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RESEARCH ARTICLE

Serum S100B but not NSE Levels are Increased in Morbidly Obese Individuals Affected by Obstructive Sleep Apnea–Hypopnea Syndrome

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Abstract
Background Obstructive sleep apnea–hypopnea syndrome (OSAHS) is considered a comorbidity associated with morbid obesity, mainly because of the large neck circumference. Depending on its severity, OSAHS can interfere in many homeostasis systems, for example, the central nervous system (CNS). Neuron-specific enolase (NSE) and S100B protein derived from astrocytes are considered sensitive biochemical markers of cerebral injury. We evaluated serum S100B and NSE levels in this study with the aim of detecting possible cerebral injury as a consequence of OSAHS.
Methods This was a transverse study with data from 25 morbidly obese patients with OSAHS. Blood samples were collected before and after polysomnography (PSG) to determine S100B and NSE protein levels. We also analyzed data evaluating depression and excessive daytime sleepiness.
Results S100B levels were higher after [0.029 (0.010–0.199) mg/l] compared to before [0.010 (0.010–0.025) mg/l] on PSG (P=0.002). S100B levels were expressed as means and IQ25–IQ75. NSE levels did not show significant differences before and after PSG.
Conclusion Our study shows a significant increase in S100B level after PSG compared to before. This suggests that there is a CNS astrocyte reaction because of possible cerebral hypoxemia in morbidly obese patients with OSAHS.

Keywords Obstructive sleep apnea–hypopnea syndrome · S100B · NSE · Cerebral lesion · Polysomnography · Apnea–hypopnea index

Abbreviations

OSAHS	obstructive sleep apnea–hypopnea syndrome
CNS	central nervous system
S100B	a protein derived from astrocyte activity
NSE	neuron-specific enolase (a glycolytic pathway enzyme from CNS)
PSG	polysomnography
AHI	apnea–hypopnea index
EDS	excessive daytime sleepiness

Introduction

Obstructive sleep apnea–hypopnea syndrome (OSAHS) is common in the middle-aged population, and obesity is an important etiologic factor in its pathogenesis. It was demonstrated to be an important independent predictor of psychosocial morbidity in subjects with severe obesity [1]. The incidence of OSAHS among morbidly obese patients is

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12- to 30-fold higher than in the general population, and one important characteristic of the morbid obesity condition is the presence of central obesity, which means large belly and neck circumferences [2]. Especially related to having a thick neck is the high incidence of OSAHS [1–3].

OSAHS is defined by the occurrence of at least five obstructive apneas, hypopneas, or both per hour while the patient is sleeping and classified by the apnea–hypopnea index (AHI). There are three levels of severity: mild (AHI=5 to 15 events per hour); moderate (AHI=15 to 30 events per hour); and severe (AHI=>30 events per hour) [3]. An apnea is defined in adults as the cessation of airflow for ten or more seconds, whereas hypopnea is the reduction (at least 30%) in airflow for ten or more seconds associated with a 4% decrease in oxygen saturation [3]. Repeated sleep apnoeic episodes may lead to behavioral and brain morphological alterations in patients with OSAHS. Accordingly, excessive sleepiness and impairment in the cognitive function are common daytime symptoms [4]. Furthermore, abnormal morphology of the brain frontal cortex, cerebellum, and hippocampus has been identified in these patients by imaging techniques [5, 6].

More recently, studies have been performed aiming to determine consequences of OSAHS on the central nervous system (CNS) through the measurement of serum neurobiochemical markers, more specifically S100B protein and neuron-specific enolase (NSE) [7, 8]. S100B is a calcium-binding protein predominantly found in cytosol of astrocytes, reaching the extracellular space through secretion or because of astrocytic lesion. Extracellular functions of S100B are particularly relevant to understanding the potential role of this protein in modulating neuropathological outcomes after brain insults. Increased production of S100B by glial activation (reactive gliosis) and/or lesion may account for a number of studies suggesting that S100B could be a diagnostic and/or prognostic marker in brain injury assessment [9, 10]. NSE is a glycolytic pathway enzyme, which the $\gamma\gamma$ isoform is predominantly found in neuronal tissue. As NSE is not physiologically secreted, increases in its peripheral levels have been specifically related to neuronal injury, as reported in traumatic brain, injury stroke, and epileptic seizures [11, 12].

The purpose of this study was to evaluate if serum levels of the neurobiochemical markers S100B and NSE are altered in morbid obese patients with OSAHS.

Patients and Methods

Patients

Clinical and laboratory assessment was performed in 25 patients (15 women and 10 men) with morbid obesity clinically evaluated in the Hospital São Lucas da PUCRS, Porto Alegre, Brazil, in the preoperative phase for obesity surgery. Patients included in this study were morbidly obese individuals presenting OSAHS clinical complaints such as excessive daytime sleepiness (EDS) [4]. Exclusion criteria were previous treatment for OSAHS and cognitive incapacity with regard to the comprehension of the consent form and questionnaires related to the study.

Patients agreed to participate in the study and signed an informed consent form. This study was approved by the Research Ethics Committee of Hospital São Lucas da PUCRS, Porto Alegre, Brazil.

Protocol

Morbidly obese patients with clinical complaints of OSAHS were invited to join the study. On the polysomnography (PSG) day, the following procedures were conducted:

- Presentation of an informed consent submission, specifying the study objectives and author contacts
- Clinical assessment, Beck Depression Inventory [13], Epworth Sleepiness Scale (EDS evaluation) [14].
- Venous blood sample (5.0 ml) drawn by a trained professional (nurse or physician), before PSG, around 9 P.M.
- Overnight PSG.
- Another blood sample drawn after PSG (around 7 A.M.)

Soon after sampling, blood sample was centrifuged at 5,000 rpm for 10 minutes, the serum was separated and stored at −70°C for further S100B and NSE measurements.

Sleep Analysis

PSG was recorded from 2,300 to 700, following international guidelines [15], in a polygraph (BrainNet, EMSA, Rio de Janeiro, Brazil). It consisted of electroencephalogram (C3-A2 and C4-A1), right and left electrooculogram, electrocardiogram, and electromyogram of the submental and anterior tibialis muscles. Respiratory parameters were assessed by inductance plethysmography (Respitrace, AMI, New York, USA) with thoracic and abdominal belts, calibrated with isovolume maneuver and nasal airflow measured by means of a pressure transducer connected to a nasal cannula. A pulse oximeter with a finger probe was used to continuously measure arterial oxyhemoglobin saturation (Protec, Brazil). Body positions and movements were detected by position sensor and video monitoring. All recordings were made by trained technicians following standard procedures and stored in a computer system. Apneas were defined as reductions in tidal volume and/or airflow to below 10% of baseline for 10 s or longer; hypopneas were defined as reductions in tidal volume and/or airflow to below 50% of baseline for 10 s or longer,

accompanied by at least 3% desaturation or at least a 3-s arousal [15].

S100B and NSE Measurements

S100B

Measurements of S100B were performed in a Lumat LB9507 luminometer (EG and G Berthold) using the immunoluminescent assay kit LIA-mat Sangtec 100 (BYK-Sangtec, Germany), which uses an antibody labeled with isoluminol. S100B standard curve was linear up to 20 mg/l, and the coefficient of variation for duplicates in all range levels of standards and samples was within 5%. S100B levels are expressed as milligrams per liter (mean± SD) [7]. The samples were carried out together in the same experiment.

Neuron-specific Enolase

NSE was measured using an electrochemiluminescent assay. It consists of a double sandwich assay that uses an anti-NSE antibody labeled with ruthenium, which is the luminescent molecule. Reactions and quantification were performed with Elecsys-2010 (Roche Diagnostics Corporation®). NSE is expressed as nanogram per milliliter (mean±SD). The coefficient of variation was less than 5% for samples and standards [7]. The samples were carried out together in the same experiment.

Statistical Analysis

Statistical analysis was performed using the SPSS 12.0 package. Continuous variables were expressed as means and standard deviation or medians and percentile/interquartile range, depending on estimated normality. Categorical data were expressed as relative and absolute frequency.

The Mann–Whitney test was used to compare continuous variables between test groups. The paired *t*-test or Wilcoxon test was used for comparison of continuous variables with repetitive measures, depending on whether or not a nonparametric technique was required. Spearman's correlation coefficient was used to determine correlations between continuous variables. Fisher's exact test was used for comparison between categorical variables. We used scatter plots and box plots for correlations.

Results

Table 1 shows characteristics of the subjects and data related to clinical laboratory evaluation.

Table 1 Characteristics of the subjects and data related to clinical and laboratory evaluation

Variable	Mean±SD
Age (years)	39.92±8.63
BMI (kg/m^2)	47.86±8.75
Beck depression inventory	12.52±8.44
EDS (epworth sleepiness scale)	12.08±5.41
AHI[a] (events/hour)	36 (8.5–74.5)
Minimum O_2 saturation (%)	72.36±14.02
Mean O_2 saturation (%)	91.04±4.25

BMI Body mass index; *Beck* Beck Depression Inventory; *EDS* excessive daytime sleepiness; *AHI* apnea–hypopnea index.
[a] Median IQ25–IQ75

Clinical depression was evaluated by Beck Depression Inventory [13], and EDS was evaluated by the Epworth Sleepiness Scale [14].

Serum NSE level (Table 2) did not show any significant variation both just before and after sleep. However, there was a significant increase in serum S100B levels after sleep, comparing with the values before (P=0.002; Table 2 and Fig. 1).

S100B levels were undetectable (below 0.02 μg/l) in 15 patients (60%) before and in 7 patients (28%) after the sleep. This difference was statistically significant (P=0.045). This difference in S100B levels (ΔS100B) was calculated to determine correlations with the variables studied. There was no correlation between ΔS100B and body mass index, Beck Depression Inventory and mean oxygen saturation.

Figure 2 shows ΔS100B correlations with AHI, minimum O_2 saturation (min O_2 sat) and EDS. There was no statistically significant correlation between ΔS100B levels and AHI (p_s=0.180), a negative correlation between ΔS100B levels and minimum oxygen saturation levels (p_s=−0.358), and a positive correlation between ΔS100B levels and EDS (p_s=0.597).

We used a group categorization of AHI, EDS, and minimum oxygen saturation indexes related to ΔS100B, as shown in Fig. 3. Categorizing AHI with a cutoff point of 30, there was a significant difference between AHI<30 and

Table 2 S100B and NSE data before and after PSG

Variable	Mean±SD	*P*
NSE before PSG (ng/ml)	9.40±2.23	0.22
NSE after PSG (ng/ml)	8.66±2.91	
S100 before[a] PSG (mg/l)	0.010 (0.010–0.025)	0.002
S100 after[a] PSG (mg/l)	0.029 (0.010–0.199)	
ΔS100B[a]	0.019 (0–0.154)	–

ΔS100B data for Spearman correlation coefficient comparison between ΔS100B and EDS, minimum O_2 saturation and AHI
NSE Neuron-specific enolase; *ΔS100B* change in S100B.
[a] Median IQ25–IQ75

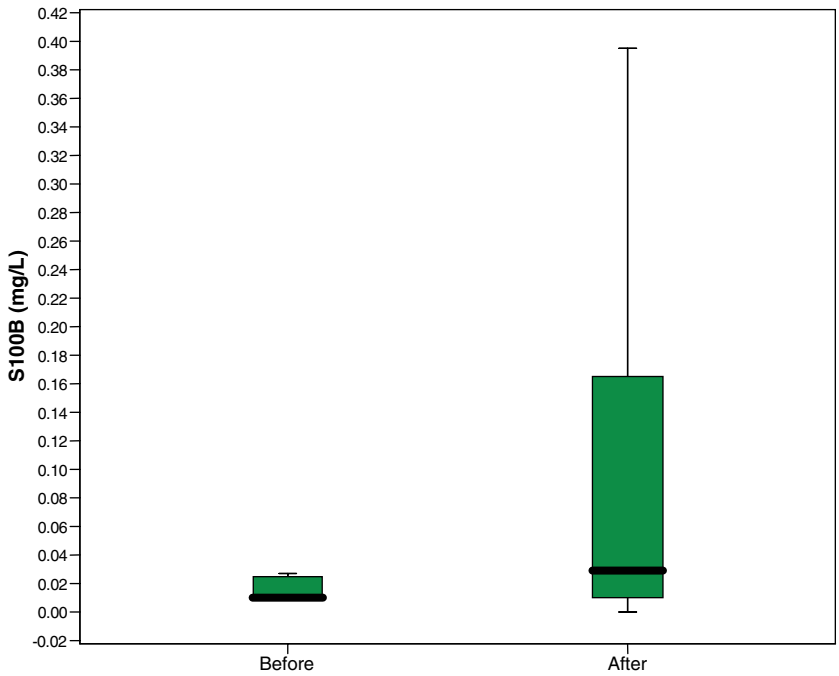

Fig. 1 S100B levels before and after PSG

AHI>30 groups after sleep for absolute mean values of S100B levels (P=0.021) and for ΔS100B (P=0.029), but not before sleep. Categorizing minimum oxygen saturation with a cutoff point of 75%, we did not find significant differences between >75% and <75% minimum oxygen saturation groups when compared to ΔS100B (P=0.152). Categorizing the EDS scale with a cutoff point of 8, we found significant differences between EDS<8 and EDS> 8 groups for S100B absolute mean levels after sleep (P= 0.001) and for ΔS100B (P<0.001), but not before sleep.

Discussion

OSAHS is a common clinical and laboratorial syndrome associated to morbid obesity, showing positive long-term results after surgery [16–18]. EDS, sleep fragmentation, and depression have been described as common symptoms in patients with OSAHS [5, 6]. Moreover, evidences have pointed that OSAHS may lead to brain neuronal damage [7, 8]. In this study, serum NSE and S100B levels, used as peripheral markers of brain injury, were measured in morbidly obese patients with OSAHS submitted to overnight PSG evaluation. Serum samples were taken after and before PSG. NSE levels presented no significant differences when compared before to after sleep, whereas the level of S100B was significantly higher after sleep (P= 0.002). Both serum NSE and S100B levels have been used in clinical and experimental investigations aiming to understand the involvement of the neural cells neurons and astrocytes in pathological conditions [19–21]. Increase of NSE levels indicates neuronal damage, whereas increase in S100B may reflect either glial damage or astrocytic reactions to neural injury (reactive astrogliosis) [22].

Intermittent hypoxia during sleep results in decreased blood oxygen saturation levels (hypoxemia) and neuronal injury by mechanisms not completely elucidated. Neuroimaging and neuropsychological findings have demonstrated usefulness, targeting the evaluation of neuropathologic effects that occur after hypoxia in patients with OSAHS [23, 24]. Besides, measurement of peripheral brain markers could provide an additional strategy trying to detect hypoxic brain damage in OSAHS. Previous works with nonobese patients have presented contradictory findings about S100B in OSAHS. Jordan et al. [8] observed no difference between controls and patients just before and after sleep. Braga et al. [7] showed significant differences in serum S100B level in OSAHS patients compared to controls after sleep. Probably ethnic difference, medications, cerebral areas affected or associated comorbidities could account for this disparity.

Although neuronal death is frequently reported either in humans with OSAHS as in animal models [25], the serum levels of neuronal marker NSE has not been able to indicate this brain damage [26]. Otherwise, brain astrocytes have very close morphological and functional relationship with neurons. High concentrations of S100B may induce neuronal cell death by apoptosis through nitric oxide release from astrocytes [27]. Conversely, S100B may exert trophic effects during developmental period, and it is also able to decrease glutamate exctitotoxicity, both effects being observed in low concentrations [28, 29]. Searching for physiological relevance of increasing S100B level in OSAHS patients, we

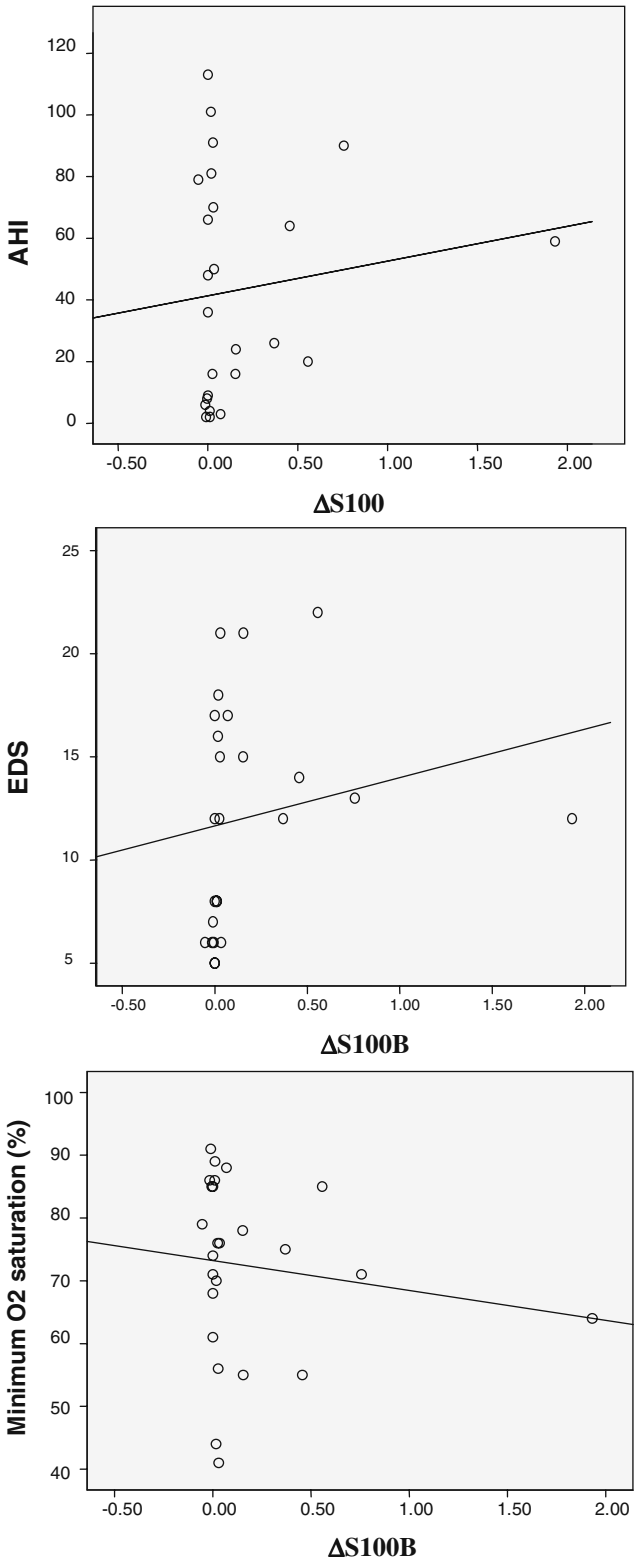

Fig. 2 Correlations between ΔS100B and AHI, EDS and minimum O_2 saturation

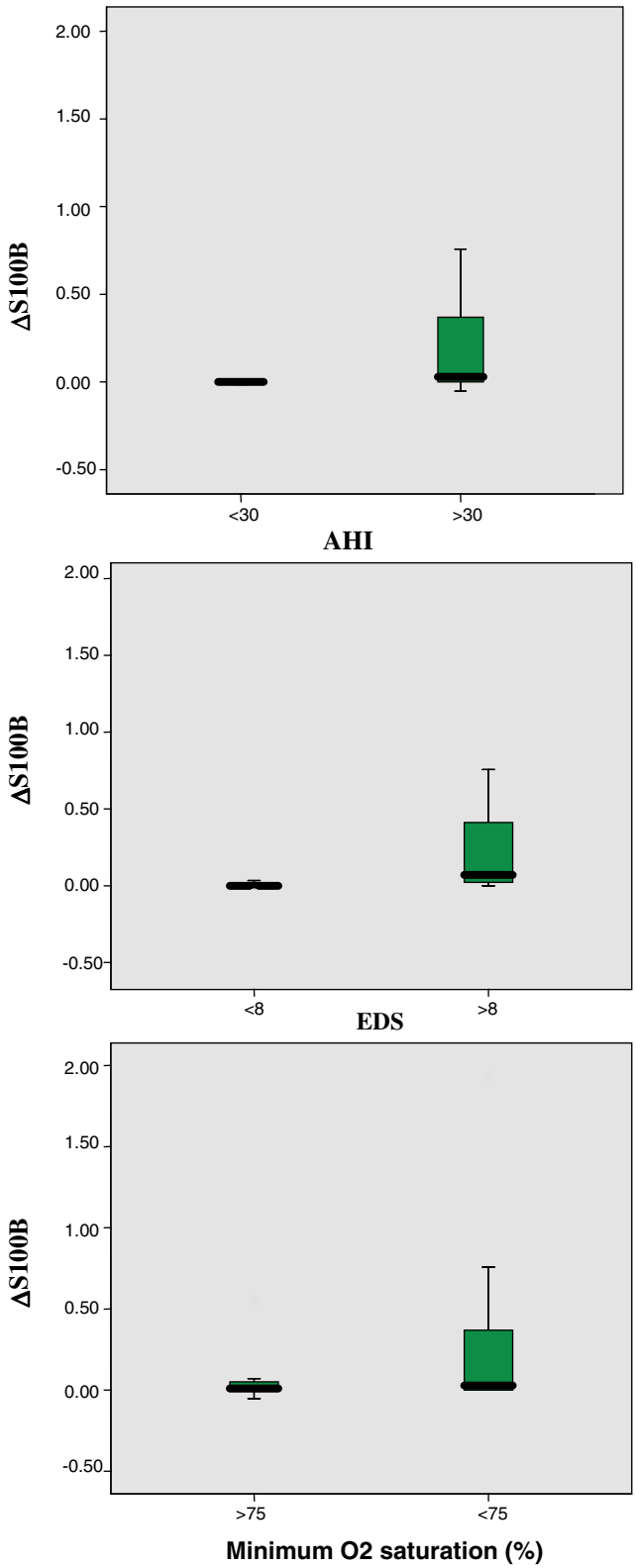

Fig. 3 Group category and comparison between ΔS100B and AHI, EDS and minimum O_2 saturation

observed in our study that it may reflect an astrocytic response trying to protect neuronal integrity once NSE level remains normal. However, the combination between serum S100B and NSE measurements with neuroimaging and spectroscopic evaluations could provide a more specific response regarding these issues.

Morbidly obese patients are more prone to have OSHAS, although functional impairment of the upper airway dilating muscles is also particularly important in the development of this pathology [2]. Cerebrovascular diseases and neuropsychiatry disorders are frequent comorbidities associated with obesity [2]. In nonobese subjects, these pathologies may cause S100B release by astrocytes [23, 27], whereas serum S100B was increased in non-obese depressive patients [30, 31]. Here in our study, we found association among depression and increase S100B level in our morbidly obese patients ("Results"). However, given that the reported symptoms of depression and anxiety in the current study were in the mild range for both groups, it is unlikely that they contributed to the observed increased S100B.

Our study showed that there is a moderate correlation between ΔS100B and minimum oxygen saturation. S100B could therefore indicate a cerebral hypoxic insult. We also showed an association between increased ΔS100B and EDS, indicating a clinical characteristic related to the pathology.

Experimental evidence has pointed to the putative influence of adipose tissue in the serum S100B level. In vitro stimulation of adipose tissue by epinephrine triggers S100B release [32]. It is important to take into account a possible influence of adipose tissue of morbidly obese subjects in the blood concentration of S100B protein, although no association was reported up to now in human studies. This is one supporting reason for S100B and NSE serum levels assessment in OSAHS postoperative patients after massive weight loss, which is already under investigation in our group.

In conclusion, we observed that there is significant difference in S100B but not NSE between *after* compared to *before* sleep levels in morbidly obese OSAHS patients. This finding could lead us to relate an astrocytic reaction to hypoxemic indexes caused by sleep hypopneas or apneas, but not neuron death. On the other hand, high S100B levels are considered toxic to the CNS, which chronically could harm neuronal tissue.

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References

1. Fritscher LG, Mottin CC, Canani S, Chatkin JM. Obesity and obstructive sleep apnea–hypopnea syndrome: the impact of bariatric surgery. Obes Surg. 2007;17:95–9.
2. Peiser J, Lavie, P, Ovnat A, Charuzi I. Sleep apnea syndrome in the morbidly obese as an indication for weight reduction surgery. Ann Surg. 1984;100:112–15.
3. Caples SM, Gami AS, Somers VK. Obstructive sleep apnea. Ann Intern Med. 2005;142:187–97.
4. Naegele B, Thouvard V, Pepin JL, Levy P, Bonnet C, et al. Deficits of cognitive executive functions in patients with sleep apnea syndrome. Sleep. 1995;18:43–52.
5. Macey PM, Henderson LA, Macey KE, Alger JR, Frysinger RC, et al. Brain morphology associated with obstructive sleep apnea. Am J Respir Crit Care Med. 2002;166:1382–7.
6. Morrell MJ, McRobbie DW, Quest RA, Cummin AR, Ghiassi R, et al. Changes in brain morphology associated with obstructive sleep apnea. Sleep Med. 2003;4:451–4.
7. Braga CW, et al. S100B and NSE serum levels in obstructive sleep apnea syndrome. Sleep Med. 2006;12:1–5.
8. Jordan W, Hagedohm J, Wiltfang J, et al. Biochemical markers of cerebrovascular injury in sleep apnoea syndrome. Eur Respir J. 2002;20:158–64.
9. Van Eldik LJ, Wainwright MS. The Janus face of glial-derived S100B: beneficial and detrimental functions in the brain. Restor Neurol Neurosci. 2003;21:97–108.
10. Donato R. Intracellular and extracellular roles of S100 proteins. Microsc Res Tech. 2003;60:540–51.
11. Kayser E, Kaiser R, Kuzmits P, et al. Clinical biochemistry of neuron specific enolase. Clin Chim Acta. 1989;183:13–32.
12. Busnello JV. Acute and chronic electroconvulsive shock in rats: effects on peripheral markers of neuronal injury and glial activity. Life Sci. 2006;78:3013–17.
13. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4:561–71.
14. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep. 1991;14:540–5.
15. Rechtschaffen A, Kales AA. A manual of standardized terminology, techniques, and scoring system for sleep stages of human subjects. Washington, DC: Public Health Service; 1968.
16. Guardiano SA, Scott JA, Ware C, Schechner SA. The long-term results of gastric bypass on indexes of sleep apnea. Chest. 2003;124:1615–19.
17. Rasheid S, Banasiak M, Gallagher SF, et al. Gastric bypass is an effective treatment for obstructive sleep apnea in patients with clinically significant obesity. Obesity Surgery. 2004;13: 58–61.
18. Buchwald H, Avidor Y, Braunwald E, et al. Bariatric surgery: a systematic review and meta-analysis. JAMA. 2004;292:1724–37.
19. Mazzini G, Schaf D, Oliveira AR, Goncalves CA, Souza DO, Portela LV. The ischemic rat heart releases S100B. Life Sci. 2005;77:882–89.
20. Willoughby KA, Kleindienst A, Müller C, Chen T, Muir JK, Ellis EF. S100B protein is released by in vitro trauma and reduces delayed neuronal injury. J Neurochem. 2004;91:1284–91.
21. Rothermundt M, Peters M, Prehn J, Arolt V. S100B in brain damage and neurodegeneration. Microsc Res Techn. 2004;60:614–32.
22. Herrmann M, Vos P, Wunderlich MT, de Bruijn CH, Lamers KJ. Release of glial tissue-specific proteins after acute stroke: a comparative analysis of serum concentrations of protein S-100B and glial fibrillary acidic protein. Stroke. 2000;31:2670–7.
23. Gale SD, Hopkins RO. Effects of hypoxia on the brain: neuroimaging and neuropsychological findings following carbon monoxide poisoning and obstructive sleep apnea. J Int Neurops Soc. 2004;10:60–71.
24. Halbower AC, Degaonkar M, Barker PB, Earley CJ, Marcus CL, Smith PL, et al. Childhood obstructive sleep apnea associates with neuropsychological deficits and neuronal brain injury. Plos Med. 2006;3:301.
25. Morrell MJ, McRobbie DW, Quest RA, Cummin AR, Ghiassi R, et al. Changes in brain morphology associated with obstructive sleep apnea. Sleep Med. 2003;4:451–4.
26. Pleines UE, Morganti-Kossmann MC, Rancan M, Joller H, Trentz O, Kossmann T. S-100 beta reflects the extent of injury and outcome, whereas neuronal specific enolase is a better indicator of neuroinflammation in patients with severe traumatic brain injury. J Neurotrauma. 2001;18:491–8.

27. Hu J, Ferreira A, Van Eldik LJ. S100beta induces neuronal cell death through nitric oxide release from astrocytes. J Neurochem. 1997;69:2294–301.
28. Tramontina F, Conte S, Gonçalves D, Gottfried C, Portela LV, Vinade L, et al. Developmental changes in S100B content in brain tissue, cerebrospinal fluid, and astrocyte cultures of rats. Cell Mol Neurobiol. 2002;22:373–8.
29. Tramontina F, Tramontina AC, Souza DF, Leite MC, Gottfried C, Souza DO, et al. Glutamate uptake is stimulated by extracellular S100B in hippocampal astrocytes. Cell Mol Neurobiol. 2006;26:81–6.
30. Arolt V, Peters M, Erfurth A, Wiesmann M, Missler U, Rudolf S, et al. S100B and response to treatment in major depression: a pilot study. Eur Neuropsychopharmacol. 2003;13:235–9.
31. Hetzel G, Moeller O, Evers S, Erfurth A, Ponath G, Arolt V, et al. The astroglial protein S100B and visually evoked event-related potentials before and after antidepressant treatment. Psychopharmacology. 2005;178:161–6.
32. Sriram K, Benkovic SA, Miller DB, O'Callaghan JP. Obesity exacerbates chemically induced neurodegeneration. Neuroscience. 2002;115:1335–46.