SUPPLEMENTAL MATERIALS

Influence of Sleep Stage on LH Pulse Initiation in the Normal Late Follicular Phase and in Polycystic Ovary Syndrome

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Evaluation of data after exclusion of a single subject with obstructive sleep apnea

One patient in the PCOS group had evidence for moderate to severe obstructive sleep apnea (OSA). This subject contributed 5 (of 35) analyzed pulses in the PCOS group. We did not exclude this subject because her sleep efficiency was excellent (96%), and it remains unknown whether OSA impacts the relationship between sleep stage and LH (GnRH) pulse initiation. The latter consideration was partly informed by a study in pubertal subjects that involved overnight admissions with and without frequent deep sleep interruption via auditory stimuli (with tactile stimuli as needed) (1). In this study, an average of 68 auditory stimuli led to a doubling of the arousal index, shorter deep sleep episodes, and a 40% decrease in time spent in deep sleep. Remarkably, marked alterations of sleep architecture did not influence LH pulse frequency and did not alter the relationship between deep sleep and LH pulse onsets (1). Nonetheless, given the possibility that our inclusion of a subject with OSA could have influenced our results, we repeated all analyses after exclusion of this particular subject. Overall, repeat analyses produced very similar results to the original, suggesting that inclusion of the single subject with OSA did not unduly influence our results.

Subject with PCOS and OSA

The PCOS subject with OSA was 30 years old and had the highest body mass index (51.1 kg/m^2) and fasting insulin (44.3 μIU/ml) of the study group. She also had the highest cycle day at the time of study (day 113). She exhibited 30 apnea/hypopnea events per hour and spent a total of 13.5 minutes with pulse oximeter readings (SpO_2) ≤ 88% (13.4 of these minutes were during REM sleep). Interestingly, she exhibited only 15 awakenings during the sleep period, her sleep efficiency was 96%, and slow wave sleep occupied 33% of total sleep time.

Summary data after exclusion of the PCOS subject with OSA

Summary data for normal subjects, the entire group of PCOS subjects (n = 7), the single PCOS subject with OSA, and the group of PCOS subjects *without* OSA (n = 6) and are shown Supplemental Table 1. P-values for comparisons between normal subjects and only those PCOS subjects without OSA are shown alongside p-values for comparisons including all PCOS subjects. Compared to normal women, PCOS women without OSA were older and demonstrated higher BMI, higher total and free testosterone, and higher fasting insulin (Supplemental Table 1). Overnight admissions occurred on cycle days 31.4 ± 24.2 (mean ± SD)—significantly different compared to women without PCOS (p = 0.0406).

Although computed p-values increased after exclusion of the subject with PCOS, most of the previously-significant p-values remained less than 0.05. However, waist-to-hip ratio was no longer statistically significant (p = 0.0932) after exclusion of the subject with OSA. In addition, LH pulse frequency lost statistical significance (p = 0.0813) after exclusion of this subject, despite minimal change in mean values (0.65 ± 0.13 vs. 0.63 ± 0.13 pulses/hour). We note that these differences in p-values at least partly reflect reduced statistical power. For example, although mean free testosterone increased slightly (from 10.4 ± 7.3 to 10.6 ± 8.0 pg/ml)—and median free T did not change—after exclusion of the subject with OSA, the p-value increased from 0.0062 to 0.0137. Similarly, exclusion of the PCOS subject with OSA did not markedly change sleep-related LH pulse frequency (0.65 ± 0.13 vs. 0.63 ± 0.13 pulses/hour); and although sleep-related LH pulse frequency appeared to be similarly elevated in PCOS compared to controls (0.63 ± 0.13 vs. 0.46 ± 0.15 pulses/hour) after excluding the subject with OSA, this difference no longer reached statistical significance (p = 0.0813).

Primary analyses of the six PCOS subjects without OSA

The total number of pulses analyzed in this group was 30, and the number of sleep-associated pulses per admission was 4.3 ± 1.1 (4 [3-5]). The total number of random time points analyzed in this group was 90. Compared to random time points, LH pulses appeared to be more than two times as likely to be initiated during NREM 3 epochs—6 of 30 pulses (20.0%) vs. 4 of 90 random points (8.9%)—but this was not statistically significant (p = 0.1115) (Supplemental Figure 1A). However, compared to the 5 minutes before randomly-determined time points, the 5 minutes preceding LH pulse initiation contained a 4.2-fold higher percentage of NREM 3 (19.3 vs. 4.6%; p = 0.0085) (Supplemental Figure 1B). No differences were observed for wake, REM, or NREM 1+2 sleep. In sensitivity analyses, NREM 3 was not demonstrably more common before LH pulses (Supplemental Figure 2).

Normal women vs. women with PCOS after excluding the subject with OSA

As shown in Supplemental Figure 3A, REM-associated LH pulse frequency was higher in women with PCOS compared to normal women (0.678 ± 0.262 vs. 0.071 ± 0.071; p = 0.0430), but other sleep stage-associated LH pulse frequencies were similar. The normalized proportion of wake epochs 0-5 minutes before LH pulses was lower in women with PCOS compared to normal women (p = 0.0426; Supplemental Figure 3B). Although no other group differences were demonstrable, the higher normalized proportion of REM epochs 0-5 minutes before LH pulses in women with PCOS compared to normal women nearly reached significance (p = 0.0543).

Post hoc analyses

REM-associated LH pulse frequency was positively correlated with total testosterone (r_s = 0.71; p = 0.0045), free testosterone (r_s = 0.58; p = 0.0292), BMI (r_s = 0.62; p = 0.0174), and fasting insulin (r_s = 0.78; p = 0.0006), but not age or cycle day. After correcting for all other PCOS group-related variables (partial correlation), REM-associated LH pulse frequency was independently predicted by total testosterone (r_s = 0.73; p = 0.0165) and fasting insulin (r_s = 0.68; p = 0.0309). Free testosterone, age, BMI, and cycle day were not independent predictors of REM-associated LH pulse frequency.

The normalized proportion of wake epochs 0-5 minutes before LH pulses was negatively correlated with free testosterone (r_s = –0.63; p = 0.0156), but not total testosterone, BMI, fasting insulin, age, cycle day. None of the PCOS group-related variables was an independent predictor of the normalized proportion of wake epochs 0-5 minutes before LH pulses, although free T was nearly significant in this regard (r_s = –0.62; p = 0.0743).

Conclusions

Repeat analyses after exclusion of a single PCOS subject with OSA produced very similar results to the original. Accordingly, this sensitivity analysis suggests that inclusion of a single subject with OSA did not unduly influence the results, especially as they relate to our primary conclusions—in women with PCOS, LH pulse initiation is not appropriately discouraged by REM sleep and may be encouraged by slow wave sleep.

Reference

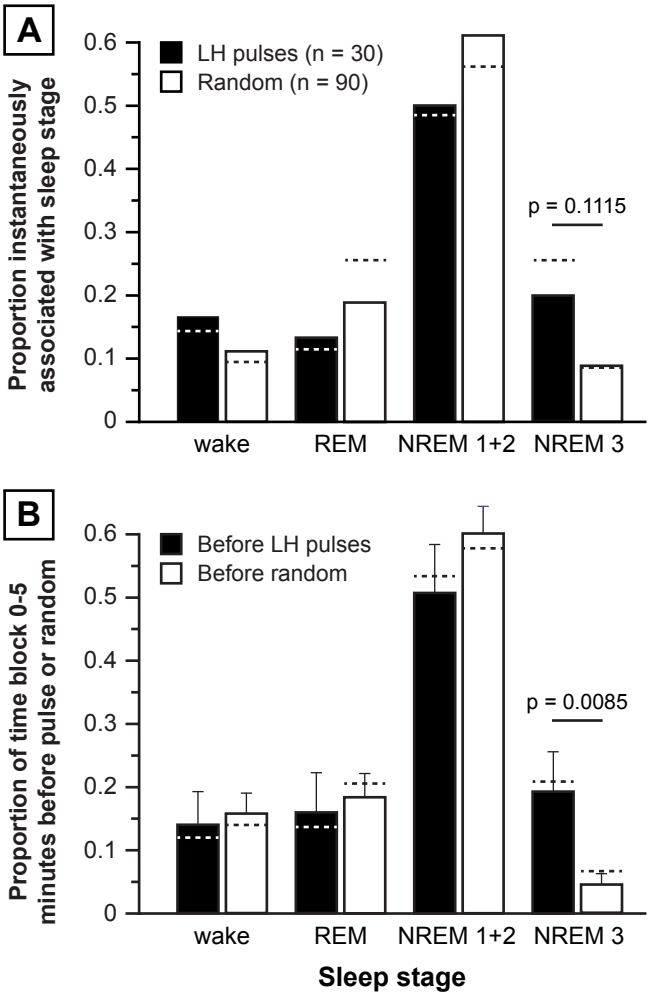
1. Shaw ND, Butler JP, Nemati S, Kangarloo T, Ghassemi M, Malhotra A, Hall JE. Accumulated deep sleep is a powerful predictor of LH pulse onset in pubertal children. J Clin Endocrinol Metab 2015; 100: 1062-1070.

Supplemental Table 1. Summary data partitioned by PCOS status and OSA status

	Normally-cycling, normoandrogenic subjects (n = 8)	All PCOS subjects (n = 7)	PCOS subject with OSA	PCOS subjects without OSA (n = 6)	p-value, normal vs. all PCOS	p-value, normal vs. PCOS without OSA
Age (years)	19.6 ± 2.7 (18.5)	26.6 ± 4.1 (27)	30	26.0 ± 4.2 (26.5)	0.0045	0.0123
Body mass index (kg/m^2)	22.9 ± 2.7 (22.4)	35.2 ± 10.4 (36.7)	51.1	32.6 ± 8.4 (33.9)	0.0205	0.0426
Body fat percentage (%)	24.8 ± 5.9 (23.2)	41.2 ± 15.6 (45.9)	56.2	38.2 ± 15.3 (43.2)	NS	NS
Waist-to-hip ratio	0.77 ± 0.06 (0.76)	0.87 ± 0.09 (0.90)	0.91	0.86 ± 0.10 (0.88)	0.0426	NS (0.0932)
Total testosterone (ng/dl)	26.3 ± 8.1 (25.2)	54.1 ± 23.3 (50.4)	52.2	54.5 ± 25.5 (50.0)	0.0033	0.0070
SHBG (nmol/l)	51.9 ± 29.6 (52)	36.1 ± 12.2 (36)	33.9	36.5 ± 13.3 (38.5)	NS	NS
Free testosterone (pg/ml)	3.9 ± 1.7 (3.5)	10.4 ± 7.3 (8.6)	9.3	10.6 ± 8.0 (8.6)	0.0062	0.0137
Estradiol (pg/ml)	98 ± 48 (78.5)	109 ± 86 (111)	34	122 ± 87 (114)	NS	NS
Progesterone (ng/ml)	0.4 ± 0.1 (0.4)	0.5 ± 0.2 (0.5)	0.3	0.5 ± 0.2 (0.5)	NS	NS
Fasting insulin (μIU/ml)	3.7 ± 3.6 (2.0)	18.2 ± 14.8 (14.4)	44.3	13.9 ± 10.1 (14.1)	0.0092	0.0176
Fasting glucose (mg/dl)	85 ± 6 (87)	90 ± 12 (90)	97	89 ± 13 (88.5)	NS	NS
Mean LH (sleep) (IU/l)	5.2 ± 3.4 (5.1)	7.0 ± 1.4 (7.4)	4.2	7.4 ± 0.7 (7.5)	NS	NS
Mean FSH (sleep) (IU/l)	3.4 ± 1.4 (3.75)	3.6 ± 1.4 (3.5)	4.0	3.5 ± 1.5 (3.4)	NS	NS
LH pulse frequency (sleep) (pulses/h)	0.46 ± 0.15 (0.45)	0.65 ± 0.13 (0.65)	0.75	0.63 ± 0.13 (0.62)	0.0401	NS (0.0813)
LH pulse amplitude (sleep) (IU/l)	3.1 ± 1.8 (2.6)	3.5 ± 2.9 (2.4)	1.4	3.9 ± 2.9 (2.7)	NS	NS
Total sleep period (hours)	6.7 ± 0.9 (7.0)	6.9 ± 0.8 (6.9)	6.6	6.9 ± 0.8 (7.1)	NS	NS
Sleep efficiency (%)	78.4 ± 18.9 (82)	80.0 ± 16.5 (85)	96	77.3 ± 16.2 (82)	NS	NS
Percent wake (%)	21.6 ± 18.9 (18)	20.0 ± 16.5 (15)	4	22.7 ± 16.2 (18)	NS	NS
Percent REM sleep (%)	11.5 ± 5.5 (13)	15.2 ± 7.9 (19)	20	14.4 ± 8.4 (16)	NS	NS
Percent sleep stage 1+2 (%)	51.3 ± 13.0 (52)	53.1 ± 7.0 (53)	43	54.7 ± 6.1 (54)	NS	NS
Percent sleep stage 3 (%)	15.5 ± 7.3 (16)	11.7 ± 11.2 (10)	33	8.2 ± 6.8 (7)	NS	NS

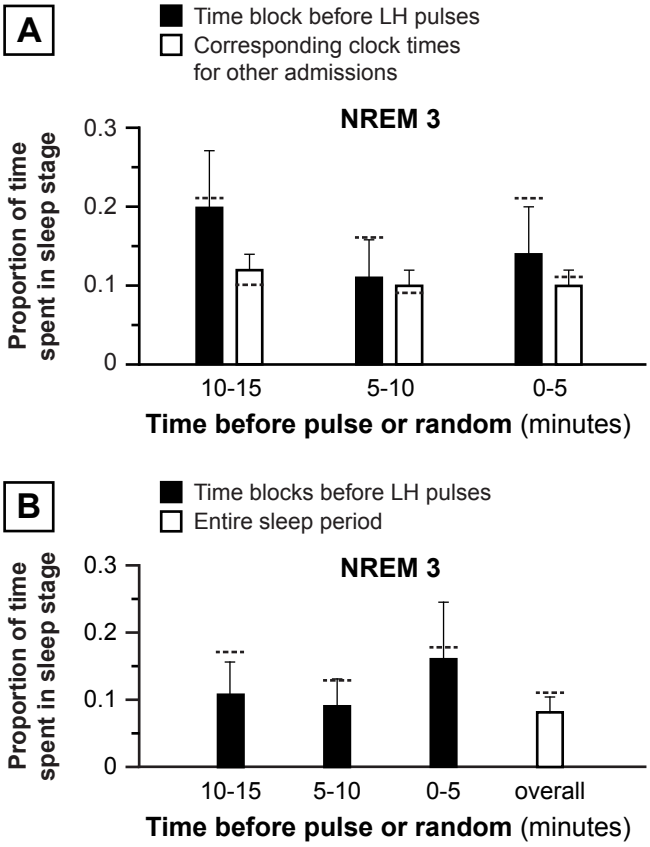
Data are presented as mean ± standard deviation (median). Exact Wilcoxon rank sum (two-sample) tests were used to compare variables; reported p-values are not corrected for multiple comparisons. Sleep-related LH pulse frequency was defined for an individual as the number of LH pulses during a sleep period divided by the total sleep period (h). For subjects with polysomnography (PSG) data for two overnight admissions, only the first admission was used for PSG-related summary statistics. Sleep efficiency is defined here as the percentage of total sleep period occupied by non-wake epochs. To convert metric units to SI units: total testosterone (ng/dl) × 0.0347 (nmol/l); free testosterone (pg/ml) × 3.467 (pmol/l); insulin (μIU/ml) × 7.175 (pmol/l); glucose (mg/dl) × 0.0555 (mmol/l); estradiol × 3.671 (pmol/l); progesterone × 3.18 (nmol/l). Abbreviations: NS, (statistically) nonsignificant (i.e., $p > 0.05$); OSA, obstructive sleep apnea; PCOS, polycystic ovary syndrome; PSG, polysomnography; REM, random eye movement; SHBG, sex hormone binding globulin.

Supplemental Figure 1



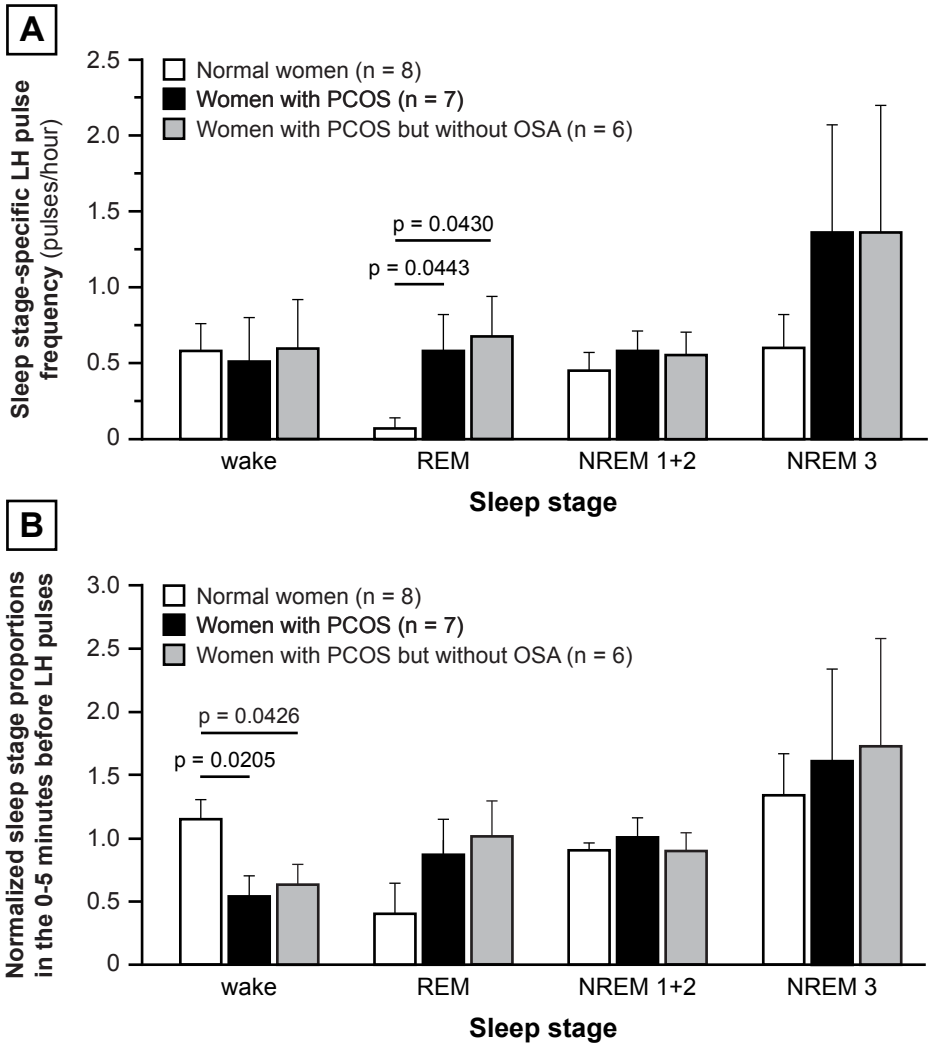
Primary analyses in women with PCOS after excluding a single subject with obstructive sleep apnea (OSA). *Panel A:* Proportion of LH pulses (solid bars) vs. random time points (open bars) instantaneously associated with various sleep stages. Dashed lines indicate proportions observed for all PCOS subjects (including the subject with OSA) as shown in Figure 5A (main text). *Panel B:* Proportion of time frame 0-5 minutes before LH pulses (solid bars) vs. random time points (open bars) occupied by each sleep stage. Data are presented as mean ± standard error of the mean. Dashed lines indicate mean values when considering all PCOS subjects (including the subject with OSA), as shown in Figure 5B (main text).

Supplemental Figure 2



Sensitivity analyses, PCOS women without obstructive sleep apnea (OSA). *Panel A:* First sensitivity analysis: Proportion of sleep stage before LH pulse initiation (solid bars) vs. average proportion of the same sleep stage occurring at the same clock times in other subjects with PCOS (open bars). Dashed lines indicate proportions observed for all PCOS subjects (including the subject with OSA) as shown in Figure 6A (main text). *Panel B:* Second sensitivity analysis: Average proportion of sleep stage before all LH pulses in an admission (solid bars) vs. proportion of the same sleep stage during the same admission's sleep period. Dashed lines indicate proportions observed for all PCOS subjects (including the subject with OSA) as shown in Figure 6B (main text). *Both panels:* Data are presented as mean ± standard error of the mean.

Supplemental Figure 3



Normal subjects vs. all PCOS subjects vs. PCOS subjects *without* obstructive sleep apnea (OSA). *Panel A:* LH pulse frequency for a given sleep stage was calculated as the number of LH pulses instantaneously associated with the sleep stage divided by the percentage of total sleep period (in hours) occupied by that sleep stage. *Panel B:* "Normalized" sleep stage proportions in the 0-5 minutes before LH pulses, defined as the average sleep stage proportion 0-5 minutes before all sleep-related LH pulses in an admission divided by the corresponding sleep stage proportion for the sleep period. *Both panels:* For subjects with two overnight admissions, results were calculated for each admission and averaged. Data are presented as mean ± standard error of the mean.