

## **Objective, but Not Subjective, Sleepiness is Associated with Inflammation in Sleep Apnea**

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**Running Head: Objective vs. Subjective EDS and OSA**

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## **Abstract**

**Study Objectives:** Objective and subjective measures of excessive daytime sleepiness (EDS) are only weakly associated. No study, however, has examined whether these two measures of EDS differ in terms of underlying mechanisms and prognostic value. Pro-inflammatory cytokines, i.e., interleukin-6 (IL-6) appear to promote sleepiness/fatigue, while the stress hormone cortisol promotes vigilance. We hypothesized that objective sleepiness is associated with increased levels of IL-6 and decreased levels of cortisol.

**Methods:** We studied 58 obstructive sleep apnea (OSA) with clinical EDS and/or cardiovascular comorbidities who underwent 8-hour in-lab polysomnography for four consecutive nights. Objective and subjective daytime sleepiness were measured by Multiple Sleep Latency Test and (MSLT), Epworth Sleepiness Scale (ESS) and Stanford Sleepiness Scale (SSS), respectively. Twenty-four-hour profiles of IL-6 and cortisol levels were assessed on the fourth day.

**Results:** The agreement between objective and subjective EDS in OSA patients was fair ( $\kappa = 0.22$ ). Objective EDS (lower MSLT) in OSA patients was associated with significantly elevated 24-hour ( $\beta = -0.34$ ,  $p = 0.01$ ), daytime ( $\beta = -0.30$ ,  $p = 0.02$ ) and nighttime ( $\beta = -0.38$ ,  $p < 0.01$ ) IL-6 levels, and significantly decreased daytime ( $\beta = 0.35$ ,  $p = 0.01$ ) cortisol levels. In contrast, subjective EDS (higher ESS/SSS) was not associated with either elevated IL-6 levels or decreased cortisol levels.

**Conclusions:** Our findings suggest that OSA with objective EDS is the more severe phenotype of the disorder associated with low-grade inflammation, a link to cardiometabolic morbidity and mortality. Compared to subjective EDS, objective EDS is a stronger predictor of OSA severity and may be useful in the clinical management of the disorder.

**Keywords:** Excessive Daytime Sleepiness, Objective Daytime Sleepiness, Sleep Apnea, Interleukin-6 and Cortisol

### **Statement of Significance**

This is the first study to examine whether objective and subjective measures of excessive daytime sleepiness (EDS) differ in terms of underlying mechanisms and prognostic value. EDS is the most common complaint of patients referred to a sleep disorders clinic and is a key feature of OSA. In clinical practice, the most common method of assessing sleepiness is subjective (e.g. Epworth Sleepiness Scale) because of its convenience and low cost. However, its predictive value, in terms of diagnosis and severity of OSA, is uncertain. In this study, we compared subjective and objective sleepiness in terms of their association with the peripheral levels of two markers, IL-6 and cortisol, which are related to sleepiness/alertness and cardiometabolic morbidities. Our findings demonstrate that in OSA patients, objective but not subjective EDS is associated with increased levels of IL-6 (low-grade inflammation) and decreased cortisol levels. These results suggest that objective EDS compared to subjective EDS is a better predictor of the severity of OSA both in terms of daytime impairment and cardiometabolic risks. Given that MSLT is a cumbersome and expensive measure of EDS, there is a need to validate easy-to-use and inexpensive methods of objective EDS to be used in the routine evaluation of OSA patients.

## Introduction

Excessive daytime sleepiness (EDS) is the most common complaint of patients referred to a sleep disorders clinic. In the general population, the prevalence of EDS is estimated to range from 8 to 30% and is associated with a significant public health burden due to medical comorbidities and occupational hazards.<sup>1, 2</sup> Furthermore, daytime sleepiness is common in patients with obstructive sleep apnea (OSA), and an important criterion for diagnosis and treatment of OSA.<sup>3</sup> The prevalence of EDS in OSA is 16-22%<sup>4-7</sup> in epidemiologic samples, and is the most common complaint in clinical samples.<sup>8</sup> Proposed underlying mechanisms appear to be associated with sleep fragmentation caused by recurrent respiratory related arousals and intermittent hypoxia.<sup>9, 10</sup>

The Multiple Sleep Latency Test (MSLT)<sup>11</sup> is considered the gold standard method for the objective measure of daytime sleepiness, whereas the Epworth Sleepiness Scale (ESS)<sup>12</sup> is the most widely used self-report questionnaire for the assessment of subjective daytime sleepiness in clinical settings, including patients with OSA. Both clinicians and health insurance agencies rely on an ESS score of 10 as a cut-off that validates the patients' complaint of daytime sleepiness and fatigue. Several studies examining the association between objective sleepiness and subjective sleepiness in experimental and clinical samples have found inconsistent results, and the correlation between these two measures is low.<sup>13</sup> However, no study has examined whether these two measures differ in terms of underlying mechanisms and/or prognostic value. It has been suggested that pro-inflammatory cytokines, such as interleukin-6 (IL-6), promote sleepiness/fatigue,<sup>14, 15</sup> whereas, cortisol, the end product of the hypothalamic-pituitary-adrenal axis, promotes vigilance and hyperarousal. We have previously hypothesized that sleepiness is associated with higher levels of pro-inflammatory cytokines and lower levels of cortisol.<sup>15</sup>

In the current study, our overall objective was to examine whether the underlying pathophysiologic mechanisms between objective vs. subjective sleepiness differ in a population of patients with OSA. Specifically, we hypothesized that objective, but not subjective, EDS is associated with higher IL-6 levels and lower cortisol levels in patients with OSA.

## **Methods**

### ***Subjects***

The study was completed by 58 research participants who had a diagnosis of OSA (37 male, 63.8%). The subjects were recruited from the Sleep Disorders Clinic and through advertisements from the community. To qualify for the study, patients with OSA had to have apnea of sufficient severity to warrant recommendation for treatment. These criteria included an apnea/hypopnea index (AHI) cut-off of  $\geq 10$  events/hour of sleep for women and  $\geq 15$  events/hour for men plus the presence of common comorbidities associated with sleep apnea, i.e., EDS and cardiovascular disorders. (i.e., hypertension or cardiac arrhythmias).<sup>5, 6</sup> Clinical sleepiness was determined by a single question of “Do you get sleepy and tired during the day? YES or NO”. If the answer was YES, he/she was determined to have clinical sleepiness. A lower threshold of AHI was chosen for women because women have on average lower indices of respiratory disturbance and they tend to manifest symptoms at a lower threshold.<sup>16</sup>

A thorough medical assessment, including physical examination, routine laboratory tests (including complete blood cell count, urinalysis, basic metabolic profile, thyroid function tests, electrocardiography, and urine drug screen), and sleep history was completed for each subject. Those who were positive for abnormal findings in the battery of clinical tests were excluded from the study. Exclusion criteria for all subjects included a history of diabetes mellitus type 2, the use

of antiglycemic agents and/or fasting glucose blood levels > 126 mg/dl at the time of screening, ongoing infections, rheumatoid arthritis, insomnia, narcolepsy, and use of medications that could affect the outcome variables (psychotropics, steroids, sympathomimetics, or sympatholytics, anti-inflammatory agents and hormone replacement therapy for women). Subjects with extreme sleep schedules or with a primary circadian disorder were excluded from the study. Patients with OSA that had used continuous positive airway pressure (CPAP) therapy previously were excluded from the study. The study was approved by the Penn State University College of Medicine Institutional Review Board and all participants provided a written informed consent.

### ***Sleep Laboratory***

All potential participants were screened in the sleep laboratory for one night for 8 hours; subjects who met the inclusion criteria were then monitored in the sleep laboratory for four consecutive nights (one adaptation and three baseline nights). During this time, the subject's sleep was continuously monitored for 8 hours (24-analogue-channel and 10-DC-channel Aurora TS amplifier system using Gamma software; Grass-Telefactor, West Warwick, RI, USA). A four-channel electroencephalogram, two-channel electro-oculogram, and single-channel electromyogram were recorded. The sleep records were subsequently scored independently according to standardised criteria. Respiration was monitored throughout the night by use of thermocouple at the nose and mouth (Pro-Tech, Murrysville, PA, USA), nasal pressure (Validyne Engineering, Northridge, CA, USA), and thoracic and abdominal strain gauges (model 1312; Sleepmate Technologies; Midlothian, VA, USA). A single-channel ECG was also recorded. All-night haemoglobin oxygen saturation was obtained from the finger (model 8600; Nonin Medical;

Plymouth, MN, USA). Anthropometric parameters were obtained and body mass index (BMI) was calculated based on height and weight measured as part of the physical examination.

During the study, patients were asked to maintain their typical daily routine, diet, and level of physical activity. During the stay in the sleep laboratory, their three daily meals were at about 07:00, 12:00 and 18:00.

### ***Daytime sleepiness***

#### Objective daytime sleepiness: Multiple Sleep Latency Test (MSLT)

MSLT was conducted immediately after night 3 (during day 4) of PSG recording (9:00, 12:00, 15:00 and 17:00) (**Table 1**). The severity of objective daytime sleepiness was evaluated using MSLT according to the standard protocol.<sup>11</sup> Lower values of MSLT indicate more objective daytime sleepiness; a clinical cut-off point of sleep latency  $\leq 8$  minutes (based on the mean of all four nap opportunities) was used to define objective EDS.<sup>11, 17</sup>

#### Subjective daytime sleepiness

##### Epworth Sleepiness Scale (ESS)

On day 1 of the study, subjective sleepiness was assessed using the ESS. The ESS is a well-validated questionnaire quantifying the self-reported disclosure of the expectation of “dozing” in a variety of situations.<sup>12</sup> Higher scores of ESS indicate more subjective daytime sleepiness; a clinical cut-off point of total ESS score  $> 10$  was used to define subjective EDS.<sup>12</sup>

## Stanford Sleepiness Scale (SSS)

We also used Stanford Sleepiness Scale (SSS) to evaluate subjective daytime sleepiness during the day 4 shortly before each trial of MSLT (09:00, 12:00, 15:00 and 17:00) (**Table 1**). Higher scores of SSS indicate more subjective daytime sleepiness.

There were 24% (n=15) and 57% (n=33) sleep apnea patients with  $MSLT \leq 8$  and  $ESS > 10$ , respectively. None of them demonstrated SOREMPs.

## *Depressive symptoms*

Depressive symptomatology was assessed both clinically and using the Beck Depression Inventory-II (BDI-II). None of the subjects met the criteria for a current episode of major depression disorder. Of the participants, three OSA subjects scored above the recommended cutoff point above 19 for moderate depression. For the purposes of the current study, we calculated a total score of modified BDI-II after excluding item 20 assessing daytime sleepiness/fatigue.

## *24-h blood sampling*

24-hour serial blood samples were collected every 60 minutes on the fourth day and night in the sleep laboratory (**Table 1**). An indwelling catheter was inserted in the antecubital vein about 30 minutes before the first blood draw. During the sleep periods, blood samples were obtained from an adjacent room by connecting external tubing to the indwelling catheter through a perforation in the wall.



### ***Hormone and cytokine assays***

Blood collected from the indwelling catheter was transferred to an EDTA-containing tube and refrigerated until centrifugation (within 3 hours). The plasma was frozen at -80°C until assay. Plasma IL-6 was measured by ELISA (R&D Systems, Minneapolis, MN) and cortisol levels were measured by specific radioimmunoassay techniques as previously described.<sup>15</sup> The lower limits of detection for IL-6 and cortisol levels were 0.094 pg/ml and 0.7 µg/dl, respectively.

### ***Statistical analysis***

We excluded night 1 to control for the “first night effect” and night 4 to control for the sleep-disturbing effect of blood drawing. Furthermore, we used the mean values of sleep variables from nights 2 and 3, instead of only night 3 (the night before MSLT and blood test) to better represent the habitual sleep pattern of the subjects. Bivariate correlations were performed in order to explore the associations between MSLT values, ESS scores, demographic and sleep variables, as well as inflammatory and stress system biomarkers. Cohen’s Kappa test was used to assess agreement between objective and subjective EDS. In order to examine whether inflammatory and stress system biomarkers predicted objective and subjective EDS, we conducted multiple linear regression models with IL-6 and cortisol values as predictors and continuous MSLT and ESS values as outcomes. Given that there is clear association between age, gender, BMI and outcome variables (sleep, IL-6, and cortisol levels), age, gender, and BMI were always entered in the models as covariables using a forced entry method, while other relevant demographic ( $p \leq 0.1$ ) and sleep ( $p \leq 0.1$ ) covariables were entered in the models using a forward method. Logistic regression models with IL-6 and cortisol values as predictors and MSLT  $> 8$  vs.  $\leq 8$  minutes and ESS  $\leq 10$  vs.  $> 10$  as outcomes were provided as a secondary analysis. We choose these cut-offs for the MSLT

and ESS scores because they are those recommended in clinical practice.<sup>12, 17</sup> Similarly to previous linear regression models, age, gender and BMI were always entered in these models as covariables using a forced entry method, while other relevant demographic ( $p \leq 0.1$ ) and sleep ( $p \leq 0.1$ ) covariables were entered in the models using a forward conditional method. The level  $p < 0.05$  was used to determine statistical significance. All analyses were conducted using SPSS 22.0 (IBM Corp., Armonk, NY).

## Results

### *Characteristics of the study population*

Our study included 58 patients with OSA of mean age  $53.73 \pm 7.02$  years, and  $n = 37$  (63.8%) were men. The correlations between unadjusted MSLT values, ESS scores, demographic and sleep characteristics are presented in **Table 2**.

### *Predictors of Objective Daytime Sleepiness*

In multiple linear regression models, after adjusting for age, gender and BMI, as well as other relevant demographic ( $p \leq 0.1$ ) and sleep ( $p \leq 0.1$ ) covariables which were shown in **Table 2**, OSA patients with lower MSLT values were associated with 1) significantly elevated 24-hour ( $\beta = -0.34$ ,  $p = 0.008$ ), daytime ( $\beta = -0.30$ ,  $p = 0.016$ ) and nighttime ( $\beta = -0.38$ ,  $p = 0.004$ ) IL-6 levels; and 2) significantly decreased daytime ( $\beta = 0.347$ ,  $p = 0.011$ ) cortisol levels (**Table 3**). Results remained the same when further adjusting for ESS scores.

When MSLT was modeled as a binary outcome ( $MSLT > 8$  minutes and  $MSLT \leq 8$  minutes), results of multiple logistic regression models were similar and in the same direction, i.e., OSA with objective EDS was associated with 1) elevated 24-hour (Odds Ratio (OR) =1.74, 95% confidence interval (CI) 1.05-2.88,  $p= 0.031$ ), daytime (OR =1.91, 95% CI 1.08-3.38,  $p= 0.026$ ) and nighttime (OR= 1.41, 95% CI 0.98- 2.02,  $p= 0.063$ ) IL-6 levels; and 2) decreased 24-hour (OR= 0.37, 95% CI 0.15- 0.92,  $p= 0.033$ ) and daytime (OR= 0.37, 95% CI 0.16-0.85,  $p= 0.020$ ) cortisol levels as compared to OSA without objective EDS. Adjusting for subjective EDS did not change the results.

**Figures 1 and 2** depict the unadjusted 24-hour serial IL-6 and cortisol levels, as well as 24-hour, daytime and nighttime IL-6 and cortisol levels in OSA patients with and without objective EDS.

#### ***Predictors of Subjective Daytime Sleepiness***

Notably, in multiple linear regression models, after adjusting for age, gender, BMI, and other relevant demographic ( $p \leq 0.1$ ) and sleep ( $p \leq 0.1$ ) covariables which were shown in **Table 2**, IL-6 or cortisol levels were not significantly associated with higher ESS scores (**Table 4**).

When ESS was modeled as a binary variable ( $ESS \leq 10$  and  $ESS > 10$ ), multiple logistic regression models provided similar results: subjective EDS defined either with the recommended cutoff of  $ESS > 10$  or even a cutoff based on the 75<sup>th</sup> percentile ( $> 16$ ) was not significantly associated with IL-6 or cortisol levels. Results remained the same when further adjusting for MSLT values.

Furthermore, we used SSS to examine the association between subjective sleepiness and IL-6 and cortisol levels on the same day as MSLT. Similarly to ESS, SSS scores were not significantly associated with either IL-6 levels or cortisol levels (all  $P$  values  $> 0.15$ ) in sleep apnea patients.

## Discussion

This is the first study to demonstrate that in patients with OSA, objective but not subjective EDS is associated with increased IL-6 levels and decreased cortisol levels, suggesting that objectively- and subjectively-measured EDS most likely reflect different central nervous system processes. Given the association of pro-inflammatory cytokines to cardiometabolic morbidity and mortality, these findings suggest that OSA associated with objective EDS is the more severe phenotype of the disorder. Furthermore, MSLT is a potentially useful test for the clinical management, diagnosis, and treatment of OSA.

The weak-to-moderate association between objective and subjective sleepiness is not well-understood. It has been proposed that the discrepancy between ESS and MSLT may be explained by a lack of sensitivity of the currently used cut-off points or by the fact that they measure different constructs, i.e., ESS estimates an overall “trait” (fixed) whereas MSLT assesses a “state” (i.e. how one reacts in a particular condition).<sup>13</sup> In our study, we also assessed whether more strict criteria of subjective sleepiness, i.e., ESS > 16 were associated with changes of the sleepiness/arousal molecules; however, even this more severe form of subjective EDS was not associated with changes of IL-6 or cortisol levels. Furthermore, the subjective Stanford Sleepiness Scale administered the same day as MSLT was not associated with either IL-6 or cortisol levels. These data combined suggested that objective and subjective EDS reflect different CNS processes. It has been suggested that MSLT assays physiologic sleep propensity associated with impaired arousal mechanisms, while the ESS captures the subjective complaint of daytime sleepiness/fatigue resulting from impaired sustained attention.<sup>18</sup>

In patients with OSA, objective but not subjective daytime sleepiness was associated with both an increase of IL-6 levels and a decrease of cortisol levels. It is known that low grade

inflammation is a preclinical risk factor for cardiometabolic morbidity<sup>19</sup> and mortality<sup>20</sup>. Also, OSA with daytime sleepiness is associated with increased risk of cardiovascular diseases, stroke, insulin resistance, lower baroreflex sensitivity, impaired heart rate variability and mortality.<sup>21-25</sup> Furthermore, CPAP therapy does not appear to be effective to reduce blood pressure in hypertensive OSA patients without objective EDS.<sup>26</sup> These findings combined suggest that objective EDS is a much stronger predictor compared to subjective EDS of the medical severity of OSA and, potentially, its association with cardiometabolic morbidity and mortality. Furthermore, it appears that the association between objective sleepiness and cardiometabolic risk is mediated through the inflammation pathway.

There are several strengths and limitations of the present study that merit discussion. Strengths include the careful selection of patients, the assessment of sleepiness with both subjective and objective tests, a rigorous experimental protocol including four consecutive nights of 8-hour recordings in the sleep lab, 24-hour blood sampling of IL-6 and cortisol levels, and the careful consideration of confounding factors. The use of a research volunteer sample, however, restricts the generalizability of the results.

Our findings have important clinical implications. First, objective EDS is a much stronger predictor of the medical severity of OSA compared to subjective EDS, which has implications in cardiometabolic morbidity and mortality. This suggests that subjective measures of EDS are of limited utility in clinical practice in determining which patients warrant immediate intervention. Anti-inflammatory agents have been shown to be of clinical utility in the treatment of EDS associated with apnea.<sup>27</sup> Second, given that the current measure of objective EDS (MSLT) is cumbersome and expensive, there is need to validate simpler, easy-to-use and inexpensive methods of objective EDS.

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Table 1. Study protocol in sleep apnea patients.

Tasks	Day 1		Day2		Day 3		Day 4	
	Day	Night	Day	Night	Day	Night	Day	Night
PSG		X		X		X		X
MSLT							X	
SSS							X	
Blood drawing							X	X

MSLT: Multiple sleep latency test; SSS: Stanford sleepiness scale.

Table 2. Demographic and sleep characteristics, IL-6 and cortisol levels and the correlations between MSLT and ESS values in patients with OSA.

	Mean $\pm$ SD	MSLT		ESS	
	N=58	r	p	r	p
Age (years)	53.73 $\pm$ 7.02	0.24	0.08	-0.12	0.37
Male (n, %)	37 (63.8 %)	-0.32	0.02	0.16	0.23
BMI (kg/ m <sup>2</sup> )	31.10 $\pm$ 5.29	-0.03	0.82	0.11	0.41
Obesity ( $\geq$ 30 kg/m <sup>2</sup> ) (n, %)	37 (63.8 %)	0.10	0.44	-0.03	0.83
m-BDI II score	6.86 $\pm$ 7.23	-0.06	0.66	-0.06	0.66
Sleep onset latency (min)	16.52 $\pm$ 9.67	0.44	<b>0.00</b>	-0.30	<b>0.02</b>
Total sleep time (min)	372.44 $\pm$ 48.88	-0.32	<b>0.02</b>	-0.04	0.74
Sleep efficiency (%)	77.44 $\pm$ 10.25	-0.31	<b>0.02</b>	-0.05	0.73
Stage 1 (%)	19.87 $\pm$ 9.61	-0.17	0.20	0.20	0.13
Stage 2 (%)	56.55 $\pm$ 11.86	0.03	0.84	0.04	0.76
Slow wave sleep (%)	10.06 $\pm$ 9.90	0.19	0.15	-0.23	<b>0.09</b>
REM (%)	13.52 $\pm$ 6.59	-0.09	0.52	-0.02	0.86
Wake after sleep onset (min)	79.16 $\pm$ 57.48	0.27	<b>0.04</b>	0.00	0.99
REM onset latency (min)	124.10 $\pm$ 71.02	0.19	0.15	-0.11	0.40
AHI (/ h)	40.93 $\pm$ 23.56	-0.14	0.30	0.13	0.31
Min SaO <sub>2</sub> (%)	79.52 $\pm$ 8.07	0.25	<b>0.06</b>	-0.22	<b>0.10</b>
Self-reported sleep onset latency (min)	23.56 $\pm$ 20.21	0.004	0.98	0.12	0.91
Self-reported sleep duration (h)	6.64 $\pm$ 1.44	0.14	0.29	-0.31	<b>0.02</b>
MSLT sleep latency (min)	10.83 $\pm$ 4.51	--	--	-0.57	0.00
ESS score	11.29 $\pm$ 4.82	-0.57	0.00	--	--
24-hour IL-6 (pg/ ml)	3.82 $\pm$ 2.14	-0.29	0.03	0.21	0.12
Daytime IL-6 (pg/ ml)	3.66 $\pm$ 2.08	-0.24	0.07	0.18	0.18
Nighttime IL-6 (pg/ ml)	4.08 $\pm$ 2.48	-0.32	0.02	0.23	0.09
24-hour cortisol (ug/dl)	8.07 $\pm$ 1.66	0.16	0.23	-0.07	0.60
Daytime cortisol (ug/dl)	8.09 $\pm$ 1.89	0.22	0.10	-0.04	0.79
Nighttime cortisol (ug/dl)	8.03 $\pm$ 1.94	0.01	0.92	-0.10	0.45

Variables are presented as Mean  $\pm$  Standard Deviation (SD). AHI: apnoea/hypopnea index; BMI: Body Mass Index; m-BDI-II: modified Beck Depression Inventory score; NREM: non-rapid eye movement sleep stage; REM: rapid eye movement sleep stage; MSLT: Multiple Sleep Latency Test; ESS: Epworth Sleepiness Scale. Daytime: 8:00- 22:00; Nighttime 23:00-7:00.

Table 3. The association between MSLT values and IL-6 and cortisol levels in patients with OSA.

Predictors	MSLT (minutes)	
N=58	$\beta$	P
24-hour IL-6 (pg/ml)	<b>-0.335</b>	<b>0.008</b>
Daytime IL-6 (pg/ml)	<b>-0.299</b>	<b>0.016</b>
Nighttime IL-6 (pg/ ml)	<b>-0.375</b>	<b>0.004</b>
24-hour cortisol(ug/dl)	0.162	0.184
Daytime cortisol(ug/dl)	<b>0.347</b>	<b>0.011</b>
Nighttime cortisol(ug/dl)	0.040	0.750

$\beta$  and p values of multiple linear regression models were calculated after adjusting for age, gender, BMI, nighttime sleep onset latency, total sleep time, wake time after sleep onset and min SaO<sub>2</sub>.  
MSLT: Multiple Sleep Latency Test; Daytime: 8:00- 22:00; Nighttime 23:00-7:00.

Table 4. The association between ESS scores and IL-6 and cortisol levels in patients with OSA.

Predictors N=58	ESS scores $\beta$	P
24-hour IL-6 (pg/ml)	0.176	0.186
Daytime IL-6 (pg/ml)	0.165	0.208
Nighttime IL-6 (pg/ ml)	0.184	0.189
24-hour cortisol(ug/dl)	-0.036	0.782
Daytime cortisol(ug/dl)	-0.006	0.962
Nighttime cortisol(ug/dl)	-0.074	0.573

$\beta$  and p values of multiple linear regression models were calculated after adjusting for age, gender, BMI, nighttime sleep onset latency, percentage of slow wave sleep, min SaO<sub>2</sub> and self-reported sleep duration. ESS: Epworth Sleepiness Scale. Daytime: 8:00- 22:00; Nighttime 23:00-7:00.

**Figure 1** Serial 24-hour plasma IL-6 levels in OSA patients with (■) and without (▲) objective daytime sleepiness (Panel A). Thick gray line on the abscissa indicates the nighttime sleep recording period. Error bars indicate standard error. OSA with objective EDS was defined as OSA patients with  $MSLT \leq 8$  min; while OSA without objective EDS was defined as OSA patients with  $MSLT > 8$  min. Daytime: 8:00- 22:00; Nighttime 23:00-7:00. Panel B, mean 24-hour, daytime and nighttime IL-6 levels in OSA with (■) and without (■) objective EDS.

**Figure 2** Serial 24-hour plasma cortisol levels in OSA patients with (■) and without (▲) objective daytime sleepiness (Panel A). Thick gray line on the abscissa indicates the nighttime sleep recording period. Error bars indicate standard error. OSA with objective EDS was defined as OSA patients with  $MSLT \leq 8$  min; while OSA without objective EDS was defined as OSA patients with  $MSLT > 8$  min. Daytime: 8:00- 22:00; Nighttime 23:00-7:00. Panel B, mean 24-hour, daytime and nighttime cortisol levels in OSA with (■) and without (■) objective EDS.

Figure 1

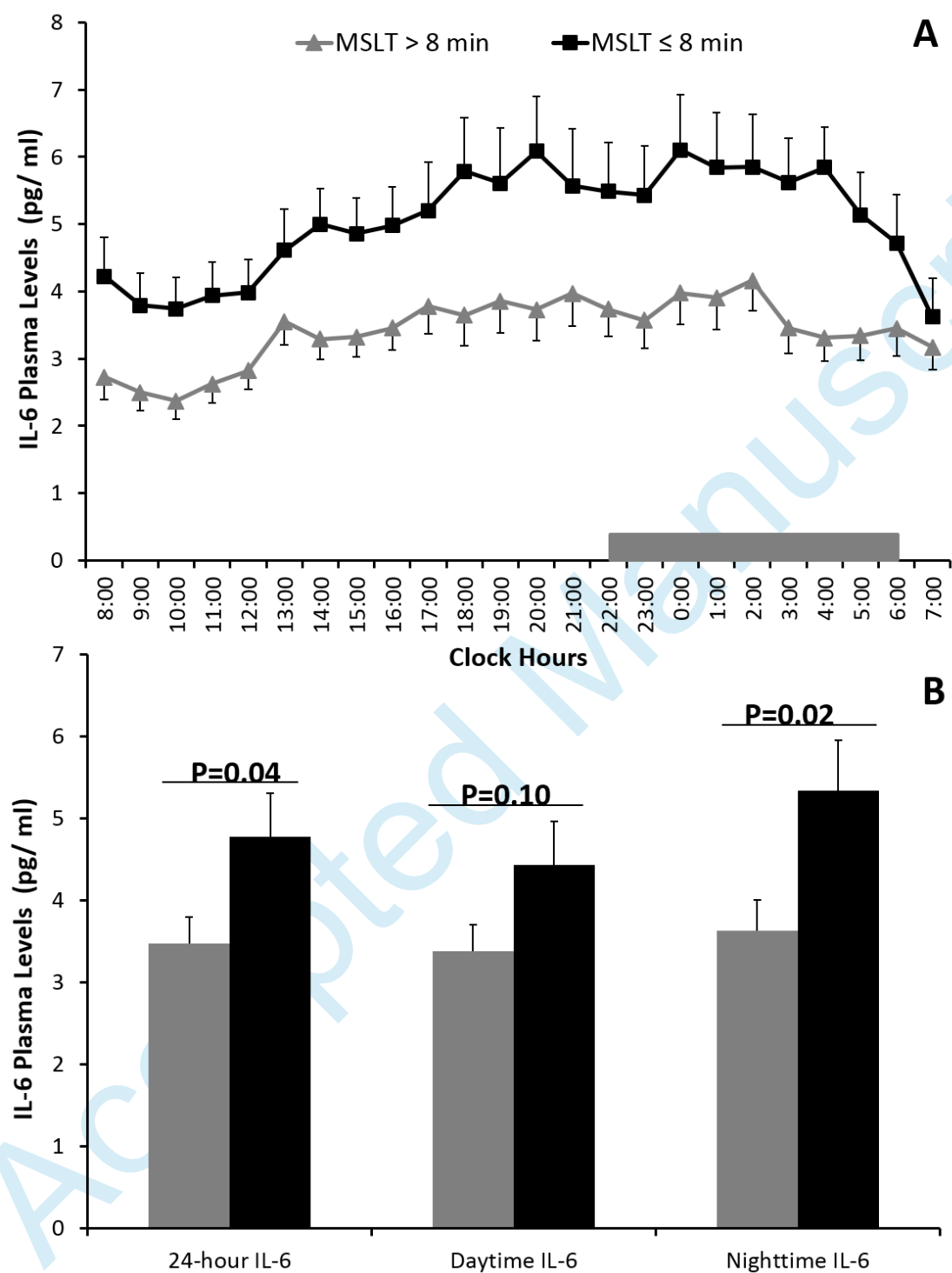


Figure 2

