Stability of oxyhemoglobin affinity in patients with obstructive sleep apnea-hypopnea syndrome without daytime hypoxemia

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Section D, Detry B, Rodenstein D, Liistro G. Stability of oxyhemoglobin affinity in patients with obstructive sleep apnea-hypopnea syndrome without daytime hypoxemia. J Appl Physiol 105: 1809–1812, 2008. First published October 23, 2008; doi:10.1152/japplphysiol.90860.2008.—A decrease in hemoglobin affinity for oxygen is considered an adaptive mechanism against tissue hypoxia. Obstructive sleep apnea-hypopnea syndrome (OSAHS) is characterized by recurrent episodes of apnea and hypopnea resulting in arterial oxygen desaturations during sleep. Maillard et al. (10) observed a right shift of the oxyhemoglobin dissociation curve (ODC) and an increase in 2,3-diphosphoglycerate (2,3-DPG) concentration ([2,3-DPG]) in 15 patients with severe OSAHS, but some had slight daytime arterial hypoxemia while breathing room air. The aim of our study was to measure the ODC and 2,3-DPG concentrations in a group of subjects normoxic during daytime referred to our sleep laboratory for suspicion of snoring or OSAHS. The patients were recruited during a period of 6 mo. All arterial and venous blood samples were taken early in the morning within 1 h of awakening following a full-night polysomnography. ODC and 2,3-DPG were analyzed in 88 patients: 56 OSAHS subjects. We conclude that patients with OSAHS who are normoxic during daytime referred to our sleep laboratory for suspicion of snoring or OSAHS. We found a significant correlation between the P50 and 2,3-DPG levels in the 88 patients: r = 0.502, P < 0.001. We observed no difference between OSAHS and non-OSAHS for the P50 and [2,3-DPG]. Finally, there was no change in these parameters measured at baseline, after 3 days and after 1 mo of treatment by nasal continuous positive airway pressure (nCPAP) on these parameters in a subgroup of patients with severe OSAHS.

MATERIALS AND METHODS

Patient selection. All the patients referred to the Cliniques universitaires St-Luc (Université catholique de Louvain, Brussels, Belgium) for suspicion of snoring or sleep apnea during a period of 6 mo were asked to participate in the study. The subjects gave an informed consent, and the protocol was approved by the Ethics Committee of our hospital. A total of 132 patients were examined and had a full-night polysomnography (PSG). A complete physical examination was followed by an ear, nose, and throat examination, which included anterior rhinoscopy, endonasal flexible endoscopy, and an electrocardiogram (ECG). All subjects had standard spirometric measurements, maximal inspiratory and expiratory flow/volume curves, a carbon monoxide transfer test (Morgan TLC; Morgan Medical, Rainham, UK), resting arterial blood gases (Ciba Corning Blood gas system 288; Ciba Corning Diagnostic, Medfield, MA), and carbon monoxide measurements (OSM III Radiometer, Copenhagen, Denmark).

The exclusion criteria were abnormal lung function tests, heart failure, diabetes, thyroid dysfunction, renal insufficiency, liver cirrhosis, anemia, and hypopnea (PaCO<sub>2</sub> < 70 mmHg) during wakefulness. Heavy smokers (>10 cigarettes/day) were also excluded. Smoking was not allowed during hospital stay, which means that patients did not smoke for 24 h before the blood samples were taken.

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A full-night diagnostic PSG was performed in each subject according
to standard criteria as described previously (1). A microphone was
 glued onto the anterior face of the patient’s neck, level with the
larynx. Airflow was monitored by three thermocouples placed in front
of the mouth and each nostril and linked to independent channels.
Body position was recorded (Pro-Tech body position sensor; Pro
Tech, Woodinville, WA) via one channel. All signals were recorded
with a digital acquisition system (OSG Brainlab, Antwerp, Belgium).
Sleep and respiratory parameters were recorded at the following
sampling rates: electrooculogram (two channels, right and left), 128
Hz; chin electromyogram (EMG) (one channel), 512 Hz; electroen-
ccephalogram (EEG) (three channels, C4-A1, C3-A2, and C4-O2), 128
Hz; ECG, 128 Hz; thoracoabdominal movements, 64 Hz; and arterial
oxygen saturation and pulse rate, 16 Hz, as previously described (13).

Snoring was designated by the characteristic microphone trace during
sleep. The oxygen desaturation index (ODI) was the number of ≥4%
total oxygen desaturations per hour of sleep. A movement arousal
(MA) was defined as the reappearance of an α-rhythm in the EEG
during a sleep epoch, accompanied by an increase in EMG, both
lasting for ≥2 s (5). The MA index (MAI) is the number of MAs per
hour of sleep. The diagnosis of OSAHS was retained if the subject had
an ODI >5. The patients were classified into two groups (non-
OSAHS or OSAHS) according to an ODI inferior or equal/superior
to 5 events per hour of sleep.

A treatment by nCPAP was offered to those patients with MAI
>30/h and ODI >20/h. After a 3-day training period for accommo-
dation to nCPAP, patients underwent a control PSG to assess treat-
ment efficacy. After a mean follow up of 1.7 mo (range 1–3 mo),
patients treated by nCPAP were reexamined, and treatment compli-
ance was checked.

Nocturnal oxygenation. Nocturnal oxygenation was assessed by the
oxygen desaturation index and by the mean SpO2 during the nocturnal
sleep time. To better describe the duration of sleep hypoxia, we
measured the time spent below 90% of SpO2. We arbitrarily fixed a
limit at 120 min to separate the patients into two groups: a hypoxic
group (time spent below 90% SpO2: >120 min) and a nonhypoxic
group (time spent below 90% SpO2: <120 min).

Oxyhemoglobin dissociation curve, organic phosphate anions mea-
surements, and arterial blood gases. Arterial and venous blood
samples were taken on the morning following PSG, while the subject
was supine and quietly breathing room air. The venous blood sample
enabled the measurement of 2,3-DPG concentration (SIGMA-kit;
Sigma Chemical, St Louis, MO) expressed in micromole/gram of Hb
as well as the plotting of the ODC by the method of Clerbaux (4). The
entire ODC on whole blood was traced under standard conditions (pH
7.40; PCO2, 40 mmHg; temperature, 37°C) and was corrected for the in-
vivo ODC by using actual values of pH, PCO2, and temperature. The
advantage of this method is that it describes the ODC at all levels of
oxygen saturation and thereby gives the resulting function of oxygen
loading and unloading (3). We report in this study the values of partial
pressure of oxygen in blood associated to a Hb oxygen saturation of
50% and 90% (P50 and P90). The measurements were repeated in
patients with OSAHS after 3 days and after 1–3 mo of nCPAP

The reference level of 2,3-DPG and the reference ODC were
determined in six healthy subjects (3 men and 3 women, 24–58 yr of
age). These subjects had normal pulmonary function tests and under-
went a nocturnal oximetry (Nonin Palmast 2500; Nonin Medical,
Plymouth, MN) to check for absence of sleep-disordered breathing,
and their blood samples were taken the next morning.

Statistical analysis. Standard parametric statistical tests were used
in this study. The comparisons between the groups were done using
the Student’s unpaired t-test and within groups, before and after
nCPAP therapy using a Student’s paired t-test. We used a linear
regression analysis to assess the correlation between some parameters
and the Spearman’s rank correlation coefficient when data were not
normally distributed. To minimize the effects of selection bias and to
account for differences in age, body mass index (BMI), and PaO2, a
subsample of men were matched on age, BMI, and PaO2 on the basis
of the smallest Euclidian distance (16).

RESULTS

One hundred and thirty-two patients were recruited in the study. Patients were excluded because of resting daytime
hypoxemia or abnormal lung function (n = 29), diabetes (n =
4), cardiac diseases (n = 6), anemia (n = 3), or thyroid
dysfunction (n = 2). The maximum Hb CO was 1.1%.

We found a significant correlation between P50 and the
2,3-DPG level in the 88 patients: r = 0.502, P < 0.001 (Fig. 1).
However, there was no correlation between 2,3-DPG concen-
tration and ODI (rs = 0.03) and between P50 and ODI (rs =
0.04). No correlation was found between P50 or 2,3-DPG level
and any of the variables (Table 1).

Table 1. Patients characteristics: non-OSAHS and OSAHS

<table>
<thead>
<tr>
<th></th>
<th>Non-OSAHS</th>
<th>OSAHS</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>44.8±11.2</td>
<td>51.9±11.9</td>
<td>0.006</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>24/8</td>
<td>48/8</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>26.6±3.6</td>
<td>30.5±5.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>14.7±1.1</td>
<td>15.1±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>PaO2, mmHg</td>
<td>89.7±7.5</td>
<td>85.3±8.8</td>
<td>0.016</td>
</tr>
<tr>
<td>FEV1, %</td>
<td>97.6±14.6</td>
<td>93.7±19.1</td>
<td>NS</td>
</tr>
<tr>
<td>FVC, %</td>
<td>94.7±12.1</td>
<td>89.7±14.5</td>
<td>NS</td>
</tr>
<tr>
<td>TST, min</td>
<td>402±80</td>
<td>367±92</td>
<td>NS</td>
</tr>
<tr>
<td>ODI</td>
<td>2.1±1.3</td>
<td>27.5±24.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAI</td>
<td>9.3±4.8</td>
<td>24.5±15.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All data, except sex, are expressed as means ± SD. OSAHs, obstructive
sleep apnea-hypopnea syndrome; BMI, body mass index; Hb, hemoglobin;
PaO2, partial pressure of oxygen in arterial blood; FEV1, forced expiratory
volume in the first second; FVC, forced vital capacity; %, percent of predicted
values; TST, total sleep time; ODI, oxygen desaturation index; MAI, move-
Comparison between non-OSAHS and OSAHS groups. Patient characteristics are detailed in Table 1. The two groups were different for age and BMI, and average PaO2, during wakefulness was lower in the OSAHS group. The sleep characteristics of the two groups are reported.

Average values of P50 and 2,3-DPG were not statistically significantly different between non-OSAHS and OSAHS patients: P50 (means ± SD mmHg): 26.5 ± 1.3 vs. 26.4 ± 1.3 (P > 0.05), 2,3-DPG (means ± SD µmol/g Hb): 15.8 ± 2.8 vs. 16.5 ± 1.9 (P > 0.05).

Effects of nCPAP. nCPAP treatment was effective after a 3-day treatment in all patients as demonstrated by PSG. We followed 13 patients treated by nCPAP, and we obtained a blood sample in 7 of them after 3 days of nCPAP treatment (Table 4). The 13 patients were examined after 1–3 mo of nCPAP treatment (Table 4). Compliance was checked using the built-in time counter of nCPAP. All these patients used their machine more than 4 h/night. Despite an effective treatment, there was no change in P50 or 2,3-DPG concentration, early or later after treatment (Table 4), and the ODC were also superimposable.

Comparison between hypoxic and nonhypoxic groups. Patient characteristics are detailed in Table 5. Hypoxic patients had a higher BMI and Hb concentration. They also had slightly less daytime PaO2 compared with nonhypoxic subjects.

All parameters of nocturnal oxygenation were significantly different although the P50 and P90 values, 2,3-DPG concentrations, and the ODC plots were similar for the two groups (Table 5).

DISCUSSION

We have shown that intermittent arterial desaturations during sleep do not induce a change in the affinity of Hb for oxygen during daytime. Hence, patients with OSAHS without daytime hypoxia do not benefit from a right shift of the ODC before the beginning of apneas and hypopneas.

The shift of the ODC to the right is considered a protective mechanism against tissue hypoxia, enabling Hb, for a given P50, to unload more oxygen to the tissues. The affinity of Hb is decreased by an increase in 2,3-DPG attributable to the effect of hypoxia on erythrocyte metabolism. We observed, as others, a strong correlation between P50 and 2,3-DPG in the population studied. Stable hypoxia of nonpulmonary origin, like hypobaric hypoxia during high-altitude stay, is associated with an increase in both P50 and 2,3-DPG concentration.

However, we did not find an increase in P50 or 2,3-DPG in patients with OSAHS, and no change was observed after nCPAP treatment. Even after matching for age, BMI, and PaO2 or after Table 3. Subgroups of patients classified according to the severity of the ODI

All data are expressed as mean ± SD.
stratification of the subjects following the level of sleep-related hypoxia, no difference was observed.

We were not able to reproduce the results of Maillard et al. (10). These authors compared 15 patients with severe sleep apnea to a group of 10 healthy subjects. They observed higher P50 and 2,3-DPG levels in the OSAHS, showing a right shift of the ODC. These values returned within the normal range after surgical or nCPAP treatment in five patients. However, as stated by the authors, they did not exclude patients with daytime “slight” arterial hypoxia. In fact, the OSAHS had a mean ± SD PaO2 of 77 ± 11 mmHg, which means that, if these values were normally distributed, the lower limit of PaO2 was <60 mmHg. By contrast, the mean PaO2 of our patients was 85.3 ± 8.8 mmHg, and we excluded patients with daytime hypoxemia. We also carefully controlled potential confounding factors like carboxyhemoglobin and inorganic ion concentrations since they affect the ODC (6).

In 1990, in a study comparing patients with sleep apnea (N = 26) with non-OSAHS subjects (N = 42), McKeon and colleagues (11) found higher blood 2,3-DPG in apneic subjects. However, daytime hypoxemia could not be excluded as a confounding factor, in view of the absence of arterial blood gases.

The highly accurate analysis method used in our study offered the analysis of the whole ODC plotting, which enables the determination not only of P50 but also of blood oxygen saturation at several levels of PO2. This method is also useful for the analysis not only of P50 but also of blood oxygen saturation in view of the absence of arterial blood gases.

The lack of increase in 2,3-DPG in patients with OSAHS may be related to different causes. Several hours of sustained hypoxia appear to be necessary to induce a significant elevation in 2,3-DPG levels, probably because these depend on the hypoxia duration and on the half-life of 2,3-DPG. The shortest period of hypoxia sufficient to induce a significant rise in blood 2,3-DPG is unknown, but it appears from this study that short hypoxic episodes in OSAHS are not sufficient to result in any significant rise in 2,3-DPG and thereby in a shift of the ODC. Data from experimental hypobaric hypoxia exposures showed a small but significant increase in DPG after 2.5 h of sustained hypoxia (15). However, one must keep in mind that other factors may produce a shift of the ODC and changes in 2,3-DPG levels; during apnea and hypopnea, the resulting alveolar hypoventilation increases arterial PCO2 and lowers the pH. A low pH shifts the ODC to the right (Bohr’s effect), but the concentration of 2,3-DPG is also decreased by acidosis (14). Therefore, the episodes of successive respiratory acidosis during sleep might neutralize the hypoxia-related induction of 2,3-DPG accumulation. Opposite effects of pH and hypoxia on 2,3-DPG were shown by Lenfant et al. (9). These authors demonstrated that the increase of 2,3-DPG at high altitude does not occur when the volunteers receive acetazolamide, which prevents the hypoxia-induced alkalosis.

Choice of monitoring parameters. We did not use the apnea index (AI) to separate patients because AI is based exclusively on the study of respiratory events (cessation of respiration), whereas the ODI takes into account the variations of SpO2 equal to or greater than 4%. We also measured the time spent below 90% of SpO2 because it better reflects actual hypoxemia. Indeed, when investigating OSAHS without preexistent respiratory pathology and with normal basal PaO2, one can observe very disturbed PSG parameters (AI and ODI), but the oscillations of the SpO2 may stay between 92 and 96%, for example.

Conclusion. This study, which included a substantial number of patients, demonstrates that patients with OSAHS without daytime hypoxemia do not have permanent oxyhemoglobin affinity changes.

REFERENCES


