appears to be partially genetically determined. In particular, twin studies have shown that there is a significant contribution of genetic variance to the
amount of slow-wave sleep (SWS; see Ref. 14), which is, interestingly, the sleep stage associated with the highest PRL secretion during sleep (28).

Although there are reasons to believe that the temporal variation of PRL secretion over the 24-h span may be influenced by genetic factors underlying the control of sleep and circadian rhythmicity, there also is evidence, albeit limited, that the overall level of PRL secretion may be partly genetically determined. Twin studies of PRL concentrations in isolated blood samples (39) or of PRL responses to pharmacological challenges, such as amphetamine or arecoline, a cholinergic agonist (16, 17), have indeed reported a significant contribution of genetic variance. Nevertheless, it cannot be inferred from these observations that PRL secretion is under genetic control during sleep or throughout the 24-h cycle. Indeed, because PRL is secreted in a pulsatile manner and undergoes a large nycthemeral rhythm with increased pulse amplitude during sleep, it is necessary to monitor plasma PRL concentrations at short-term intervals throughout the entire 24-h cycle to investigate the relative contribution of genes vs. the environment in the control of the levels and temporal organization of PRL secretion.

The present study was thus designed to determine whether genetic or environmental factors affect the basal PRL secretion during waking and during sleep by sampling blood every 15 min throughout an entire sleep-wake cycle from 10 monozygotic (MZ) and 10 dizygotic (DZ) twin pairs. A detailed analysis of the 24-h profiles of plasma PRL was performed using measures of rhythm amplitude, overall waveshape, and quantification of the characteristics of PRL pulsatility. Sleep was recorded polygraphically. All hormonal and sleep parameters were submitted to a statistical procedure specifically designed for the analysis of twin studies, which provides a partition of the total variance into the contributions of genetic and environmental factors (2, 3, 40).

SUBJECTS AND METHODS

Subjects

Twins were selected from the Twin Register of the University of Antwerp (Belgium) and the Twin Register of the Vrije Universiteit Brussel (Brussels, Belgium). Criteria for eligibility included normal health, absence of personal or family history of endocrine or psychiatric disorder, no sleep complaints, and regular sleep-wake schedules. Subjects who had traveled across time zones during the past 3 mo were excluded. Twin pairs including a member taking drugs or a shift worker were excluded. The volunteers were paid for their participation in the study, and informed consent was obtained from all subjects and from their parents if they were under 18 yr of age. Other details of subject recruitment have been reported elsewhere (14, 15).

A total of 10 MZ pairs (age 24.4 ± 4.5 yr; mean ± SD) and 10 DZ pairs (age 25.5 ± 4.8 yr; mean ± SD) was studied. All subjects had reached stage Tanner V of sexual development. The twins were classified as MZ and DZ after analysis of different genetic markers, including HLA and ABO, Rh, MnSs, Kk, Lea, Leb, Fya, Fyb, Jka, Jkb, and P1 blood groups. In the MZ group, 8 of the 10 twin pairs were living together. In the DZ group, 4 of the 10 twin pairs were cohabiting. All subjects were living in the same geographical area (within a 50-km radius).

Experimental Protocol

All subjects were studied in the Sleep Laboratory of the Department of Psychiatry, Erasme Hospital, University of Brussels, Belgium. Both members of each pair were studied simultaneously but in separate rooms located in the immediate vicinity of each other. On admission, all subjects had a physical examination and routine laboratory tests. All were found to be in normal health. After one night of habituation, sleep was recorded during four consecutive nights. On the day preceding the last night of recording, a catheter was inserted into a forearm vein between 1200 and 1400. Blood sampling at 15-min intervals was started 1 h after catheter insertion and lasted for 25 h. Data collected during the first hour of sampling were discarded to avoid artifactual effects related to the venipuncture stress. During the night, the catheter was connected to plastic tubing extending into the adjacent room, and sampling was performed without disturbing the subject. The line was kept patent with a slow drip of heparinized saline. All subjects were ambulatory during the daytime and had normal breakfast (0800), lunch (1230), and dinner (1900). Daytime naps were not allowed. The subjects were asked to retire around 2300 and were allowed to wake up spontaneously in the morning. During bedtime hours, the lights were turned off.

PRL Assay

Duplicate determinations of plasma PRL concentrations were performed using a radioimmunoassay procedure. All samples from the same twin pair were analyzed in the same assay (1). The intra-assay coefficient of variation averaged 10% throughout the range of PRL concentrations measured. The interassay coefficient of variation was below 15%. The limit of sensitivity of the assay was 0.7 µg/l.

Determination of PRL Secretory Rates

The secretory rates of PRL were derived from plasma concentrations using deconvolution analysis. This procedure assumes that the kinetics of PRL disappearance follow a single exponential. The half-life used was 25 min, as previously used and justified by Veldhuis and co-workers (35, 36). The distribution space was taken to be 1,587 ml/m² of body surface area, a value derived from the metabolic clearance rate estimated by Cooper et al. (7) in normal humans. No assumption was made for the shape of the secretory pulses.

Sleep Analysis

The polygraphic recordings of sleep were scored at 20-s intervals in the wake stage, during stages I-IV, and during REM according to standardized criteria (21). Sleep onset and morning awakening were defined, respectively, as the first and last 20-s intervals scored II-IV or REM. The twins included in the present study were a subset of a larger sample of 26 pairs of twins for whom a detailed analysis of genetic effects on sleep parameters has been previously reported (14). As PRL secretion is influenced strongly by sleep, we repeated for the subset of subjects included in the present study the analysis of genetic effects for the following parameters: sleep period (i.e., time spent from sleep onset to morning awaken-
ing), total sleep time (i.e., sleep period – duration of wake periods), sleep efficiency (i.e., sleep period/total recording time), sleep maintenance (i.e., total sleep time/sleep period), duration of stages I and II, duration of SWS (stages 3 and 4), duration of REM sleep, duration of intrasleep waking periods, and REM sleep latency (time spent from sleep onset to the first epoch scored REM).

Analysis of 24-h PRL Profiles

The quantitative characteristics of each individual profile of plasma PRL, which were used as inputs to the statistical analysis of genetic variance, were estimated separately for the nycthemeral (i.e., 24-h) rhythm and for the pulsatile variations.

The characteristics of the nycthemeral variation were quantified by calculating a best-fit curve using a robust locally weighted regression proposed by Cleveland (5) with a window width of 2 h. The acrophase and nadir were defined, respectively, as the time of occurrence of maximum and minimum in the best-fit curve. The amplitude of the best-fit curve was defined as 50% of the difference between the maximum and the minimum. The timing of the acrophase was expressed relative to the sleep onset. The value of the acrophase/nadir was defined as the level attained by the best-fit curve at its maximum/minimum. The relative value of the acrophase was calculated as the value of the acrophase divided by the value of the mean PRL level during waking.

Significant pulses of plasma PRL were identified using ULTRA, a computerized algorithm for pulse detection and quantification (30). The general principle of this algorithm is the elimination of all peaks for which either the increment (difference between the peak and the preceding trough) or the decrement (difference between the peak and the next trough) does not exceed a certain threshold related to measurement error. A threshold of two times the intra-assay coefficient of variation (i.e., 20%) was used to quantify plasma PRL pulses. For each significant pulse, the amplitude was defined as the difference between the level at the peak and the level at the preceding trough.

Each of the 40 plasma PRL profiles included in the present study was characterized quantitatively by the following parameters: the 24-h mean PRL level, the mean PRL level during sleep (i.e., during the polygraphically defined sleep period), the mean PRL level during sleep normalized for sleep maintenance, the mean level during wake (mean of PRL value between 1000 and 2200, a time interval that did not include either the onset of the nocturnal PRL rise or the postawakening period of return to daytime concentrations), the value of the nadir, the value of the acrophase, the relative value of the acrophase, the timing of the acrophase relative to sleep onset, the amplitude, the total number of pulses over the 24-h period, the number of pulses during wake and during sleep, the mean pulse amplitude over the 24-h period, and the mean pulse amplitude during wake and during sleep.

Deconvolution of plasma concentrations allowed us to estimate PRL secretory rates, and the following parameters characterizing levels and temporal organization of PRL secretion were determined: the amount of PRL secreted during the 24 h, the amount of PRL secreted during wake (1000 and 2200), the amount of PRL secreted during the sleep period, the amount of PRL secreted during the sleep period normalized for sleep maintenance, and the amount of PRL secreted during the first three hours of sleep per minute of SWS.

Statistical Analysis of Genetic Variance

The twin values for each of the parameters quantifying sleep as well as plasma and secretory PRL profiles were submitted to the method for analysis of twin data developed by Christian and co-workers (2–4, 40).

When applied to small twin samples such as those in the present study, this analysis is relatively sensitive to outlying values. Therefore, for each parameter, the outlying values were identified using a two-tailed test (11, 12) with a significance level of 0.05. When a significant outlier(s) was identified, the analysis was repeated after the outlying pair(s) was excluded. A genetic effect on the variability of a given parameter was considered as significant if the appropriate estimate of genetic variance was significant with P < 0.05 and if the result was not critically dependent on the inclusion of significant outliers.

The method of Christian and co-workers (3, 40) for analysis of twin data is applicable only if the means for the parameter under study are not significantly different between MZ and DZ twins. When the analysis is applicable, the next step is the examination of possible MZ-DZ differences in the variance of the parameter under study. If the F-test for significance of differences in variances has a P > 0.20, thus indicating that the variances are similar in both groups, a genetic effect is considered significant if the “within-pair estimate of genetic variance” has a P < 0.05. If the F-test has a P < 0.20, a trend for a difference in variance between the MZ and DZ groups is present, and the within-pair estimate of genetic variance may be biased. In this case, another estimate, the “among-component estimate of genetic variance” must be used, and a genetic effect is considered significant if this estimate has a P < 0.05.

These estimates of genetic variance are appropriate if there are no MZ-DZ differences in environmental covariance, but this assumption cannot be tested. Among the outputs of the analysis are the intraclass correlation coefficients, which are a measure of the similarity in the parameter of interest in the MZ and DZ twin pairs. The existence of a significant genetic effect is reflected typically in a higher intraclass correlation in the MZ than in the DZ group. If, however, there is a significant intraclass correlation in the MZ group but not in the DZ group, the existence of a higher environmental covariance in the MZ group, rather than of a genetic effect, should be suspected, since a genetic effect should be reflected in a significant level of correlation among the DZ twins, who are related genetically as siblings. It is also possible that relatively small DZ intraclass correlations may reflect the presence of gene interactions (4, 9). To examine the first possibility, the method of Christian and colleagues includes a test to exclude the existence of a MZ-DZ difference in environmental covariance. In the present study, differences in environmental covariance could be associated with the higher proportion of twins living together in the MZ group. Therefore, a two-factor analysis of variance of the within-pair differences in the parameter under consideration, using zygosity and cohabitational status (i.e., living together or not) as cofactors, was also performed.

Unless otherwise indicated, all group values are reported as means ± SD.

Global Estimation of Similarity of Twin Patterns of PRL Secretion

A global measure of the overall within-pair similarity in the 24-h patterns of plasma PRL concentrations was obtained by calculating the coefficient of cross-correlation between simultaneous plasma PRL values. This measure is independent of the mean level of each twin profile but reflects the concordance, both in timing and magnitude, of 24-h and pulsatile variations.
Fig. 1. Profile (24 h) of plasma prolactin (PRL) in representative pairs of monozygotic (MZ; A) twins and dizygotic (DZ; B) twins. Dashed lines: best-fit curves; bars: sleep period, as determined by polygraphic recording. Note similarity of nycthemeral waveshape in MZ but not DZ twins. Coefficient of cross-correlation between the two profiles is given for each pair.

Fig. 2. Profile (24 h) of plasma prolactin (PRL) in representative pairs of MZ (A) twins and DZ twins (B). Dashed lines: best-fit curves; bars: sleep period, as determined by polygraphic recording. Note similarity of nycthemeral waveshape in MZ but not DZ twins. Coefficient of cross-correlation between the two profiles is given for each pair.
RESULTS

Representative 24-h plasma PRL profiles in MZ and DZ twin pairs are shown in Figs. 1–3. The best-fit curves are plotted in dashed lines.

All individual profiles exhibited the typical sleep-dependent pattern of PRL concentrations, with higher levels during sleep and lower levels during the waking period. Overall, nearly one-half of the total 24-h secretory output of PRL (46.3 ± 6.4%) occurred during the average 7h48 sleep period. Plasma PRL pulses occurred throughout the 24-h cycle. Visual examination suggests a greater similarity of waveshape of PRL profiles in MZ than in DZ twins. This was confirmed by the calculation of the within-pair coefficient of cross-correlation, which was significantly higher in MZ twins (0.76 ± 0.08) than in DZ twins (0.52 ± 0.25; P < 0.01).

Each parameter quantifying the nycthemeral and pulsatile variations of plasma and/or secretory PRL profiles, as well as each sleep parameter, was submitted individually to the analysis of genetic variance. For all parameters, there were no differences in means between the MZ and DZ groups (P > 0.05), and the analysis of Christian and co-workers (3, 40) was thus applicable. A summary of the statistical analysis is given for PRL parameters in Table 1 and for sleep parameters in Table 2. There were no significant effects of cohabitation for any of the parameters.

For both MZ and DZ groups, the distribution around the line of identity of individual values for the 24-h secretion, the secretion during wake, the secretion during sleep normalized for sleep maintenance, the amplitude of the 24-h rhythm, the level of the nadir, and the level of the acrophase are shown in Fig. 4.

Parameters Characterizing the Overall 24-h PRL Profiles

The 24-h mean PRL level and amount of PRL secreted during the 24 h. No outlier was detected. Both the MZ and the DZ intraclass correlations were significant and similar in size, indicating that there are within-pair environmental factors influencing the 24-h mean PRL level and the total amount of PRL secreted during the 24 h (Fig. 4). The estimate of genetic variance was nonsignificant.

Amplitude of the 24-h plasma PRL profile. When the amplitudes of the overall 24-h variation were examined, one of the DZ twins (DZ2–2) was identified as an outlier (P < 0.025). When this subject was excluded, the intraclass correlations were 0.63 (P < 0.10) for the MZ group and 0.49 for the DZ group, but, as may be seen in Fig. 4, the difference in variance between the two groups was still present (P = 0.04). Therefore, estimations of the genetic component had to be based on the among-component estimate of genetic variance, which tended to be significant (P = 0.07), indicating a trend for a genetic effect on the overall amplitude of the 24-h variations of plasma PRL.

Absolute value of the acrophase of the 24-h plasma PRL profile. In 36 out of the total of 40 subjects, the acrophase of PRL concentrations occurred during sleep.

Fig. 3. Profile (24 h) of plasma prolactin (PRL) in representative pairs of MZ twins (A) and DZ twins (B). Dashed lines: best-fit curves; bars: sleep period, as determined by polygraphic recording. Note similarity of nycthemeral waveshape in MZ but not DZ twins. Coefficient of cross-correlation between the two profiles is given for each pair.
In four subjects (who were not siblings), the acrophase was delayed until shortly after awakening. The same subject (DZ2–2) who was identified as an outlier for the absolute rhythm amplitude also was an outlier for the value of the acrophase ($P, 0.025$), but not for the value of the nadir. Thus his elevated rhythm amplitude was related to a higher acrophase, rather than a lower nadir. When this outlier was excluded, the intraclass correlations were $0.70$ ($P, 0.05$) for MZ twins and $0.54$ for DZ twins. As shown in Fig. 4, a significant difference in variance between the two groups was present ($P = 0.08$). A tendency ($P = 0.08$) for the existence of a genetic effect on the value of the acrophase was detected.

### Table 1. Summary of analysis of genetic variance of PRL profiles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of Outliers</th>
<th>Intraclass Correlation</th>
<th>P Level for MZ-DZ Difference in Variance</th>
<th>P Level for Estimates of Genetic Variance</th>
<th>Heritability Estimate [2-(RMZ$^2$ - RDZ$^2$)]</th>
<th>P Level to Exclude a Difference in Environmental Covariance</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MZ</td>
<td>DZ</td>
<td>MZ</td>
<td>DZ</td>
<td>Within pair</td>
<td>Among component</td>
<td>Value</td>
</tr>
<tr>
<td>Combined effects of wake and sleep</td>
<td>0</td>
<td>0</td>
<td>0.83*</td>
<td>0.73*</td>
<td>0.94</td>
<td>0.26</td>
<td>0.42</td>
</tr>
<tr>
<td>Amount of PRL secreted during 24-h cycle</td>
<td>0</td>
<td>0</td>
<td>0.78*</td>
<td>0.71*</td>
<td>0.76</td>
<td>0.25</td>
<td>0.56</td>
</tr>
<tr>
<td>Absolute value of acrophase</td>
<td>0</td>
<td>1</td>
<td>0.70*</td>
<td>0.54</td>
<td>0.08</td>
<td>0.81</td>
<td>0.08</td>
</tr>
<tr>
<td>Relative value of acrophase, %wake mean</td>
<td>1</td>
<td>0</td>
<td>0.66†</td>
<td>0.64*</td>
<td>0.81</td>
<td>0.54</td>
<td>0.42</td>
</tr>
<tr>
<td>Amplitude</td>
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<td>0.63†</td>
<td>0.49</td>
<td>0.04</td>
<td>0.90</td>
<td>0.07</td>
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<tr>
<td>Mean concentration</td>
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<td>0</td>
<td>0.85*</td>
<td>0.62†</td>
<td>0.74</td>
<td>0.04</td>
<td>0.46</td>
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<tr>
<td>Amount of PRL secreted during wake</td>
<td>0</td>
<td>0</td>
<td>0.86*</td>
<td>0.68*</td>
<td>0.70</td>
<td>0.05</td>
<td>0.52</td>
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<tr>
<td>Absolute value of nadir</td>
<td>0</td>
<td>0</td>
<td>0.81*</td>
<td>0.51</td>
<td>0.36</td>
<td>0.01</td>
<td>0.56</td>
</tr>
<tr>
<td>PRL secretion during sleep</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean concentration</td>
<td>0</td>
<td>0</td>
<td>0.72*</td>
<td>0.73*</td>
<td>0.93</td>
<td>0.57</td>
<td>0.48</td>
</tr>
<tr>
<td>Mean concentration relative to sleep maintenance</td>
<td>0</td>
<td>0</td>
<td>0.81*</td>
<td>0.62†</td>
<td>0.91</td>
<td>0.14</td>
<td>0.43</td>
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<tr>
<td>Amount of PRL secreted during sleep</td>
<td>0</td>
<td>0</td>
<td>0.71*</td>
<td>0.52</td>
<td>0.46</td>
<td>0.47</td>
<td>0.23</td>
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<tr>
<td>Amount of PRL secreted relative to sleep maintenance</td>
<td>0</td>
<td>2</td>
<td>0.75*</td>
<td>0.61</td>
<td>0.05</td>
<td>0.89</td>
<td>0.04</td>
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</table>

For intraclass correlation, $n = 10$ monozygotic (MZ) and 10 dizygotic (DZ) pairs for combined effects of wake and sleep prolactin (PRL) secretion during wake; $n = 9$ MZ and 8 DZ pairs for PRL secretion during sleep. RMZ and RDZ, intraclass correlation for MZ and DZ twins, respectively. *P < 0.05 and †P < 0.10.

In four subjects (who were not siblings), the acrophase was delayed until shortly after awakening. The same subject (DZ2–2) who was identified as an outlier for the absolute rhythm amplitude also was an outlier for the value of the acrophase ($P < 0.025$), but not for the value of the nadir. Thus his elevated rhythm amplitude was related to a higher acrophase, rather than a lower nadir. When this outlier was excluded, the intraclass correlations were 0.70 ($P < 0.05$) for MZ twins and 0.54 for DZ twins. As shown in Fig. 4, a significant difference in variance between the two groups was present ($P = 0.08$). A tendency ($P = 0.08$) for the existence of a genetic effect on the value of the acrophase was detected.

### Table 2. Summary of analysis of genetic variance of sleep parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of Outliers</th>
<th>Intraclass Correlation</th>
<th>P Level for MZ-DZ Difference in Variance</th>
<th>P Level for Estimates of Genetic Variance</th>
<th>Heritability Estimate [2-(RMZ$^2$ - RDZ$^2$)]</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>MZ</td>
<td>DZ</td>
<td>MZ</td>
<td>DZ</td>
<td>Within pair</td>
<td>Among component</td>
<td>Value</td>
</tr>
<tr>
<td>Sleep period</td>
<td>0</td>
<td>0</td>
<td>0.70*</td>
<td>−0.09</td>
<td>0.52</td>
<td>0.09</td>
<td>0.05</td>
</tr>
<tr>
<td>Sleep maintenance</td>
<td>0</td>
<td>0</td>
<td>0.48</td>
<td>−0.16</td>
<td>0.50</td>
<td>0.26</td>
<td>0.08</td>
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<tr>
<td>Duration of stages I and II</td>
<td>0</td>
<td>0</td>
<td>0.77*</td>
<td>0.29</td>
<td>0.92</td>
<td>0.05</td>
<td>0.25</td>
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<tr>
<td>Duration of stages III and IV</td>
<td>0</td>
<td>0</td>
<td>0.84*</td>
<td>0.39</td>
<td>0.10</td>
<td>0.30</td>
<td>0.04</td>
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<tr>
<td>Duration of REM sleep</td>
<td>0</td>
<td>0</td>
<td>0.37</td>
<td>0.19</td>
<td>0.51</td>
<td>0.56</td>
<td>0.30</td>
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<tr>
<td>Duration of wake</td>
<td>0</td>
<td>1</td>
<td>0.46</td>
<td>0.29</td>
<td>0.00</td>
<td>0.97</td>
<td>0.10</td>
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<tr>
<td>REM sleep latency</td>
<td>0</td>
<td>0</td>
<td>−0.01</td>
<td>0.26</td>
<td>0.37</td>
<td>0.86</td>
<td>0.65</td>
</tr>
</tbody>
</table>

REM, rapid eye movement; $n = 9$ MZ and 8 DZ pairs for intraclass correlation. *P < 0.05 and †P < 0.10.
factors modulated the relative increase in nocturnal PRL concentrations over daytime values.

Estimations of plasma PRL pulsatility. Neither environmental nor genetic effects could be evidenced for PRL pulse frequency and/or amplitude over the 24-h cycle, during wake or during sleep.

PRL Concentrations and PRL Secretion During Wake

Mean plasma PRL level and amount of PRL secreted during wake. No outlier was detected. The variances for these two parameters were similar in both groups, and the within-pair estimates of genetic variance were significant for both parameters (Table 1 and Fig. 4). The possibility of MZ-DZ difference in environmental covariance could be excluded. Thus plasma PRL concentrations and amount of PRL secreted during the daytime are partially genetically determined.

Value of the nadir of the 24-h PRL profile. There were no significant outliers in either group (Fig. 4). The within-pair estimate of genetic variance was highly significant ($P = 0.01$), and the possibility of a difference in environmental covariance could be excluded, indicating a genetic effect for the value of the PRL nadir.

Sleep Parameters

In three subjects (MZ8–1, DZ3–1, and DZ10–1), technical difficulties related to the blood sampling procedure resulted in major disruptions of sleep. Sleep efficiency in these individual studies was only around 50% (51.3, 53.9, and 48.2%, respectively), i.e., much lower than for normal laboratory recordings in healthy young men. The corresponding twin pairs were not included in the analysis of sleep parameters, since poor within-pair reproducibility was a direct result of the
experimental procedure. In the remainder of the subjects, sleep efficiency averaged 82.3 ± 14.0% (range 68–96%).

For all sleep parameters, the analysis of Christian and co-workers (3, 40) was applicable because differences in means between the MZ and DZ groups were nonsignificant (P > 0.05). Cohabitation effects were nonsignificant (P > 0.10) for all sleep parameters. The results of the genetic analysis of variance are given in Table 2. No significant outliers were detected for any of the parameters. Figure 5 illustrates the distribution around the line of identity of individual values for SWS, stages I and II, REM sleep, and the wake stage.

Neither environmental nor genetic effects could be demonstrated for sleep efficiency, amount of wake, amount of REM sleep, or for REM sleep latency. For all of these parameters, intraclass correlations were indeed nonsignificant for both the MZ and the DZ groups (Table 2).

For the sleep period, the intraclass correlation was significant for the MZ group but close to zero in the DZ group. The possibility of a higher environmental covariance in the MZ group could not be rejected, suggesting the existence of more common environmental factors in the MZ than the DZ group.

A significant genetic effect was found for the duration of stages III and IV (SW sleep), and a similar trend was observed for the duration of stages I and II (Table 2 and Fig. 5). Thus non-REM sleep, but not REM sleep or sleep fragmentation, appeared to be partially under genetic control.

PRL Secretion During Sleep

Mean plasma PRL level and amount of PRL secreted during sleep. No outlier was detected for either parameter. For the mean PRL concentration during sleep, the MZ and the DZ intraclass correlations were both significant (Table 1; P < 0.05) and similar in size, indicating the predominance of environmental, rather than genetic, factors.

When the amount of PRL secreted during sleep was mathematically derived from the plasma concentrations, the intraclass correlation for the MZ group was essentially identical to that observed for the mean PRL level (0.71 vs. 0.72), but, for the DZ group, the intraclass correlation for the amount of PRL secreted (r = 0.52) tended to be smaller than for the mean plasma level (r = 0.73). Because a common constant half-life was used for all subjects, the greater within-pair variability of amount of PRL secreted vs. mean plasma PRL level in DZ twins reflects a greater variability in body surface area. The results of the analysis do not demonstrate either a genetic or an environmental effect on amount of PRL secreted during sleep.

Mean plasma PRL level during sleep and amount of PRL secreted during sleep normalized for sleep maintenance. Because PRL secretion is influenced by sleep quality, the analysis was repeated after normalizing the mean PRL level during sleep and the amount of PRL secreted during sleep for sleep maintenance, i.e., the percentage of the sleep period actually spent asleep.

Fig. 5. Scatter plots of individual sleep parameters in MZ twins and DZ twins. Solid lines represent lines of identity. Outliers are not represented. SWS, slow-wave sleep; REM, rapid eye movement.
For the mean plasma PRL level during sleep normalized for sleep maintenance, intraclass correlations were significant for both MZ and DZ twins and tended to be higher for MZ twins (0.81 vs. 0.62). A weak tendency (Table 1; \( P < 0.14 \)) for a genetic contribution was detected, suggesting, when sleep quality is controlled for, that nocturnal PRL concentrations may be under partial genetic control.

Similar conclusions were obtained for the amount of PRL secreted normalized for sleep maintenance (Fig. 4).

Other indexes of PRL secretion during sleep. The analysis of genetic variance also was performed for a number of other indexes of PRL secretion during sleep, including amount of PRL secretion above daytime level, amount of PRL secretion above daytime level normalized for sleep maintenance, amount of PRL secreted per minute of SWS during the first three hours of sleep, and timing of the acrophase relative to sleep onset. For none of these parameters could evidence for either environmental or genetic factors be obtained.

DISCUSSION

The analysis of parameters quantifying the 24-h profiles of plasma PRL and PRL secretory rates in MZ and DZ twins permitted determination of whether genetic and/or environmental factors influence the levels and temporal variability of human PRL secretion during wake and during sleep. Both nycthemeral (i.e., 24-h) and pulsatile variations were characterized from plasma concentrations measured at 15-min intervals. The results indicate that baseline daytime PRL concentrations are partially genetically determined and that the amplitude and overall waveshape of the daily PRL secretory profile also are influenced by genetic factors.

In contrast, the mean concentrations and total secretory output during the sleep period appeared to be dependent on common within-pair environmental factors. Consistent with the fact that nearly one-half of the total daily production of PRL occurs during sleep, the overall 24-h concentrations and total secretion were found to be influenced by common factors in the environment. When the environmental effect of sleep was controlled for by taking into account interindividual variations in sleep fragmentation (by normalizing for sleep maintenance), the estimations of genetic variance approached statistical significance, suggesting that the secretory response to a standardized sleep/circadian stimulus also is partly genetically programmed. No evidence for either environmental or genetic effects on the frequency and amplitude of PRL pulses could be demonstrated either for the entire 24-h period or for the wake or sleep periods.

Previous studies investigating the existence of a genetic component in the regulation of PRL secretion in humans were all conducted during the daytime period. An early study examining plasma PRL concentrations in single blood samples collected in the morning in pubertal MZ and DZ male and female twins did not find evidence of a genetic control of basal PRL secretion (39). Whether this negative result reflects a greater influence of environmental factors on PRL secretion at this specific stage of sexual maturation or the failure to adequately estimate daytime PRL concentrations from a single blood sample cannot be determined. In contrast, studies in adults who were exposed to pharmacological challenge with amphetamine (16) or arecoline (17) showed significantly higher concordance of PRL responses in identical twins. These results are in accordance with our findings of partial genetic control of the PRL acrophase (i.e., a response to physiological nocturnal stimulation) and of nearly significant estimates of genetic variance for mean PRL concentrations and amount of PRL secretion during sleep when normalized for sleep maintenance.

Besides providing evidence for genetic influences on basal PRL secretion and responsivity to stimulation, the present results also indicate that the temporal organization of PRL secretion over the 24-h cycle is partly genetically determined. Indeed, cross-correlation analyses clearly indicated a higher level of intrapair reproducibility of the waveshape of the 24-h PRL profile in MZ than in DZ twins. Consistent with this estimation of the overall similarity of the diurnal variation, calculations of estimates of genetic variance suggested the existence of genetic factors in the control of the amplitude of the rhythm.

In contrast, PRL concentrations during the entire 24-h period and during sleep appeared to be primarily determined by environmental factors. Because sleep plays a major role in the control of PRL secretion over the 24-h cycle (18, 24, 25, 33), these results suggest that environmental factors influencing sleep, a state that is influenced strongly by life habits as far as timing, duration, and quality are concerned, also influence nocturnal PRL secretion. Indeed, although a nearly significant estimate of genetic variance was obtained for the absolute value of the PRL acrophase (suggesting that the secretory capacity of the lactotrophs to endogenous stimulation is partially genetically determined), when the value of the acrophase was expressed relative to daytime baseline concentrations to quantify the relative magnitude of maximal nocturnal stimulation, environmental influences appeared predominant.

As previously reported (14) in these healthy young men, genetic factors were found to contribute significantly to the variability in SWS duration but not in REM sleep duration or amount of wake or sleep efficiency. Because SWS is the sleep stage associated with the highest PRL secretory activity during sleep (28), it is possible that the trend for significant estimates of genetic variance found for nocturnal PRL concentrations normalized for sleep maintenance reflects genetic influences on SWS. A number of additional calculations attempting to address this question were performed but failed to provide conclusive results.

The pituitary secretes PRL in pulses of stable frequency but modulated amplitude, giving rise to a sleep-related increase in overall secretion and, consequently, to the well-established 24-h rhythm of plasma PRL concentrations (27, 37). When the pulsatile component of PRL secretion was quantified (i.e., by estimating number and amplitude of pulses), no evidence for
either environmental or genetic effects could be demonstrated. It is likely that the relatively small sample size in the present study did not permit us to address the impact of zygosity on the highly reproducible characteristics of PRL pulsatility.

In conclusion, the present study indicates that both basal daytime concentrations of PRL and the temporal organization of PRL secretion over the 24-h cycle are partially genetically determined in normal young men. These genetic determinants should in turn influence the multiple processes modulated by PRL, including reproduction and humoral as well as cell-mediated immune function.

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REFERENCES


