Proton Magnetic Resonance Spectroscopy Study of Brain Metabolism in Obstructive Sleep Apnoea Syndrome before and after Continuous Positive Airway Pressure Treatment.

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Study Objectives: Obstructive sleep apnoea syndrome (OSAS) causes sleep related oxygen desaturation, excessive daytime sleepiness (EDS), and cognitive impairment. The role of hypoxic brain damage, sleep fragmentation, and the associated comorbidities (hypertension, vascular disorders) in the pathogenesis of cognitive deficits remains controversial. The aim of this study was to evaluate the cerebral metabolism of OSAS patients in vivo before and after CPAP treatment.

Design and Patients: Fourteen OSAS patients without cardiovascular or cerebrovascular impairment underwent the same protocol before and after 6 months of CPAP including: overnight videopolysomnography (VPSG), Multiple Sleep Latency Test (MSLT), and within the next 2 days neuropsychological and ¹H-MRS evaluations. Single voxel ¹H-MRS was performed in the parietal-occipital cortex, and absolute concentrations of N-acetyl-aspartate (NAA), creatine, and choline were measured, acquiring spectra at multiple echo-times and using water as internal standard. Ten matched controls were also studied.

Results: OSAS patients had a mean RDI of 58/hr, a mean arousal index of 57/hr, and a mean nadir SpO₂ of 71%. Before CPAP, all patients showed a normal global cognitive functioning, with only a small number of pathological tasks in working memory and attention tests in a minority of patients.

CPAP therapy was effective in resolving sleep apnoea and normalizing sleep structure, and improving EDS and neuropsychological alterations. Before CPAP treatment cortical [NAA] in OSAS (11.86 mM±0.80, mean±SD) was significantly lower than in controls (12.85±0.93; P = 0.01) and positively correlated with minimum SpO₂ during sleep (r = 0.69; P = 0.006) and MSLT scores (r = 0.62; P = 0.01). Cortical [NAA] reduction persisted after therapy (11.94±1.33; P = 0.87 versus pre-CPAP).

Conclusions: OSAS patients have cortical metabolic changes consistent with neuronal loss even in the absence of vascular comorbidities. Metabolic changes persisted after CPAP in the absence of EDS, nocturnal arousals, and major cognitive deficits, likely related to hypoxic damage prior to CPAP treatment.

Keywords: Obstructive sleep apnoea syndrome, proton magnetic resonance spectroscopy, N-acetyl-aspartate, nasal continuous positive airway pressure, polysomnography, chronic hypoxia, follow-up.

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INTRODUCTION

OBSTRUCTIVE SLEEP APNOEA SYNDROME (OSAS) IS ESTIMATED TO AFFECT 2%–4% OF THE MIDDLE-AGED NORTH AMERICAN POPULATION¹ AND 11% OF THE elderly.² It is characterized by repetitive complete (apnea) or partial (hypopnea) upper airway obstructions during sleep, resulting in oxygen desaturation and terminating in arousals disrupting normal ventilation and sleep architecture.³ OSAS represents an independent risk for complications such as systemic hypertension, ischaemic heart disease, left heart failure, cardiac arrhythmias, and stroke.⁴

OSAS causes excessive daytime sleepiness (EDS), cognitive dysfunction, and mood disorders, all affecting quality of life. Impaired vigilance, attention, concentration, short- and long-term

memory, executive and motor functions have been documented in OSAS.^{5,6} However, conflicting findings emerge from a meta-analysis on the neuropsychological effects of obstructive sleep apnea in untreated patients. General intelligence, including basic verbal and visual-perceptual abilities were unaffected, whereas vigilance, executive functions, and motor coordination were moderately to markedly impaired. The effect of OSA on visual skills, motor skills, and memory functioning was inconsistent.⁶

Continuous positive airway pressure (CPAP) applied through a nasal mask is the first line of treatment in OSAS. By preventing upper airway collapse, CPAP normalizes sleep patterns and EDS and improves quality of life.^{7,8} The positive impact of CPAP has mainly been detected on vigilance, attention, and memory deficits that are likely related to sleep fragmentation.^{8,9} Other neuropsychological changes, especially in the executive, motor, and visuoconstructive domains, tend to persist despite CPAP treatment, probably due to irreversible hypoxemic damage.^{8,9}

Neuropsychological and functional imaging studies addressing the problem of cognitive deterioration in OSAS have rarely considered the impact of comorbidities such as hypertension, vascular disease, and diabetes mellitus in determining neurological dysfunction. This has often led to inconsistent and controversial results.

Proton magnetic resonance spectroscopy (¹H-MRS) is a sensitive, non-invasive technique that assesses and monitors biochemical changes in brain areas of interest. This technique allows direct quantification of brain metabolites, including N-acetylaspartate (NAA). Most studies have indicated that NAA is a putative neu-

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ronal marker¹⁰ located in neurons and neuronal processes. Reduced NAA concentration is typically seen in neurodegenerative, inflammatory, or vascular disorders.¹⁰ The partial reversibility of NAA deficit after therapy¹¹ or during recovery from acute brain pathology¹² indicates that reduced brain NAA may be not only related to neuronal loss, but also to neuronal dysfunction.

¹H-MRS studies on OSAS patients have assessed cerebral metabolism in different brain regions, yielding inconsistent results. A significant decrease in the NAA/Cho ratio associated with slight decrease in NAA/Cr, and increase in Cho/Cr was found in the posterior periventricular white matter.¹³ However, a later study detected a significant reduction in both NAA/Cr and Cho/Cr in the frontal white matter.¹⁴ Investigation of the left hippocampus revealed increased NAA/Cr, suggesting reduced creatine in OSAS patients.¹⁵ The inconsistent results of these studies may be related to both MRS methodological differences and the clinical heterogeneity of the patient population recruited.

We used ¹H-MRS to evaluate brain metabolism in OSAS patients strictly selected for the absence of cardiovascular and cerebrovascular comorbidities. Absence of comorbidities was required to avoid potential confounding factors and to evaluate only brain metabolic features directly resulting from recurring apnoea/hypopnea during sleep.

The MRS acquisition protocol was designed to assess brain concentrations of NAA, choline, and creatine¹⁶ to avoid uncertainties in interpreting metabolite ratios, namely possible changes in creatine concentration.¹⁵ The same study protocol applied before and after 6 months of CPAP included videopolysomnography (VPSG) with Multiple Sleep Latency Test (MSLT), neuropsychological testing, and ¹H-MRS assessment.

METHODS

Subjects

Fourteen OSAS patients with mean body mass index (BMI) of 32.3±4.6 and EDS, as assessed by means of non-structured clinical interview, were recruited from the Sleep Disorders Centre of the Dipartimento di Scienze Neurologiche of the University of Bologna, Italy. To qualify for the study, subjects had to have an EDS (Epworth Sleepiness Scale, ESS) score ≥10; normal wake and sleep systolic and diastolic blood pressure; no diabetes mellitus or hyperlipidaemia; no cardiovascular disorders; and no history or current evidence of drug abuse or neurological disease. Patients with abnormalities on neurological examination or structural brain lesions on brain CT and conventional MRI were excluded. Patients were asked to refrain from drugs throughout the study and were drug free at the time of examination. Ten age- and sex-matched healthy controls were recruited on the basis of the same exclusion criteria used for the patients. Healthy subjects had mean BMI 25±2, range 22-28; mean diastolic (DBP) 72±4, range 60-82; systolic blood pressure (SBP) 120±4, range 105-133; normal ESS scores; and normal brain MRI.

The approval from the Policlinico S.Orsola Hospital Ethics Committee and written informed consent from each participant were obtained.

Study Design

All OSAS patients completed a standardized study protocol including: a diagnostic overnight VPSG with MSLT the follow-

ing day; neuropsychological tests (performed between 15:00 to 18:00) and ¹H-MRS (performed between 14:00 to 16:00) evaluations within the following 2 days; VPSG with CPAP titration; and subsequent MSLT within one week. The same protocol study was performed again after 6 months of CPAP treatment.

Sleep Study

Nocturnal VPSG Procedures

A diagnostic overnight VPSG was carried out in all OSAS patients, after an adaptation night in the sleep laboratory. VPSG with CPAP titration was then performed, and patients were prescribed CPAP. Patients were reevaluated with VPSG 6 months later, and compliance with prescribed CPAP treatment was evaluated by means of self-reported use, expressed as the average time with CPAP in relation to total night time spent in bed, assessed with a patient diary.

VPSG data were analysed in 30-s epochs according to Rechtschaffen and Kales,¹⁷ and sleep structure and sleep efficiency (percentage of total sleep period spent asleep) were calculated. Snoring was evaluated by means of tracheal microphone. An abnormal breathing event during sleep was defined as apnoea (a complete cessation of airflow lasting ≥10 seconds) or hypopnoea (a discernible reduction of airflow accompanied by a decrease of at least 4% in oxyhaemoglobin saturation (SpO₂)).¹⁸⁻²⁰ Obstruction was confirmed by respiratory effort recorded by thoracic-abdominal strain gauge, increased negative endoesophageal pressure by intraesophageal balloon, and intercostalis electromyogram. The average number of apnoea-hypopnoea episodes per hour of sleep was calculated as the respiratory disturbance index (RDI). Transient arousals were scored as visible EEG arousals lasting ≥2 seconds and not associated with any stage/state change in the 30-s epoch scoring.²¹ The arousal index (AI) (number of arousals per hour of sleep) was calculated. Mean SpO₂ value, mean diastolic (DBP) and systolic blood pressure (SBP), and heart rate (HR), and their minimum and maximum values were calculated in relation to total sleep period (TS) for each patient.

Objective and Subjective Sleepiness Assessment

Diagnostic overnight VPSG, CPAP titration, VPSG, and CPAP retitration VPSG after 6 months of CPAP treatment were all followed by an MSLT to evaluate objective sleepiness.²² Each MSLT (5 sessions) was performed beginning not earlier than 2 hours after morning awakening.

Before diagnostic VPSG and after 6 months of CPAP treatment the Epworth Sleepiness Scale (ESS)²³ for measuring subjective daytime sleepiness was administered to each patient.

Neuropsychological Evaluation

At baseline before and 6 months after CPAP all patients were administered a battery of neuropsychological tests assessing: 1) global cognition by Mini Mental Status Examination (MMSE),²⁴ corrected for age and education according to Italian standardization (cut-off: 23.8)²⁵ and the Brief Mental Deterioration Battery (BMDB) with its Final Result (FR)^{26,27} (normal values above 0); 2) memory by 15 Rey's words with immediate and delayed recall (verbal learning of word lists and delayed free recall), immediate visual memory (visual recognition), digit span forward (immediate verbal memory) and backwards (working memory)

Figure 1—A) Localization of medial brain parietal-occipital grey matter volume of interest (VOI). B) Proton MR spectra from an OSAS patient (case no. 4) compared to a sex- and age-matched control (TE= 35ms; TR= 4000ms). The NAA (N-acetyl-aspartate) peak is lower in the patient.

task), Corsi's cube span (immediate visuo-spatial memory); 3) executive functioning by phonemic fluency (verbal production ability), analogies (logical-deductive ability), Weigl's sorting test (categorical thinking); 4) constructional praxis by copy design (visuo-construction ability); 5) attention via attention barrage by visual selective attention task and 6) psychomotor performance by computerized auditory and visual simple and complex reaction times (vigilance and selective attention tasks), finger tapping test (manual dexterity), "watch test" (sustained attention). These tests were chosen because they explore the cognitive domains previously assessed in the literature in OSAS patients.⁶ The same tests were standardized in the Italian population and in our own normal control samples^{26,27} and have been adopted in other sleep dysfunction studies.²⁸

¹H-MRS Protocol

Brain MR imaging and spectroscopy studies were carried out on a 1.5 Tesla clinical whole body magnet (General Electric Medical Systems, Milwaukee, WI, USA) with a quadrature birdcage headcoil (25 cm of diameter), at S. Orsola-Malpighi Hospital, Bologna. ¹H-MRS was conducted from 14:00 to 16:00 in all patients. The MRI protocol consisted of axial T1-weighted Spin-Echo (SE) images (Echo Time, TE=14 ms; Repetition Time, TR=500 ms; FOV=24 cm; 20 slices of 4 mm of thickness; gap=1mm) and axial fluid-attenuated inversion recovery (FLAIR) (TE=97ms, TR=8000ms, FOV=24 cm, 20 slices of 5 mm of thickness, gap=1 mm). Single voxel spectroscopy was performed with the point resolved spectroscopy sequence (PRESS). A volume of interest (VOI) of 18 cm³ was placed in the brain medial parietal-occipital grey matter (Figure 1A). The VOI included both parietal and occipital cortex. The occipital cortex was included for 2 reasons: a) there is some evidence that there is a reduction in thickness in the occipital cortex of OSAS patients;²⁹ b) to obtain a good quality MRS data set with a scan protocol duration below 1 hour (i.e. high signal to noise ratio), we selected a relatively large VOI, and part of the occipital cortex had to be included in the VOI.

The water signal was suppressed by the CHESS (Chemical Shift Selective) sequence. Absolute quantification was made by applying the method developed by the "EEC Concerted Action

BIOMED I programme".¹⁶ This method uses water as internal standard and involves the acquisition of spectra at long TRs for both metabolites and water (to separate brain from cerebrospinal water).^{16,30} Absolute concentrations of NAA, creatine-phosphocreatine (Cr), and choline (Cho) were measured by acquiring spectra at 5 echo times (TE = 35, 70, 100, 144, 288 ms; TR = 4 s; number of acquisitions = 32). Brain water content from the selected VOI was obtained by acquiring single spectra, without water suppression, at TE=20, 30, 40, 50, 60, 80, 100, 300, 600, 900, 1000 ms (TR=15 s). The water peak area was calculated for each spectrum with Fourier transform and the Lorentzian fit (Levenberg-Marquardt) method. Data were processed with a bi-exponential fit to separate brain from cerebrospinal water to extrapolate the value of cerebral water signal intensity at TE = 0. Peak areas for NAA at 2.02 ppm, for Cr at 3.03 ppm, and for Cho at 3.22 ppm were calculated using the time domain fitting program AMARES/MRUI (<http://carbon.uab.es/mrui>), as previously described.³¹ Spectroscopic analyses were performed by one spectroscopist (R. L., 15 years' experience) who was blinded to the subject's condition.

Statistical Analysis

For the ¹H-MRS data, Student's unpaired t-test was used to compare OSAS patients at baseline and healthy controls. The OSAS patients before and after CPAP were compared using Student's paired t-test. Linear regression analysis was used to calculate correlation coefficients. Statistical significance was taken as P < 0.05. For the neuropsychological data the nonparametric test for 2 dependent samples of Wilcoxon with 0.01 level of significance was used.

RESULTS

Sleep Studies

Demographic and clinical/PSG characteristics of OSAS patients are shown in tables 1-2. At baseline all OSAS patients had an ESS score ≥ 10 . Mean wake SBP/DBP and HR (average of 3 consecutive determinations at 10-minute intervals) were 130.7/84.4

Table 1—Main demographic and clinical data from OSAS patients.

Case n°/Sex	Age	BMI	Snoring duration (yrs)	Sleep apnoea duration (yrs)	EDS duration (yrs)	MMSE	BMDB
1/M	54	33.5	34	10	2	27.97	2.35
2/M	51	32.5	35	3	3	29.97	3.01
3/M	54	25.8	15	9	2	29.97	1.54
4/M	57	31.8	10	4	2	28.97	2.52
5/M	44	32.7	30	10	1	28.31	2.92
6/M	46	33.4	30	12	2	29.62	2.58
7/M	50	37.3	32	1	1	28.99	2.37
8/M	45	29.6	15	4	4	28.21	2.16
9/M	57	27.8	40	2	2	28.74	1.86
10/M	54	27.1	34	12	3	28.97	2.38
11/M	51	40.6	20	2	2	25.97	2.13
12/M	43	31.4	25	3	2	28.62	1.64
13/M	35	40.5	10	3	5	27.10	2.43
14/M	37	28.0	10	7	4	27.75	2.68
Mean	48	32.3	24	6	3	28.51	2.32
±SD	±7	±4.6	±11	±4	±1	±1.09	±0.43

BMI= body mass index, EDS=excessive daytime sleepiness, MMSE= mini mental status examination, BMDB: Final result (FR) of Brief Mental Deterioration Battery

mm Hg and 71.8/m. All patients presented severe sleep fragmentation with a mean AI of 57 (range 12-81), mean RDI of 58 (range 11-90), mean SpO₂ of 86±4 % (range 77±7 – 90±2), mean nadir SpO₂ of 71 % (range 46-88), and oscillations in HR (mean: 64 ± 8 beats/min, range 40-120) and in arterial pressure (mean DBP: 72 ± 12 mm Hg, range 46-122; mean SBP: 122±18.3 mmHg, range 77-192). At MSLT all patients had mean sleep latency values <10 minutes and sleep onset REM periods (SOREMPs) were seen in 4 patients; one patient had 1 SOREMP, two patients had 2 SOREMPs, and another had 3 SOREMPs (Table 2).

CPAP titration prevented apnoeas, hypopnoeas, and oxyhemoglobin desaturation, and normalized respiratory related arousals

and cardiovascular oscillations at a mean nCPAP level of 11 cm H₂O (range 8-13). Average sleep latency values at MSLT were higher than baseline, with SOREMP in only one patient.

At CPAP retitration performed after a mean period of 6 months (self-reported estimated use of CPAP >80% of total night time), sleep structure and respiratory effort related indices were normal. Mean AI was 5±3 (range: 2-9), RDI 1.3±3 (range: 0-3) and SpO₂ 96±1, with fewer cardiovascular oscillations than during diagnostic VPSG (mean HR: 61±4 beat/min, range 53-69; mean DBP: 72±5 mmHg, range 62-83; mean SBP: 121±7 mmHg, range 108-136). Mean ESS was 5±2 (range: 2-9), mean MSLT was 12 min 8 s±4 min 40 s (range: 4 min 54 s to 20 min). All parameters reported showed an improvement compared with pre-CPAP values in all cases (P <0.001).

No patient complained of tiredness or drowsiness, as assessed by means of nonstructured clinical interview, and ESS global score was ≤9, (mean: 5; range: 2-9) with a mean BMI of 31±5 (not significantly different from pre-CPAP treatment BMI) and mean wake SBP/DBP and HR of 123/81 mm Hg and 69/m.

Neuropsychological Evaluation

At baseline, all patients had normal general cognitive indexes. When considering the number of pathological neuropsychological tasks (PNT), baseline evaluation detected a small number of PNT (2.0%) in 4 patients in tasks exploring immediate verbal and spatial memory and auditory and visual attention tasks; one patient had 3 PNT and one patient 2 PNT. Six months after CPAP, the total number of PNT decreased to 1.1%; patients with PNT were still 4 (two the same as baseline), all with one PNT (almost the same as at baseline) (Table 3). Comparison of the entire OSAS group in the first versus the second evaluations showed significantly improved 15 Rey's words with immediate recall (P = 0.008) and FR of BMDB (P = 0.01). The same differences were detected when excluding the patients with less severe (<30) RDI.

Table 2—Main baseline and post-CPAP PSG findings from OSAS patients.

Case n°	RDI	PSG SpO ₂ mean (%)	Baseline			ESS score	Post-CPAP		
			PSG SpO ₂ minimum (%)	MSLT (minutes)			CPAP level-mean SpO ₂	MSLT (minutes)	ESS Score
1	62	77±7 (56-92)	56	3 min 12 s + 1 SOREMP	13		8 cm H ₂ O - 95%	16 min 30 s	4
2	82	89±3 (56-94)	56	2 min + 2 SOREMPs	10		10 cm H ₂ O - 96%	20 min	5
3	43	89±3 (79-94)	79	9 min 30 s	12		8.5 cm H ₂ O - 95%	18 min 45 s	5
4	53	87±4 (64-93)	64	4 min 37 s	13		8.5 cm H ₂ O - 96%	9 min	6
5	75	83±5 (69-94)	69	5 min 54 sec + 3 SOREMPs	13		9 cm H ₂ O - 95%	17 min 11 s	3
6	80	80±7 (46-95)	46	1 min 15 s	14		9 cm H ₂ O - 95%	11 min	2
7	90	89±2 (80-94)	80	4 min 45 s	12		11 cm H ₂ O - 95%	6 min 37 s	5
8	11	90±1 (88-92)	88	8 min 6 s + 2 SOREMPs	11		10 cm H ₂ O - 96%	8 min + 1 SOREMP	5
9	68	87±4 (76-94)	76	8 min 30 s	13		15 cm H ₂ O - 95%	15 min 30 s	9
10	70	84±5 (66-94)	66	3 min 52 s	13		9.5 cm H ₂ O - 95%	11 min	4
11	29	90±2 (82-94)	82	3 min 42 s	12		12 cm H ₂ O - 96 %	8 min 24 s	8
12	17	88±3 (80-94)	80	7 min 45 s	13		10 cm H ₂ O - 96 %	10 min	4
13	63	88±6 (80-94)	80	9 min 30 s	18		10 cm H ₂ O - 96 %	13 min	5
14	68	84±5 (72-92)	72	4 min 30 s	14		9 cm H ₂ O - 96 %	4 min 54 s	5
Mean ± SD	58±24	86±4	71±12	3 min 31 s±2 min 44 s	13±2			12 min 8 s±4 min 40 s	5±2

RDI=respiratory disturbance index, PSG SpO₂ = PSG mean nocturnal oxyhaemoglobin saturation, MSLT= Multiple sleep latency test, SOREMP= sleep onset REM periods, ESS= Epworth Sleepiness Scale.

¹H-MRS Study

Figure 1B displays representative spectra from an OSAS (case n. 4) and a control subject showing reduced NAA in the patient. Baseline cortical NAA concentration was significantly lower in OSAS patients as a group compared with controls ($P = 0.01$), whereas no significant difference was found in the Cr and Cho concentrations (Table 4, Fig. 2). In OSAS patients the cortical [NAA] was positively correlated both with minimum SpO₂ during diagnostic VPSG ($r = 0.69$; $P = 0.006$) and the diagnostic MSLT score ($r = 0.62$; $P = 0.01$): patients with lower cortical [NAA] showed lower minimum SpO₂ and shorter sleep latency on MSLT (Fig. 3). No correlations were found between any cortical metabolite concentrations and the other clinical and PSG variables (data not shown). After 6 months of CPAP therapy despite the absence of EDS, nocturnal arousals, and O₂ desaturations, cortical [NAA], [Cr], and [Cho] were unchanged: cortical [NAA] (post-CPAP) = 11.94 ± 1.33 , $P = 0.87$; [Cr] (post-CPAP) = 10.57 ± 0.94 , $P = 0.96$; and [Cho] (post-CPAP) = 2.05 ± 0.15 , $P = 0.90$.

DISCUSSION

In our OSAS patients, the baseline PSG demonstrated severe sleep fragmentation and oxygen desaturation, and the MSLT and ESS showed respectively objective and subjective diurnal somnolence. CPAP therapy improved sleep parameters and nearly normalized EDS. One of the limitations of the study was the reliance on purely subjective data when assessing the compliance with CPAP treatment.

At baseline all patients showed normal cognitive general functioning (i.e. normal values of MMSE and FR of BMDB, as shown in Table 1) and only a minority presenting a small number of minor pathological tasks, like working memory (digit span backwards) and some psychomotor tasks (attentional tests) (Table 4). The absence of impairment in general cognitive functioning in our patients may be related to our strict selection criteria for the OSAS sample, in particular the exclusion of patients with cardiovascular, cerebrovascular, or metabolic comorbidities, thus excluding secondary causes of mental deterioration. The neuropsychological tests, especially the Brief Mental Deterioration Battery, employed to evaluate global cognition have shown high specificity and sensitivity in differentiating dementia patients, even those with early onset and short duration of cognitive impairment, from normal controls.^{24,25} There was no suggestion of retest effect on the neuropsychological results after CPAP, and the effect was kept to a minimum by the considerable interval of time elapsing between the 2 evaluations (6 months). Ferini-Strambi et al⁹ failed to find any significant difference between the cognitive test results at intervals of 15 days and 4 months.

Figure 2—Mean N-acetyl-aspartate (NAA) concentration in the group of OSAS patients pre- and post- CPAP compared to healthy volunteers. Error bars indicate standard deviation.

In contrast with the absence of global cognitive impairment, baseline cortical NAA concentration was lower than in controls and persisted unchanged after therapy despite the improvement of PSG parameters compared to pre-CPAP values in all cases ($P < 0.001$).

In keeping with our findings, MRI and PET/SPECT studies demonstrated brain functional abnormalities in OSAS. Macey et al, using MRI voxel-based morphometry (VBM), found grey matter loss in the frontal, parietal, occipital and temporal cortex, and anterior cingulate gyrus, hippocampus, and cerebellum of 21 OSAS patients. The degree of grey matter loss correlated to disease severity.²⁹ Diminished grey matter in the left hippocampus was detected by VBM in a smaller sample of patients.³² In contrast, another VBM study failed to detect areas of grey matter volume change in patients with severe OSAS.³³ A preliminary 18-FDG PET study detected reduced glucose metabolism in the right premotor and anterior cingulate cortical areas,³⁴ and a preliminary SPECT 99m-TcHMPAO study found reduced perfusion in the temporal-parietal and parietal regions, that improved or normalised after CPAP therapy.³⁵

Previous ¹H-MRS studies have thus demonstrated contrasting results, likely related to factors such as different acquisition techniques and localisations prescribed or different patient samples. The ¹H-MRS study of heterogeneous groups of OSAS patients with or without different comorbidities, using the 2-dimensional chemical shift technique, showed a decrease of NAA/Cho, associated with a small reduction in NAA/Cr and increase in Cho/Cr in the posterior periventricular white matter.^{13,36} The extent of NAA/Cho reduction was weakly correlated to the apnoea-hypopnoea index.³⁶ However, these findings were not confirmed in 2

Table 3—Pathological Neuropsychological Tasks (PNT) results in OSAS patients before and after CPAP

	Total PNT N (%)	Case number with n ≥ 1 PNT (%)	Number of pts. with 3 PNT	Number of pts. with 2 PNT	Number of pts. with 1 PNT	Type of task (number of patients)
Baseline	7/350 (2.0%)	4, 3, 9, 10 (28.5%)	1	1	2	CRT (2), DSB (1), SS (1), Barr S (1), CVART (1), CVARTe (1)
After CPAP	4/350 (1.1%)	4, 3, 11, 12 (28.5%)	0	0	4	CRT (1), SRTe (1), DSB (1), Barr S (1), SVARTe (1)

CRT: complex reaction times; DSB: digit span backwards; SS: spatial span; Barr S: barrage score; CVART: complex visual reaction times; CVARTe: complex visual reaction times errors; SRTe: simple reaction time errors; SVARTe: simple visual attention reaction times errors.

Figure 3—Correlation between cortical N-acetyl-aspartate (NAA) concentration and minimum SpO₂ during nocturnal sleep (A) and MSLT score (B) in OSAS patients.

later ¹H-MRS single-voxel studies of OSAS patients that pointed either to a reduction in NAA and Cho (frontal white matter)¹⁴ or in Cr content (left hippocampus).¹⁵ Most ¹H-MRS studies undertook no neuropsychological assessment and post-therapy re-examination to verify the potential reversibility of biochemical changes.

Our ¹H-MRS results indicate that in OSAS subjects the concentration of cortical NAA may be reduced in the absence of structural lesions, as assessed by means of brain MRI obtained before and after 6 months of CPAP treatment in each patient, and before the development of major cognitive impairment. The persistence of low cortical NAA concentration after therapy indicates a neuronal loss rather than a neuronal dysfunction, most likely related to the repeated episodes of hypoxia. Interestingly, high brain lactate has been detected using ¹H-MRS in some OSAS patients studied during sleep.³⁷

The role of hypoxia in determining cortical damage is support-

ed by a number of experimental models.^{38,39} Exposure to intermittent hypoxia, like that occurring in OSAS, led to neurobehavioral impairment in the absence of significant sleep disruption.³⁸ Intermittent hypoxia in developing rat brain is associated with cognitive deficits like impaired spatial learning and hyperactivity³⁹ similar to the clinical findings observed in some pediatric OSAS patients.⁴⁰ The neurobehavioral deficits of adult rats are correlated to degenerative changes in the cortex and hippocampus.³⁸

Our study showed that the extent of cortical neuronal damage in OSAS patients was consistently related to the minimum SpO₂ occurring during nocturnal sleep. The absence of any other significant correlations between cortical NAA reduction and other clinical and VPSG variables further supports the role of intermittent hypoxia, rather than arousals and sleep fragmentation, on the metabolic cerebral changes observed in our patients.

In conclusion, the cortical NAA reduction found in our sample of OSAS patients without cardiovascular disease supports the central pathogenetic role of cerebral hypoxia in obstructive apnoea. This biochemical alteration, unchanged after effective CPAP therapy, suggests irreversible cerebral damage in OSAS patients. To elucidate the natural history of brain involvement in the sleep apnoea syndrome, longitudinal ¹H-MRS studies of patients from the early stages of the disease will help to define the window of reversibility of metabolic changes in the brain.

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Table 4—Cortical metabolite concentrations (mmol/Kg wet weight) in pre-CPAP OSAS patients and in healthy controls.

Case n°	[NAA]	[Cr]	[Cho]
1	11.55	11.37	2.10
2	11.01	11.09	1.94
3	12.07	10.65	1.98
4	10.78	10.39	2.01
5	12.23	11.69	2.19
6	10.67	9.02	1.84
7	12.92	12.76	2.02
8	12.51	10.55	2.15
9	12.59	9.37	1.99
10	12.38	10.99	2.07
11	11.85	10.56	2.18
12	12.70	10.63	2.33
13	12.23	10.64	1.74
14	10.61	7.97	2.08
Mean ± SD	11.86±0.80	10.55±1.17	2.04±0.15
Controls (n. =10)	12.85±0.93	10.58±0.77	2.13±0.17
P values	0.01	NS	NS

[NAA] = N-acetyl-aspartate, [Cr] = creatine, [Cho] = choline, NS = not significant

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