Pupil Miosis Within 5 Minutes in Darkness Is a Valid and Sensitive Quantitative Measure of Alertness: Application in Daytime Sleepiness Associated With Sleep Apnea

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Study Objectives: The regulation of arousal and pupillary functions may be intimately linked via activity in the nucleus locus coeruleus. In this preliminary study, we tested the validity of the gradual pupillary miosis during 5 minutes in darkness, as a quantitative physiologic index of the arousal state of the brain.

Design: Cross-sectional assessment of 2 groups with between-group comparison and correlational analyses within the patient group.

Participants: Eleven unmedicated male patients recently diagnosed with obstructive sleep apnea (OSA) with no comorbid conditions who had undergone polysomnography to assess OSA severity and sleep variables, and 11 sex- and age-matched healthy controls.

Interventions: Sampling of the resting pupil diameter (RPD) over 5 minutes in darkness in the morning and in the afternoon hours, using an infrared video pupillometer.

Measurements and Results: The RPD was smaller, indicating a lower level of arousal, in the patient group compared with controls in both the morning and the afternoon; the RPD showed a significant circadian reduction in the afternoon only in the patient group. Within the patient group, the RPD correlated negatively with Epworth Sleepiness Scale scores and Arousal Index and positively with the lowest oxygen saturation during the night. Controlling for the effect of body mass index, the relationship between RPD and subjective sleepiness was lost, whereas the relationship with most of the objective indexes of OSA severity was improved.

Conclusions: The 5-minute pupillary miosis in darkness holds promise as a simple, fast-to-administer, valid, and sensitive test for the objective assessment of excessive daytime sleepiness.

Keywords: Pupil, arousal, excessive daytime sleepiness, sleep apnea

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INTRODUCTION

EXCESSIVE DAYTIME SLEEPINESS IS A RECOGNIZED PUBLIC HEALTH PROBLEM THAT CAN AFFECT ALERTNESS AND COGNITIVE FUNCTIONS AND THEREFORE work and academic performance. Excessive daytime sleepiness is also a major cause of car and working accidents. Up to approximately 1 in 8 adults experience serious daytime sleepiness, with obstructive sleep apnea (OSA) being one of the most common underlying causes, although recent evidence points to the importance of metabolic factors and depression.[1] The incidence of OSA ranges between 4% and 9%, with predominance in middle-aged men, and increasing with age.[2-4] Although the severity of the OSA is assessed objectively with full-night polysomnography (PSG), the severity of the associated excessive daytime sleepiness is assessed subjectively with the Epworth Sleepiness Scale (ESS) and objectively with the Multiple Sleep Latency Test (MSLT).[5,6] The MSLT has become the international gold standard for the objective assessment of sleepiness,[7] but it is also an expensive, intrusive, laborious, and not easily repeatable test, which, in addition, does not correlate well with subjective scales of sleepiness[8] or with a number of variables expected to influence sleepiness (e.g., the frequency of respiratory disturbances during sleep).[9] For the above reasons, it is worthwhile to investigate the validity of other objective methods of assessing daytime sleepiness.

The ideal test for the assessment of sleepiness should be based on physiology and should be fast, simple, easy to use, not intrusive, easily repeated, and cost effective. Recent converging evidence suggests that these requirements may actually be met by a test based on the physiology of the pupil. In a dark and quiet environment, the pupils of healthy, well-rested subjects initially remain large and steady, but, eventually, in the absence of external stimulation, they begin to decrease in size, and, finally, they start to oscillate—a phenomenon originally termed pupillary fatigue waves.[10] These events are accentuated and occur faster in healthy sleep-deprived subjects and patients with sleep disorders.[11,12] Pupil size in darkness is controlled by the nucleus locus coeruleus in 2 ways: first, the noradrenergic neurons of the locus coeruleus innervate sympathetic spinal column nuclei and exert an excitatory influence on the peripheral sympathetic nervous system, including the iris dilator muscle; second, an important central role is played by an inhibitory tone on the parasympathetic neurons of the pupilloconstrictor Edinger-Westphal nucleus directly from the ascending noradrenergic systems of the medulla and the locus coeruleus in cats[13,14] and humans.[15] Thus, the reduction of pupil size in darkness is believed to be the result of reduced inhibition of the Edinger-Westphal nucleus as a result of reduced locus coeruleus activity. Equally, the ensuing spontaneous pupillary fluctuations in darkness are believed to be the result of variable inhibition of the Edinger-Westphal nucleus as a result of ensuing fluctuations

in locus coeruleus activity.[15] In line with this is the clinical finding that drugs that "switch on" the locus coeruleus increase the levels of arousal and the pupillary diameter, with a reduction in the ensuing pupillary oscillations, whereas drugs that "switch off" the locus coeruleus decrease the levels of arousal, cause pupillary miosis in darkness, and increase the number of ensuing pupillary oscillations.[16] Therefore, it appears that the regulation of arousal and pupillary functions are intimately linked via activity in the locus coeruleus, a structure implicated in the regulation of attention and arousal,[17,18] as well as maintenance of wakefulness[19,20] and pupillary control.[21]

Technologic advances in the last decade in the area of computerized infrared pupillometry have allowed the accurate continuous monitoring and recording of pupil size and oscillations in darkness. Examination of a full, uninterrupted, 11-minute pupillometric record of healthy sleep-deprived subjects reveals that, as the subjects drift into sleepiness, their pupils become gradually miotic during the first 3 to 5 minutes, and, subsequently, the pupillary oscillations appear.[11,21] Although it would appear feasible to measure the degree of arousal of sleepy subjects by quantifying the gradual pupillary miosis in the first 3 to 5 minutes of recording, research has focused only on the quantification of the ensuing pupillary oscillations obtained with an 11-minute pupillometric recording in darkness.[22] Although this technique is more convenient than the MSLT, it is still rather complicated and thus far has been excluded from the routine evaluation of sleepiness. Limiting the measurements on the gradual pupillary miosis has, besides simplicity, 2 important advantages: firstly, methodologic problems due to signal transformations would be redundant, and, secondly, the test would be substantially shorter. i.e., 5 versus 11 minutes. This would increase the feasibility of the technique for application to patients with excessive daytime sleepiness, who often cannot stay awake with their eyes open for 11 minutes in the dark and unstimulating environment of the pupillometric chamber.

This is a preliminary study designed to test the validity of the gradual pupillary miosis during 5 minutes in darkness as a quantitative physiologic index of the arousal state of the brain. For this reason, we sampled the resting pupil diameter (RPD) of patients with OSA over a period of 5 minutes in darkness and compared the results with those of age-matched healthy controls. The predictions were that (a) the RPD would be smaller in the patients with OSA compared with healthy controls, (b) RPD would correlate inversely with objective (PSG) indexes of disease severity, and (c) RPD would correlate inversely with subjective measures of sleepiness within the patient group. We chose to use a subjective measure of trait (ESS) rather than acute sleepiness (e.g., the Stanford Sleepiness Scale) because the latter does not correlate with pupillary variables[11,23] or the MSLT,[8] probably due to lack of insight into one's own acute sleepiness state, a problem that correlates positively with the level of sleepiness in normal subjects[24] and is typical for narcoleptic patients.[25] In contrast, scoring of the ESS is much more stable and reliable, as indicated by its satisfactory test-retest reliability.[26] Moreover, the scale can discriminate very well between normal and pathologic sleepiness,[7,8] and it is widely used for the routine diagnostic assessment of excessive daytime sleepiness associated with OSA. Lastly, we were interested in exploring whether RPD correlates with trait aspects of sleepiness.

METHODS

Subjects

The study was approved by the Ethics Committee of the University of Crete, and all participants gave their written informed consent prior to screening. We restricted our sample to unmedicated patients (age range: 30-50 years) with a recent (< 10 days) diagnosis of OSA and with normal laboratory findings (see below) who were not yet under any type of treatment. Exclusion criteria were the presence of common comorbid conditions such as hypertension, cardiopulmonary disease, diabetes mellitus, and a body mass index (BMI) $\geq$ 32, which we regarded as extraneous sources of variance in the estimation of OSA severity. Following elimination of these extraneous sources of variance, we took provisions to include patients with a full range of disease severity (from mild to severe) in order to obtain the "true" disease-related variability and to maximize the chances of detecting correlations between RPD and indexes of disease severity. Additional exclusion criteria were unrelated active clinically significant disease, ocular conditions or operations, a history of drug abuse, a positive urine drug-screening test, excessive caffeine consumption (> 5 cups per day), or the use of any prescribed or over-the-counter medication that affects the autonomic nervous system. Eleven male patients were recruited from the Sleep Disorders Unit, Department of Thoracic Medicine, Medical School, University of Crete, on the basis of the above criteria.

Clinical assessment and testing of the patients included routine hematology (full blood count), blood chemistry (thyroid and liver function tests, urinalysis, and electrolytes) and urine drug-screen test. In addition, BMI was calculated; neck, waist, and hip circumferences were measured; and a 12-lead electrocardiogram was performed. Daytime sleepiness was assessed subjectively using the ESS.[7] Overnight PSG started at 10:30 pm and ended at 6:30 AM. Recordings were made with an Alice-4 18-channel polygraph (Alice-4 Respironics, Pittsburgh, PA) and included monitoring of electroencephalogram (C3/A2, C4/A1 and Cz/Oz, sampling rate: 100 Hz, band-pass filters 2-40 Hz, notch filter at 50 Hz), electrooculogram, genioglossus and anterior tibialis electromyograms, electrocardiogram, oxygen saturation, nasal airflow with nasal thermistors and nasal cannula pressure transducer, thoracic and abdominal movements, microphone snoring sounds, and body position (all signals were recorded according to the 10-20 international electrode placement system).[27] Sleep stages were scored visually,[27] and microarousals were defined according to standardized criteria.[28] Respiratory-event analysis and the apnea-hypopnea index were scored visually and calculated according to international criteria; hypopnea was defined as a reduction of thoracoabdominal effort of at least 50% with an associated oxygen desaturation of at least 4%; apnea was defined as a cessation of airflow at the nose and mouth lasting at least 10 seconds. These episodes were classified as obstructive on the basis of the presence of paradoxical movements of the rib cage and of the abdomen. The severity of OSA was established according to the American Sleep Disorders Association clinical and laboratory criteria[29] by an investigator (SS) who was blind to the results of pupillometric assessment (see below). Outcome measures were arousal index, which referred to the number of arousals per hour of sleep; the apnea-hypopnea index, which referred to the number of apneas and hypopneas per hour of sleep; the total sleep time; the amount of slow-wave sleep, rapid eye movement (REM)

sleep, and non-REM sleep (measured in actual time in minutes); sleep efficiency; and lowest oxygen saturation and desaturation. The patients' characteristics are shown in Table 1. Patients were instructed to maintain their normal patterns of caffeine and nicotine consumption the morning of the experimental testing.

Eleven healthy male controls matched precisely (1:1) for age were also included in the study, following the same routine hematologic and blood chemistry, an electrocardiogram, and a urine drug-screening test. Inclusion criteria for the healthy controls was informed consent, and exclusion criteria were presence or history of any sleep disorder based on a clinical interview and/or an ESS score > 5, hypertension or any active clinically significant disease, abnormal laboratory findings, obesity, ocular conditions or operations, a history of drug abuse or a positive urine drug-screening test, excessive caffeine consumption (> 5 cups per day), or the use of any prescribed or over-the-counter medication that affects the central and the autonomic nervous system. These subjects were instructed to maintain their usual sleep-wake schedules (subjective time in bed [mean ± SD]: 6.7 ± 0.9) for a few days before the study. The characteristics (mean ± SD) of the healthy control group were age 40.18 ± 5.72 years, which was identical to that of the patient group, a BMI of 27.2 ± 1.3 kg/m², and an ESS score of 2 ± 2. Independent samples t tests showed that the control group had a significantly lower BMI (t = 2.8; p < .01) and lower ESS scores (t = 3.95; p < .001), as compared with the patient group.

Tests and Apparatuses

Pupillometry

Pupillometry took place 1 to 3 days after the clinical, laboratory, and diagnostic PSG assessment. Because we were interested in the relationship between RPD and trait aspects of the disease, we did not consider it necessary to include a second PSG the night immediately preceding the pupillometric measurements. The pupillometric recordings took place in a dark, sound-attenuated room, following demonstration of equipment and instructions to subjects but without previous adaptation to the procedures, which might increase the soporific nature of the technique. A binocular infrared video pupillometer (PROCYON P2000D, Procyon, London, UK, sampling rate: 25 Hz, spatial resolution: >0.05 mm, accuracy > ± 3%) was used to monitor RPD in darkness. RPD was measured twice in the same day, once at 10:00 AM (RPD_{am}) and once at 2:00 PM (RPD_{pm}) to capture the reported circadian effect on pupil size[30] and a possible group-by-time of day interaction. Subjects sat on the pupillometry stool, and they had to lean forward to position themselves at the pupillometer, and, allowing for adjustments of the height of the pupillometry stool, this position was consistent between participants. Subjects were instructed to sit comfortably but stay still and look through the eye tubes of the pupillometer for 5 minutes. They were also advised to stay awake and resist sleep. Blinking was allowed, but subjects were reminded to do so as little as possible, with a standardized verbal instruction at the onset of each 20-second period, and this verbal instruction had the additional benefit of minimizing the soporific nature of the technique. The video image of the pupils provided instantaneous feedback for camera focus during recording. Pupil diameter was sampled for 15 consecutive 20-second periods, and, thus, the total monitoring time was 300 seconds. The outcome measures were the average RPDs for each 1 of the 15 twenty-second periods and the collapsed RPD for the entire 300-second re-

Table 1—Characteristics of 11 Men Diagnosed With Obstructive Sleep Apnea

Pt.	Age, y	Weight, kg	BMI, kg/m²	ESS score	AHI	AI	Severity
1	42	98	32.00	3	13	29	Mild
2	36	83	28.07	2	21	20	Mild
3	41	78	26.06	15	19	33	Moderate
4	38	90	28.68	5	40	20	Moderate
5	46	99	31.60	19	65	58	Severe
6	35	93	32.18	6	72	34	Severe
7	36	86	27.14	6	33	40	Severe
8	49	80	27.01	15	63	42	Severe
9	40	96	32.07	6	80	60	Severe
10	48	88	27.77	18	79	65	Severe
11	31	118	32.00	18	29	22	Severe
Mean	40.2	91.8	29.5	10	46.7	38.5	
SD	5.7	11.5	2.5	7	25.5	16.3	

Pt refers to patient; BMI, body mass index; ESS, Epworth Sleepiness Scale AHI, apnea-hypopnea index; AI, arousal index.

cording. Data were stored for off-line cleaning from spontaneous blinks, scoring, and statistical analysis. Cleaning from blinks and other artifacts (signal disruptions due to eye closures or out-of-focus camera) was performed via visual inspection of the records and removal of the artifacts using the manufacturer's software. Recording periods were excluded if excessive presence of artifacts was observed disrupting more than two thirds of a 20-second recording period (i.e., excessive blinking or eye closure for > 13 seconds) in both eyes. No recording periods were discarded based on these criteria.

Data Reduction and Analysis

The collapsed RPD data for the entire 300-second recording entered the statistical analysis. Because of the well-established inverse relationship between age and pupil size,[21] a mixed model, 2-way analysis of covariance with age as the covariate and with time of day (10:00 AM, 2:00 PM) as the within-subject factor and group (patients, healthy controls) as the between-subject factor was used to analyze the RPD data. A separate analysis of covariance including BMI as a second covariate was also performed. Kolmogorov-Smirnov tests of normality showed normal distribution of scores for all variables; therefore, Pearson correlations and multiple linear regression analyses were used, as appropriate, to examine the relationship between RPD_{am} and RPD_{pm} on the one hand and subjective (ESS) and objective (PSG) indexes of disease severity on the other. Separate partial correlations were used to control for the effect of BMI and neck, waist and hip circumference.

RESULTS

Comparison of Results From Patients and Controls

Figure 1 shows the RPD_{am} (10:00 AM) and the RPD_{pm} (2:00 PM) values as they were sampled for 15 consecutive 20-second periods, for the 2 groups (left panel) and the collapsed RPD_{am} and RPD_{pm} for the entire 300-second recording (right panel). The 2 × 2 (group × time-of-day) analysis of covariance of these data with age as the covariate showed a significant main effect of group

Table 2—Correlation Matrix between RPD and Subjective and Objective Indexes of OSA Severity in 11 Patients

	ESS	AI	AHI	LSatO$_2$	DeSatO$_2$	TST	REM	NREM	SWS	SE
RPDAM	-.53	-.66[a]	-.46	.55[b]	-.44	-.35	-.28	-.36	-.25	-.30
RPDPM	-.63[a]	-.67[a]	-.49	.70[a]	-.46	-.47	-.43	-.46	-.24	-.30
After partialling out the effects of BMI, neck, waist and hip circumference										
RPDAM	-.48	-.78[a]	-.71[b]	.77[a]	-.63	-.61	-.16	-.63	.07	-.62
RPDPM	-.62	-.80[a]	-.74[b]	.91[a]	-.65	-.74[b]	-.40	-.74[b]	.05	-.63

RPD refers to resting pupil diameter; ESS, Epworth Sleepiness Scale; AI, arousal index; AHI, apnea-hypopnea index; LSatO$_2$, lowest oxygen saturation; DeSatO$_2$, desaturation; BMI, body mass index; TST, total sleep time; REM, rapid eye movement; NREM, non-rapid eye movement; SWS, slow-wave sleep.
Values represent Pearson correlation coefficients: [a]$p < .05$, [b]$p < .08$

($F_{1,19}$ = 4.62; $p < .05$) and a significant group × time-of-day interaction ($F_{1,19}$ = 5.83; $p < .05$). When BMI was also taken as the covariate, the group main effect remained ($F_{1,18}$ = 4.79; $p < .05$), but the interaction effect was no longer significant ($F_{1,18}$ = 3.47; $p = .08$).

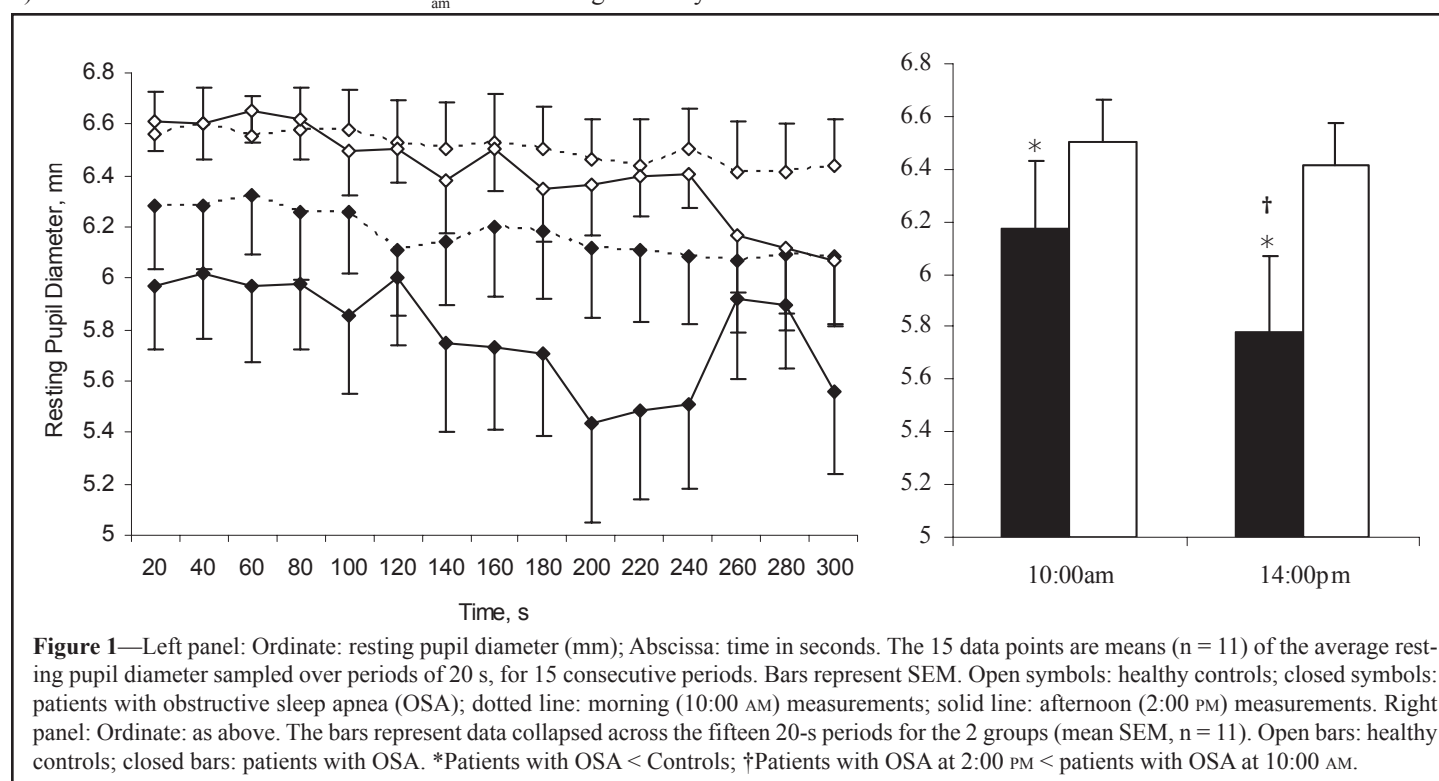### Relationship of RPD to Indexes of Disease Severity, Within the Patient Group

The Pearson correlation coefficients between RPD on the one hand and ESS scores and PSG indexes on the other are shown in Table 2. The RPD$_{pm}$ correlated negatively with the ESS score and arousal index and positively with the lowest oxygen saturation of the overnight PSG recording. When the effect of BMI and indexes of body fat were partialled out, however, the relationship of RPD and ESS scores was lost, whereas the relationship with most of the objective PSG indexes of disease severity improved (see Table 2). This table also shows that the RPD$_{am}$ correlated significantly with only the arousal index, but partialling out the effects of body fat improved the relationship with several other PSG indexes. Although the PSG indexes were highly intercorrelated (data not shown), only the lowest oxygen saturation correlated negatively with ESS scores (r = -0.66, df:11, $p < .028$).

Because RPD$_{pm}$ correlated better than did RPD$_{am}$ with ESS scores and PSG indexes of disease severity, we attempted to determine which variables predicted the greatest amount of variance of the RPD$_{pm}$ scores using linear multiple regression analysis, with RPD$_{pm}$ as the dependent variable and ESS scores and all of the PSG indexes as the independent variables. Entering all the above independent variables revealed a nonsignificant model ($F_{1,9}$ = 2.42, $p > .1$), but a backward regression analysis revealed that 86.3% (adjusted R^2 = 0.726) of the variance of the RPD$_{pm}$ was significantly ($F_{5,5}$ = 6.3, $p < .05$) predicted by arousal index (β = -1.1 ± 0.016, t = -3.92, $p = .011$; partial correlation r = -0.87), ESS scores (β = -0.96 ± 0.042, t = -3.24, $p = .023$; partial correlation r = -0.82), total sleep time (β = 0.95 ± 0.011, t = 2.69, $p = .043$; partial correlation r = 0.77), and slow-wave sleep (β = 0.86 ± 0.055, t = 2.88, $p = 0.034$; partial correlation r = 0.7) together.

Because of the high intercorrelation between the PSG variables, we repeated the regression with only the key PSG variables AHI and lowest oxygen saturation and also including ESS, age, and BMI as the independent variables. Entering these independent variables revealed a significant model ($F_{5,5}$ = 5.55, $p < .05$) that explained 84.7% (adjusted R^2 = 0.695) of the variance of the RPD$_{pm}$. A backward regression analysis revealed that 83.9% (adjusted R^2 = 0.769) of the variance of the RPD$_{pm}$ was significantly ($F_{3,7}$ = 12.13, $p < .004$) predicted by age, BMI, and lowest oxygen saturation, but only age and oxygen saturation were significant predictors (β = -.54, t = -2.86 $p = .024$; partial correlation r = -0.73 and β = 0.45, t = 2.47, $p = .043$; partial correlation r = 0.68, respectively). Age and lowest oxygen saturation together accounted for 78.4% of the variance.

Figure 1—Left panel: Ordinate: resting pupil diameter (mm); Abscissa: time in seconds. The 15 data points are means (n = 11) of the average resting pupil diameter sampled over periods of 20 s, for 15 consecutive periods. Bars represent SEM. Open symbols: healthy controls; closed symbols: patients with obstructive sleep apnea (OSA); dotted line: morning (10:00 AM) measurements; solid line: afternoon (2:00 PM) measurements. Right panel: Ordinate: as above. The bars represent data collapsed across the fifteen 20-s periods for the 2 groups (mean SEM, n = 11). Open bars: healthy controls; closed bars: patients with OSA. *Patients with OSA < Controls; †Patients with OSA at 2:00 PM < patients with OSA at 10:00 AM.

DISCUSSION

Comparison of Results From Patients With Results From Controls

Tonic levels of pupil diameter were already lower from the first 20-second period in our group of patients with OSA, as compared with the age-matched group of healthy controls, and remained so at all times, as suggested by the significant group effect; this suggests lower tonic arousal levels in the patients. The significant group × time-of-day interaction indicates that pupil size decreased significantly in the afternoon, compared with in the morning, only in the patient group. Indeed, pupil diameter was becoming progressively smaller from the first to the 15th period, and this was much more pronounced in the afternoon measurements for the patient group (see Figure 1 left). A midafternoon circadian nadir in the levels of alertness in both patient and control groups has been previously demonstrated with MSLT,[31] and such a circadian effect is the likely cause underlying our observation in the patient group. It is noticeable that a similar circadian effect in pupil size was not evident in our control group, in contrast with a previous report by Merritt et al,[30] who found a significantly greater decrease in pupil size at 2:00 PM, compared with 10:00 AM, in a group of healthy subjects. The most likely reason for this discrepancy is that we refer to cumulative pupil size after recording for 5 minutes only, whereas Merritt et al refer to cumulative pupil size from the sixth to the 11th minute of a pupillometric record, discarding the first 5 minutes of recording.[30] It is quite possible that, in contrast with our group of patients with OSA, our healthy nonsleepy subjects maintained steady levels of alertness during the 5 minutes of recording, and, had we monitored pupil size for 11 minutes, we could have demonstrated a small circadian effect in the pupils of our control group. We followed up this hypothesis with a small methodologic study (data not shown but available on request) on 12 healthy subjects of the same mean age as that of our OSA group. We found that RPDs in the first and last 5 minutes of recording were highly correlated in both the morning and the afternoon sessions and that RPD in the afternoon but not in the morning session was significantly smaller in the last compared with the first 5 minutes of recording, suggesting indeed the presence of a circadian effect with more-prolonged recording. All of the above, taken together, support the validity of this test, show that it can capture tonic arousal levels, and, in the patient group at least, demonstrate the sensitivity of RPD to circadian changes in the levels of alertness that parallel previous MSLT findings. Although 5 minutes of recording may not be sensitive to a circadian effect in healthy subjects, our findings still indicate that recording RPD over the first 5 minutes is sufficient to distinguish between patients with excessive daytime sleepiness associated with OSA and controls, especially when the test is performed at the midafternoon circadian nadir.

Relationship of RPD With Indexes of Disease Severity, Within the Patient Group

The correlations shown in Table 2 suggest that (a) the greater the amount of arousal during sleep, as indicated by the high arousal index, the smaller the pupils of the patients in the morning and in the afternoon and (b) patients with smaller pupils in the afternoon report greater subjective daytime sleepiness and have greater amounts of arousal and lower oxygen saturations during sleep. After we controlled for the effects of BMI and neck, hip,

and waist circumference, the relationship between pupil diameter and subjective sleepiness was lost, whereas the relationship with most of the objective indexes of OSA severity was improved (see Table 2). These results suggest that body fat tissue alone is an important determinant of subjective daytime sleepiness, independent of the presence and the severity of sleep apnea, which is consistent with previous findings[1,32-34] in obese patients. Considering that 1 of our selection criteria was a relatively low level of obesity (BMI < 32), these results demonstrate the sensitivity of pupillometry. It is difficult to know whether obesity has a direct effect on RPD or whether its effect on RPD is mediated through a reduction in arousal (alertness) levels. When BMI was included as a covariate in the analysis of covariance, the group main effect remained, suggesting that BMI cannot account for the reduced tonic levels of pupil diameter and, by extension, for the reduced tonic levels of arousal in the OSA group. However, the significant group × time-of-day interaction was lost, suggesting that BMI may account, to some extent, for the observed circadian reduction in arousal at 2:00 PM in the OSA group. It has to be mentioned, however, that there was still a tendency for a significant interaction effect (p = .08) and that this interaction might have remained significant had our sample size been larger. On the other hand, there is indirect evidence suggesting that obesity itself may have an effect on pupil size: the number of postsynaptic α_2 adrenoceptors in obese rats are reduced[35] and the (α_2-mediated) growth hormone response to clonidine is reduced in obese individuals,[36] suggesting the possibility of an underresponsive central α_2 adrenoceptor system in obesity. This could lead to smaller pupils, considering that the tonic inhibitory effect of the locus coeruleus on the pupilloconstrictor Edinger-Westphal nucleus is mediated via postsynaptic α_2 adrenoceptors.[13,15] Further research is required on this issue, investigating the RPDs of obese subjects with and without OSA and/or excessive daytime sleepiness.

The multiple regression analysis meaningfully showed that, the greater the subjective sleepiness (as indicated by high ESS scores) and the worse the quality and amount of sleep (as indicated by the high arousal index and the smaller amount of total sleep time and slow-wave sleep in the subjects), the smaller the pupil diameter of the subjects in the afternoon. These are noteworthy findings in light of the failure of the MSLT[8] to correlate with subjective measures of sleepiness or with the frequency of respiratory disturbances during sleep.[9] Restricting the independent PSG variables to only the apnea-hypopnea index and the lowest oxygen saturation and also including ESS scores, age, and BMI in the regression model revealed that, regardless of the number of respiratory events (as assessed by the apnea-hypopnea index), the greater the age of the patients and the lower their lowest oxygen saturation, the smaller were their pupils. The inverse relationship between age and pupil size in healthy individuals is strong and well known,[21] and it appears that it holds true for patients with OSA as well. However, the presence of this strong relationship did not prevent an association between pupil diameter and lowest oxygen saturation to reveal itself. The likely explanation for the age-related decline in pupil size is that it is due to an age-related reduction in neuronal numbers in the nucleus locus coeruleus,[37,38] the main source of tonic inhibition of the pupilloconstrictor Edinger-Westphal nucleus.[13-15,21] Indeed, the pupils of patients with a very recent diagnosis of Alzheimer's disease are reduced over and above of what is expected by age alone,[39] consistent with the disease-related accelerated global neuronal loss, which is already

profound in the locus coeruleus in the early stages of the disease.[40,41] Equally, it is quite conceivable that hypoxia in OSA may cause a reduction in central arousal levels and in pupil size alike, through a global reduction of neuronal function. It is interesting in this respect that among all PSG variables, only the lowest oxygen saturation correlated both with RPD and with ESS scores.

The cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are elevated in OSA, and their plasma levels correlate positively with measures of excessive daytime sleepiness, such as nocturnal sleep disturbance and hypoxia, as well as with BMI.[42] Other studies have shown that TNF-α and IL-6 are elevated in OSA independent of obesity[32,43] and that BMI positively correlates with TNFα and IL-6 levels, suggesting that these 2 cytokines may play a role in daytime sleepiness experienced by obese individuals in the absence of OSA.[43] All of our results taken together suggest the intriguing possibility that reduced levels of pupil diameter in OSA reflect reduced arousal levels in this condition, which may be mediated, at least in part, via the inhibitory effects of these cytokines on the arousal-regulating noradrenergic nucleus locus coeruleus. Indeed, TNF-α has been shown to reduce noradrenaline levels within the locus coeruleus[44] and also to inhibit noradrenaline release from axon terminals in the rat.[45]

The gradual pupillary miosis within the first few minutes in a dark and quiet environment reflects the gradual reduction in the central arousal state of the brain and is due to an intimate physiologic link between regulation of arousal and pupil size.[13,22] In this study, we used a single measure, the average RPS, over 5 minutes of recording in darkness as an immediate quantitative measure of arousal. This test yielded lower tonic levels of pupil diameter in patients with OSA, compared with sex- and age-matched controls, and this suggests lower tonic levels of arousal in the patients. The differences in tonic arousal levels became more apparent in the afternoon circadian nadir; furthermore, although pupillometry did not always take place immediately following the diagnostic PSG, pupil size correlated meaningfully with objective indexes of disease severity and subjective trait sleepiness within the patient group. The above, taken together, suggest that (a) the 5-minute pupil test captures tonic arousal levels, (b) a reduced level of tonic arousal may be related to OSA severity, and (c) a reduced level of tonic arousal may be related to a high level of trait subjective sleepiness. This is in line with recent models that emphasize the importance of arousal in the expression of sleepiness.[45] Our results link well with a recent report showing that pupil diameter in groups of subjects with different levels of partial sleep deprivation and mean and median ages similar to those of our OSA patient group correlated inversely with subjective sleepiness and performance errors in simulated driving.[46] Although the findings of the present study will need to be independently replicated, they do suggest that this test has very good face validity and it is feasible to perform in patients with excessive daytime sleepiness, as none of the patients found the procedure difficult to tolerate and none of them fell asleep.

The clearest limitations of this preliminary study are the small number of subjects and the fact that the controls were not matched for BMI, whereas the correlation of the 5-minute pupil test with MSLT and subjective measures of acute sleepiness has yet to be demonstrated. Future research directions include larger groups of patients and sleep-deprived healthy controls at different time points in the same day, using additional subjective measures of sleepiness (e.g., the Stanford Sleepiness Scale) and in parallel with the MSLT. If monitoring of resting pupil size in darkness is to be more regularly incorporated in future studies, it is important to optimize and standardize the technique and to have normative data across different age groups, controlled at least for BMI. This would allow for the categorization of individuals in terms of pupil size in an epidemiologically meaningful way. This physiologic test reflects different aspects of central arousal than does the MSLT and may be complementary to the latter, is inexpensive, quick to administer, easy to repeat, and straightforward to score with no signal transformations and no mathematical algorithms required and, therefore, holds promise as a suitable clinical tool for the objective assessment of excessive daytime sleepiness and its severity, disease monitoring, and response to treatment.

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