Sodium oxybate increases prolactin secretion in narcolepsy patients and healthy controls

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Abstract

Objective: Hypocretin deficiency causes narcolepsy and may affect neuroendocrine systems, including TSH, ACTH and LH secretion. Symptoms can be treated effectively with sodium oxybate (SXB) in many patients. This study was performed to compare prolactin (PRL) secretion in patients and matched controls and establish the effect of SXB administration on PRL and sleep in both the groups.

Design: Open label intervention. Blood was sampled before and after 5 days of SXB treatment. The study was performed at the Leiden University Medical Centre, Leiden, The Netherlands.

Methods: Subjects were admitted to the clinical research centre on both occasions.

Patients or participants: Eight male hypocretin-deficient narcolepsy with cataplexy patients and eight controls matched for sex, age, body mass index, waist-to-hip ratio and fat percentage were enrolled.

Interventions: SXB two times 3 g per night for five consecutive nights.

Results: Patients and controls underwent 24 h blood sampling at 10 min intervals for measurement of PRL concentrations. The PRL concentration time series was analysed with a new deconvolution programme, approximate entropy (ApEn) and Cosinor analysis. Sleep was polygraphically recorded. Basal and pulsatile PRL secretion, as well as pulse regularity and frequency, ApEn and diurnal parameters were similar in patients and controls. SXB treatment caused similar nocturnal increase in PRL secretion, advance of the acrophase and decrease in ApEn in patients and controls. Slow wave sleep was increased to a similar extent in patients and controls.

Conclusion: This detailed study did not demonstrate altered PRL secretion in hypocretin-deficient narcolepsy patients during the basal state or during SXB administration. Therefore, hypocretin signalling is unlikely to be a regulator of the lactotrophic system.

European Journal of Endocrinology 164 363–370

Introduction

Narcolepsy is a debilitating disorder characterised by excessive daytime sleepiness (EDS), cataplexy, hypnagogic hallucinations, sleep paralysis and nocturnal sleep disturbances (1). In recent years, obesity and hormonal alterations have been recognised as additional features of the narcoleptic syndrome (2).

Interest in energy homeostasis in narcolepsy increased after the discovery of hypocretin deficiency as the cause of this disorder since hypocretins (orexins) are known to influence feeding behaviour, wakefulness and energy expenditure (3–6). Hypocretins are also involved in the control of the secretion of various hormones (7–11).

Prolactin (PRL) is a polypeptide hormone, which is primarily produced in the anterior pituitary gland (12). Apart from its role in lactation and reproduction, PRL also takes part in the regulation of body energy homeostasis (12, 13). Prolonged hyperprolactinaemia is often accompanied by weight gain in humans, which can be ameliorated by normalisation of serum PRL (13). The major physiological regulator of PRL release is dopamine from tubero-infundibular origin. Hypothalamic dopamine inhibits the basally high secretory tone of pituitary lactotrophs by binding to D2 receptors expressed in their cell membranes (14, 15). Hence, alterations in the dopaminergic system, as described previously in the post-mortem brains of narcoleptic humans, may cause changes in PRL secretion (16). However, earlier reports on PRL secretion in narcolepsy patients have been inconclusive, showing either increased, decreased or normal levels (2, 17–19). These discrepancies are likely due to the use of only few baseline measurements of hormone levels, small sample size, relatively poor matching or too long blood sampling intervals, which are not adequate to assess either the pulsatile nature of PRL secretion or its total daily production rate.
Sodium oxybate (SXB) or gamma-hydroxybutyrate (GHB) is effective in the treatment of narcolepsy. It reduces cataplexy, improves nocturnal sleep quality and higher doses of SXB may also reduce EDS (20). Although the exact mode of action is still unclear, SXB acts on gamma-aminobutyric acid type B (GABAB) and has an impact on dopamine and serotonin release (21, 22). Therefore, SXB treatment may be expected to alter PRL secretion. Indeed, there are reports that SXB administration may increase PRL secretion (23, 24). Several reports have been published on PRL and sleep; however, the precise association between PRL release and sleep still remains to be elucidated (23–28).

We hypothesised that changes in hypothalamic hypocretin signalling in narcoleptic patients may give rise to altered PRL secretion. In addition, we aimed to determine the effect of SXB administration on PRL secretion and sleep, both in a healthy and in a hypocretin-deficient state. Therefore, using a combination of polysomnography and state-of-the-art endocrine techniques, we compared PRL secretion between hypocretin-deficient narcoleptic subjects and matched controls both at baseline and after five nights of SXB administration.

Subjects and methods

Subjects

Eight male narcolepsy patients with definite cataplexy were included, who fulfilled the diagnostic criteria of the 2nd edition of the International Classification for Sleep Disorders (29). All narcolepsy patients were hypocretin-1 deficient and free of medication for at least 2 weeks before the study. Only one of the patients received prolonged SXB administration in advance. He did stop taking SXB 20 days prior to the study. One patient took SXB in the past for a short period of time and did not take SXB 20 days prior to the study. One patient took SXB 20 days prior to the study. One patient was tapered off antidepressants. Five patients were not taking any drugs at the time of enrolment. All consecutive male patients eligible for the study were asked to participate. Eight healthy controls, matched for sex, age, body mass index (BMI), waist-to-hip ratio (WHR) and fat percentage, were included for comparison. Bioelectrical impedance analysis (Bodystat, Douglas, Isle of Man, UK) was used to estimate fat percentage. Subjects were eligible for study after exclusion of hypertension, any known history of pituitary, psychiatric or neurological disease (other than narcolepsy), alcohol or drug abuse, recent weight change (>3 kg weight change within the last 3 months), a sleep disorder history assessed through clinical interview (controls) and endurance sports. Routine laboratory measurements as described previously (31). These segments were condensed from the 30 s scoring intervals by calculating the percentage of time spent in stages I and II non-rapid eye movement (REM) sleep, slow wave sleep (SWS) and REM sleep.

Assays

Serum PRL concentrations were measured with a sensitive time-resolved immunofluorometric assay with a detection limit of 0.04 µg/l (Delfia, Wallac Oy, Turku, Finland). The study was approved by the ethics committee of the Leiden University Medical Centre. Written informed consent was obtained from all subjects.

Protocol

Subjects were admitted to the Clinical Research Centre for 24 h blood sampling. A cannula was inserted into the antecubital vein 45 min before the start of blood sampling at 1200 h. Blood samples were collected with S-monovetten (Sarstedt, Etten-Leur, The Netherlands) from a three-way stopcock attached to a 0.9% NaCl and heparin (1 U/ml) infusion (750 ml/24 h) to keep the cannula from clotting. Sampling was performed through a long line to prevent sleep disruption by investigative manipulations. For PRL measurements, blood was collected in serum tubes at 10 min intervals. After clotting, the blood was centrifuged within 30 min of sampling (1250 g at 4°C for 20 min). Subsequently, plasma was divided into separate aliquots in Sarstedt tubes and stored at −80°C until assay. Three standardised meals were served at 0830, 1300 and 1800 h (Nutridrink, Nutricia, Zoetermeer, The Netherlands; 1.5 kcal/ml, 2100 kcal/day; macronutrient composition per 100 ml: protein, 6 g; fat, 5.8 g; carbohydrate, 18.4 g). Subjects remained (semi)supine except for bathroom visits. Daytime naps were allowed.

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Turku, Finland). The assay was calibrated against the 3rd WHO standard 84/500, 1 ng/ml = 36 mU/l. The intra-assay coefficient of variation varied from 3.0 to 5.2%, while the inter-assay coefficient of variation was between 3.4 and 6.2% (in the 0.1–250 µg/l concentration range). In order to minimise inter-assay variability, samples from each patient and matched control were analysed in the same run.

Calculations and statistics

Deconvolution analysis

PRL concentration time series were analysed using a recently developed automated deconvolution method, which was mathematically verified by direct statistical proof and empirically validated using hypothalamo-pituitary sampling and simulated pulsatile time series (32). For PRL, the fast half-life was represented as 3.5 min constituting 37% of the decay amplitude and the slow half-life was represented as an unknown variable between 20 and 50 min (33, 34). All candidate pulse-time sets were deconvolved. Statistical model selection was then performed to distinguish among the independently framed fits of the multiple candidate pulse-time sets using the Akaike information criterion (35). The parameters (and units) are frequency (number of bursts per total sampling period, λ of the Weibull distribution), regularity of inter-pulse intervals (unitless γ of Weibull), slow half-life (min), basal and pulsatile secretion rates (concentration units/session), mass secreted per burst (concentration units) and waveform shape (mode or time delay to maximal secretion after objectively estimated burst onset, min).

Approximate entropy

Approximate entropy (ApEn) (1, 20%), was used as a scale- and model-independent regularity statistic to quantify the orderliness (regularity) of PRL release. Higher ApEn denotes greater disorderliness (irregularity) of the secretion process. Mathematical models and clinical experiments have established that greater irregularity signifies decreased feedback control with high sensitivity and specificity (both > 90%) (36).

Diurnal variation

The waveform of individual PRL profiles was quantified by a best-fit curve obtained using a locally weighted regression procedure with a regression window of 4 h and a Gaussian kernel (37). The values (and timings) of the acrophase and the nadir were defined as the levels (the timings) corresponding to the maximum and the minimum of the best-fit curve respectively. The amplitude was defined as 50% of the difference between the acrophase and the nadir values.

Statistical analysis

Data are presented as mean± S.E.M., unless otherwise specified. Statistical comparisons were made with either Student’s t-test or repeated measures ANOVA when appropriate. Pearson’s correlation coefficient was applied to assess all correlations. Cross-correlation analysis was applied to assess the association between PRL levels and the percentage of time spent in various sleep stages in the preceding 10 min sampling interval, taking into account all of the sampling intervals during sleep (38). Statistical calculations were performed using Systat software (version 11, Systat Software, Inc., San Jose, CA, USA) and SPSS (release 17.0, SPSS, Inc., Chicago, IL, USA). All tests were two-tailed and significance level was set at 0.05.

Results

Narcoleptic patients and controls did not differ with respect to gender (male), age (38.0 ± 4.7 vs 37.9 ± 4.1, P=0.984 respectively), BMI (28.1 ± 1.6 vs 27.4 ± 1.4, 18
16
14
12
10
8
6
4
2
12 15 18 21 24
Time
Basal
Treatment
Oxybate

Figure 1 Mean 24 h plasma prolactin concentration ± S.E.M., before and after the SXB administration in narcolepsy patients (n=8) and controls (n=8). The downward arrows indicate ingestion of SXB in treatment condition. The dark horizontal bar indicates the lights off period.
Table 1  Deconvolution of serum prolactin concentration profiles in narcolepsy patients and healthy controls. Data are shown as mean ± S.E.M. Each group consisted of eight subjects. Comparisons were made using Student’s t-test (column 8) and two-way ANOVA for repeated measurements. Secretion data are expressed in mass units per litre hormone distribution volume.

<table>
<thead>
<tr>
<th></th>
<th>Narcolepsy</th>
<th>Controls</th>
<th>Narcolepsy versus controls</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>SXB</td>
<td>Baseline</td>
</tr>
<tr>
<td>Pulse frequency (no/24 h)</td>
<td>23.5±0.9</td>
<td>16.6±1.6</td>
<td>21.3±0.8</td>
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<tr>
<td>Half-life (min)</td>
<td>39±4.7</td>
<td>33±2.5</td>
<td>39±4.1</td>
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<tr>
<td>Basal secretion (µg/l per 24 h)</td>
<td>91±16.8</td>
<td>115±18</td>
<td>144±59</td>
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<tr>
<td>Pulsatile secretion (µg/l per 24 h)</td>
<td>93±8.0</td>
<td>155±17.2</td>
<td>105±15.0</td>
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<tr>
<td>Total secretion (µg/l per 24 h)</td>
<td>184±20.0</td>
<td>220±32.0</td>
<td>249±73</td>
</tr>
<tr>
<td>Mean pulse mass (µg/l)</td>
<td>3.99±0.37</td>
<td>6.69±1.15</td>
<td>4.89±0.55</td>
</tr>
<tr>
<td>Mode (min)</td>
<td>19±2.0</td>
<td>20.6±1.8</td>
<td>18.2±1.7</td>
</tr>
<tr>
<td>λ (events/24 h)</td>
<td>21.4±0.7</td>
<td>15.1±1.5</td>
<td>19.3±0.7</td>
</tr>
<tr>
<td>γ (dimensionless)</td>
<td>2.19±0.17</td>
<td>2.05±0.11</td>
<td>2.18±0.12</td>
</tr>
</tbody>
</table>

*P<0.05.

P=0.742), fat percentage (23.6±2.1 vs 23.4±1.7, P=0.946) and WHR (0.92±0.03 vs 0.90±0.02, P=0.579). As expected, in narcolepsy patients the mean BMI was in the overweight range. Ingestion of SXB was well tolerated and apart from mild drowsiness no other side effects were reported.

The mean 24 h PRL concentration in narcolepsy patients was 5.13±0.47 µg/l at baseline and 5.65±0.52 µg/l during the SXB administration (P<0.001, see Fig. 1). In controls, it was 6.78±1.68 and 7.40±1.66 µg/l respectively (P<0.001). The PRL concentration strongly increased shortly after the administration of each dose of SXB. In patients, an increase from 4.56±0.26 to 12.08±1.61 µg/l occurred (P<0.001), and in controls PRL increased from 6.28±1.72 to 14.07±2.85 µg/l (P<0.001) during the first SXB administration. After the second dose, serum PRL increased from 5.12±0.36 to 12.26±2.74 µg/l in patients (P<0.001) and from 7.28±1.94 to 13.82±1.86 µg/l in controls. The effect of SXB was not statistically different between patients and controls.

Deconvolution analysis

At baseline, there were no significant differences in basal, total or pulsatile PRL secretion between narcolepsy patients and controls (Table 1). However, there was a clearly stimulatory effect of SXB on PRL secretion. Basal secretion increased slightly after SXB administration, whereas total PRL secretion increased with 20% in patients and nearly 25% in controls, most likely resulting from an increase of more than 60% in mean pulse mass in both the groups (Table 1).

Diurnal variation

The acrophase of serum PRL concentrations shifted after SXB administration to 1.5 h earlier in controls and 3 h earlier in narcolepsy patients (Table 2). Similarly, the nadir advanced in both the groups after the SXB administration. The amplitude increased significantly in both the groups indicating a greater degree of diurnal variation in PRL secretion after SXB treatment. Intergroup differences as well as group×treatment interaction effects were not significant, indicating a similar effect of SXB on both narcolepsy and control subjects.

Approximate entropy

The ApEn values decreased in both the groups after the SXB administration, reflecting greater regularity of PRL release. There were no differences in baseline ApEn values nor was the group×treatment interaction effect significant (Fig. 2).
Sleep recordings

On average, compared with controls, narcolepsy patients spent significantly less time awake both during basal conditions and SXB treatment (Table 3). During the day (defined as the lights on period between 0730 and 2300 h), narcolepsy patients spent significantly less time awake, while significantly more time was spent in non-REM sleep, regardless of treatment (Table 3). The SXB administration resulted in a significant decrease in stages I and II non-REM and REM sleep over 24 h in both the groups (P = 0.011 and 0.009 respectively), while it significantly increased the time spent in SWS (P = 0.001). During the day, SXB treatment reduced the time spent in stages I and II non-REM and REM sleep (P = 0.038 and 0.041 respectively), while it tended to increase wakefulness (P = 0.098). The percentage of SWS during the night more than doubled in both the groups in response to SXB treatment (narcolepsy: 6.5 ± 1.9 vs 16.5 ± 3.0%; controls: 7.1 ± 1.9 vs 18.5 ± 2.4%; P = 0.001 for treatment effect), whereas there were trends for decreases in the percentages of stages I and II non-REM and REM sleep. The cross-correlation between SWS and PRL release strongly increased after SXB treatment in both narcolepsy patients (−0.03 ± 0.03 vs 0.47 ± 0.12) and controls (0.09 ± 0.06 vs 0.50 ± 0.07), P ≤ 0.001 for treatment effect (Fig. 3). However, the SXB administration did not significantly affect the cross-correlation between PRL release and REM sleep in either narcolepsy patients (0.12 ± 0.05 vs 0.04 ± 0.03) or controls (0.22 ± 0.04 vs 0.10 ± 0.07), P = 0.070 for treatment effect. Similarly, the cross-correlation between PRL secretion and the percentage of time spent in stages I and II non-REM sleep did not change after SXB treatment (0.10 ± 0.09 vs 0.19 ± 0.07 in narcolepsy patients and 0.47 ± 0.06 vs 0.37 ± 0.10 in controls, P = 0.957 for treatment effect).

Table 3 Sleep patterns before and after sodium oxybate administration. Data are shown as mean ± S.E.M. The data are presented as percentages of sleep stages during the 24 h of study, before and after the SXB administration. Unpaired t-tests were used to assess the differences between the two groups.

|                  | Baseline       | Patients |  |  | Controls |  |  |
|------------------|----------------|----------|  |  |  |  |  |
|                  | Wake total (%) | 60.8 ± 2.9 |  |  | 68.7 ± 2.0 |  | 0.044* |
|                  | Wake day (%)   | 79.4 ± 4.2 |  |  | 95.6 ± 2.1 |  | 0.004* |
|                  | Wake night (%) | 25.8 ± 5.7 |  |  | 18.4 ± 4.0 |  | 0.310 |
|                  | Stages I/II total (%) | 29.1 ± 1.4 |  |  | 25.0 ± 2.4 |  | 0.155 |
|                  | Stages I/II day (%) | 14.6 ± 3.0 |  |  | 2.5 ± 1.6 |  | 0.003* |
|                  | Stages I/II night (%) | 55.1 ± 2.5 |  |  | 65.6 ± 5.7 |  | 0.114 |
|                  | SWS total (%)   | 3.7 ± 0.7 |  |  | 2.5 ± 0.7 |  | 0.239 |
|                  | SWS day (%)     | 2.1 ± 0.6 |  |  | 0.03 ± 0.03 |  | 0.005* |
|                  | SWS night (%)   | 6.5 ± 1.9 |  |  | 7.1 ± 1.9 |  | 0.843 |
|                  | REM total (%)   | 6.3 ± 1.8 |  |  | 3.7 ± 0.8 |  | 0.191 |
|                  | REM day (%)     | 2.9 ± 1.4 |  |  | 0.8 ± 0.5 |  | 0.203 |
|                  | REM night (%)   | 12.6 ± 3.0 |  |  | 8.8 ± 1.8 |  | 0.305 |

Discussion

This is the first study in which advanced endocrinological modelling has been applied to accurately assess the secretory dynamics of PRL secretion and its response to SXB challenge in narcolepsy patients. As PRL secretion dynamics was similar in hypocretin-deficient narcolepsy patients and healthy controls, our findings indicate that hypocretin is unlikely to be a major physiological regulator of PRL secretion. We showed that the SXB administration markedly increased PRL secretion and that it enhanced the association between PRL release and SWS. As SXB is known to modulate dopamine release, the major regulator of PRL secretion, these findings suggest that changes in the tubero-infundibular dopaminergic output could underlie the effect of SXB on PRL release. These effects of SXB are unlikely to involve the hypocretin system, since SXB treatment-stimulated
PRL release did not significantly differ between narcolepsy patients and controls.

PRL secretion is under inhibitory control of dopamine released from the tubero-infundibular dopaminergic neurons (TIDA) (12). Other inhibitors of PRL release include somatostatin and neuropeptide Y (NPY), while TRH, serotonin, oestrogen, oxytocin, as well as stress and light stimulate PRL secretion (12). GABA has a dual effect on PRL secretion: by inhibiting TIDA neurons it stimulates PRL secretion whereas it inhibits PRL release via a non-dopaminergic pathway (12). The effect of hypocretins on PRL release is still subject to debate. A study in male rats showed that i.c.v. administration of hypocretin-1 (orexin A) reduces plasma PRL through a pathway that appears to be partly independent of the dopaminergic system (11). Fasting upregulates hypocretin-1 and NPY, which in turn stimulates TIDA neuronal activity and inhibits PRL secretion (39). This effect of hypocretin-1 on dopamine, however, seems to be indirect and mediated through NPY (40). As we found no indications for altered PRL secretion in a hypocretin-deficient state, our findings do not support a role of hypocretin in the regulation of PRL secretion. Conversely, our data also do not provide evidence for the possibility that disrupted PRL release contributes to sleep disturbance in narcolepsy.

In contrast to earlier reports in the late seventies we did not find any evidence for decreased PRL secretion in narcolepsy patients (18, 19). This discrepancy is likely due to the application of suboptimal analytical techniques in previous studies. Moreover, it is important to note in this context, that, for obvious reasons, hypocretin deficiency was not established in patients included in these early studies. A more recent study reported elevated levels of PRL in 13 narcolepsy patients (7 with typical cataplexy) compared with controls. However, in this study, which included predominantly women, the groups were not matched for BMI, and PRL levels were measured only on a single time point (17).

As PRL secretion exhibits a marked circadian variation, is more variable in women, and is positively associated with body weight, differences in these factors may have been responsible for the differences in PRL levels between narcoleptics and controls in this study (12).

SXB administration resulted in a marked increase in PRL secretion in both narcoleptics and controls. This finding is well in line with previous reports. A nearly threefold increase in PRL within 15 min of a 2.5 g GHB injection and a fivefold increase after 1 h were reported in healthy young men (23). Likewise, van Cauter et al. (24) reported a dose-dependent increase in PRL secretion after the SXB administration in healthy humans. The mechanism through which SXB stimulates PRL secretion is still unclear. SXB can influence dopaminergic, serotonergic and GABAB signalling, and activation of these systems could initiate PRL release (21, 22). Systemic administration with low amounts of SXB generally induces hyperpolarisation of dopaminergic structures with a reduction in dopamine release, thereby enhancing PRL secretion (22). In addition, SXB increases the turnover of serotonin, a PRL-releasing factor, most likely due to an increase in available tryptophan, a precursor of serotonin (15, 22). Additionally, GABAB receptor stimulation may also have a stimulatory effect on serotonin and PRL release (22). Moreover, GHRH increases GH and PRL secretion (41). It is conceivable that SXB stimulates GHRH release and thereby promotes both GH and PRL. However, since dopamine is the major regulator of PRL secretion, the potentiating effect of SXB on PRL release is most likely due to its action on the hypothalamic TIDA neurons.

In accordance with a previous study, we did not find an association between PRL release and SWS during the basal state (25). However, after SXB administration the cross-correlation between PRL levels and SWS substantially increased. As the effect of SXB administration on PRL release is likely due to its inhibition of the activity of TIDA neurons, this finding suggests that the suppression of the hypothalamic dopaminergic system may also be responsible for the enhancing effect of SXB on SWS. Alternatively, a mechanism upstream of the TIDA system with effects on both sleep regulation and hypothalamic dopaminergic activity may be involved. In any case, this mechanism is unlikely to include the hypocretinergic system since the facilitating effect of SXB on the cross-correlation between PRL and SWS was similar in narcoleptics and controls. Further studies are needed to elucidate the exact nature of this novel regulatory circuit.

A potential limitation of this study is the relatively small number of participants. However, this limitation is partly offset by the application of accurate assessment of

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**Figure 3** Cross-correlation coefficients between PRL levels and SWS. Sodium oxybate treatment resulted in a substantial increase in the coupling between PRL release and SWS as evidenced by a significant increase in the cross-correlation ($P < 0.001$ for treatment effect).
hormone secretory dynamics, which would have been unfeasible in a larger group of subjects due to both the expensive and laborious nature of the experiments. Additionally, our subjects were very closely matched.

In conclusion, we found no evidence for altered PRL secretion in hypocretin-deficient narcolepsy patients either during the basal state or after the SXB administration. Therefore, hypocretin signalling is unlikely to be a major regulator of the lactotrophic system. Moreover, our findings suggest that the marked stimulatory effect of SXB on PRL release and SWS is mediated through its influence on the hypothalamic dopaminergic system.

Declaration of interest

G J Lammers and S Overeem have served as paid members of the UCB advisory board and received lecture fees from the UCB. None of the other authors have financial conflicts of interest.

Funding

This study was supported by UCB pharma.

Author contribution statement

C E H M Donjacour: study design, data collection, data analysis, interpretation of results and preparation of the manuscript. N A Aiz: data collection, data analysis, interpretation of results and preparation of the manuscript. M Frolich: data analysis, interpretation of results and preparation of the manuscript. S Overeem: study design and preparation of the manuscript. G J Lammers: study design, interpretation of results and preparation of the manuscript. H Pijl: study design, interpretation of results and preparation of the manuscript.

Acknowledgements

We are greatly indebted to the volunteers who participated in this study. In addition, we thank E J M Ladan-Eygenraam, M van Dijk-Besling, H G Haasnoot-van der Bent, J van Vliet-de Regt and P J van Someren for technical assistance during the study.

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Received 2 December 2010
Accepted 10 December 2010