Abnormal lipid peroxidation in patients with sleep apnoea

A Barcelò, C Miralles, F Barbé, M Vila, S Pons, A.G.N. Agustí


ABSTRACT: The prevalence of cardiovascular diseases is increased in patients with the obstructive sleep apnoea syndrome (OSAS). The fall and rise of arterial oxygenation that follows each apnoea may increase lipid peroxidation and contributes to explaining this association. In the present study, the authors determined lipid peroxidation in patients with OSAS and the effect of treatment with continuous positive airway pressure (CPAP).

Fourteen male patients with severe OSAS (59±5 apnoea h⁻¹ (±SEM) and 13 healthy nonsmoking, male volunteers of similar age were studied. Patients were studied at diagnosis and after treatment with CPAP for more than 1 yr (>4 h night⁻¹). A venous blood sample was obtained early in the morning after fasting all night. In patients with OSAS, a sample before and during sleep was also obtained. Low density lipoprotein (LDL) particles were isolated by sequential ultracentrifugation. Their level of oxidation was determined by the thiobarbituric acid assay (TBARs), and their susceptibility to oxidation by the lag phase measurement.

Patients with OSAS showed higher TBARs (28.1±2.8 versus 20.0±1.8 nmol malondialdehyde mg⁻¹ LDL protein⁻¹, p=0.02) and shorter lag phase values (83.8±3.4 versus 99.7±3.4 min, p=0.005) than controls. These differences were not due to the smoking status of the patient. Likewise, these values did not change significantly throughout the night yet, the lag phase value was significantly improved by treatment with CPAP (124.9±8.5 min; p<0.001).

These results indicate that obstructive sleep apnoea syndrome is associated with abnormal lipid peroxidation and that this is improved by chronic use of Continuous positive airway pressure. These results can contribute towards explaining the high prevalence of cardiovascular diseases seen in Obstructive sleep apnoea syndrome. Eur Respir J 2000; 16: 644–647.

The obstructive sleep apnoea syndrome (OSAS) is a frequent disease characterized by the occurrence of numerous episodes of absence of respiratory flow (apnoea) during sleep [1]. Each episode of apnoea is followed, in most patients, by a marked decrease in arterial oxygen saturation (SaO₂) that is rapidly normalized when ventilation resumes (hypoxia-reoxygenation) (H/R) [1]. Treatment with continuous positive airway pressure (CPAP) prevents the occurrence of apnoeas and the H/R episodes that follow them [2].

The prevalence of cardiovascular diseases is increased in OSAS [3–5]. The mechanisms underlying this association are unclear [6–8]. Because atherosclerosis is a hallmark of these vascular diseases, it is conceivable that OSAS may somehow contribute towards enhancing atherogenesis [6]. High plasma low density lipoprotein cholesterol (LDL) levels favour this process, especially if LDL particles have been previously oxidized [9, 10]. The authors hypothesized that this may occur in OSAS due to the repetition of H/R episodes throughout the night. Accordingly, a study that investigated: 1) the plasma levels of oxidized LDL (and its susceptibility to oxidation) in patients with untreated OSAS, as compared to healthy subjects was designed. Because the duration of apnoeas and the severity of the H/R episodes increases throughout the night [11, 12], these same variables were analysed before, during and after sleep; and 2) the effects of long-term treatment (>1 yr) with CPAP upon these indices of lipid peroxidation were also examined.

Methods

Subjects and ethics

Fourteen patients with OSAS and 13 healthy volunteers were studied. In patients, the diagnosis of OSAS was established by full polysomnography (Ultrasom Nicolett, Wis., USA). Each patient was studied twice: at diagnosis and after being effectively treated for at least 1 yr with CPAP (REM Star; Respiromics®, Murrysville, PA, USA). Five patients who did not use the device for a minimum of 4 h night⁻¹ (compliance was checked by the timer built up in the CPAP device) and two patients who refused to return to the laboratory were excluded from the second study. Thirteen healthy, nonsmoking, non-obese controls of a similar age, in whom OSAS was excluded clinically using criteria presented by KAPUNIAI et al. [13] were also studied. None of the patients had personal or familial history of cardiovascular disease or diabetes and none received
medication. This investigation was approved by the Ethics Committee of the institution (the Hospital Universitari Son Dureta). All participants signed their consent after being fully informed of the nature and characteristics of the study.

Protocol

Patients with OSAS (n=14) arrived at the institution at ~9 p.m., after fasting for at least 6 h. A heparinized venous catheter (Abbocat®-T, Dublin, Ireland) was inserted into an antecubital vein to allow serial blood sampling through the night without disturbing sleep. Three different sample were obtained from the patients (30 mL each) in ethylenediamine tetraacetic acid (EDTA) (1 g·L⁻¹): before going to sleep (sample A), after 5±1 h of sleep (sample B) and 1 h after awakening (sample C). This latter sample was followed by a fourth (10 mL, without anticoagulant) for general biochemical assessment (glucose, cholesterol, etc.) (sample D). Patients remained fasted during the study. In controls and patients studied after CPAP treatment, only two venous blood samples (samples C and D) were obtained at ~9 a.m., after fasting all night. Blood samples were immediately centrifuged during 15 min at 2500 revolution per minute (Jouan S.A., model CR4 22, Saint-Mannheim, Germany). LDL (density 1.019±1.063 g·mL⁻¹) was isolated by sequential ultracentrifugation [14]. A Shepadhex G-25 column (PD-10™, Pharmacia Biotech, Uppsala, Sweden) eluted with phosphate buffered saline (PBS) was used to eliminate salts from LDL samples. These were later filtered through 0.45 μm filters (Millipore, Cedex, France). Protein content was determined using the Bradford method [15]. To assess the level of LDL oxidation the thiobarbituric acid assay (TBA) was used. In brief, samples were heated with TBA under acidic conditions, and the amount of chromogen formed by the reaction of thiobarbituric acid reactive substances (TBARS) (a) and lag phase values (b) determined in sample C in controls and in patients with OSAS. At diagnosis, patients with OSAS showed a higher TBARS concentration (28.1±2.8 versus 20.0±1.8 nmol MDA·mg⁻¹ LDL protein, p=0.02) and more susceptibility to LDL oxidation (shorter lag phase value) (83.8±3.4 versus 99.7±3.4 min, p=0.005) than controls. This latter measurement, however, corresponds only to a subset of patients (fig. 1b), as only those in whom the available volume of LDL ensured adequate reproducibility of the measurement were included. TBARS concentration and lag phase values were similar in smoker and nonsmoker patients. In keeping with a recent study [21], no significant correlation between TBARS or lag phase values and: age, BMI, apnoea-hypopnoea index, mean or lowest SaO₂ value at night was found. In contrast, the TBARS concentration was negatively related to the lag phase values (Rho -0.72, p=0.03). The abnormal TBARS and lag phase values determined in patients with

Measurements

The lipid profile of the serum samples was determined by routine laboratory techniques (Hitachi 717; Boehringer Mannheim; Germany). LDL (density=1.019±1.063 g·mL⁻¹) was isolated by sequential ultracentrifugation [14]. A Shepadhex G-25 column (PD-10™, Pharmacia Biotech, Uppsala, Sweden) eluted with phosphate buffered saline (PBS) was used to eliminate salts from LDL samples. These were later filtered through 0.45 μm filters (Millipore, Cedex, France). Protein content was determined using the Bradford method [15]. To assess the level of LDL oxidation the thiobarbituric acid assay (TBA) was used. In brief, samples were heated with TBA under acidic conditions, and the amount of chromogen formed was measured at 532 nm (Shimadzu, UV-160, Kyoto, Japan). The absorbance was expressed in malondialdehyde (MDA) equivalents·mg⁻¹ of LDL protein using a standard curve for MDA [16]. To ensure reproducibility of data, measurements were obtained in triplicate. The susceptibility of LDL to oxidation was investigated by the so-called lag phase, a standard technique in the field [17, 18]. The kinetics of conjugated dienes during LDL oxidation was measured continuously by recording the absorbance at 234 nm every 5 min during a 4.5 h period. The intersection with the horizontal axis at a tangent to the slope of the propagation phase was used to calculate the lag phase (min) [19, 20].

Results

All patients suffered from severe OSAS (mean apnoea-hypopnoea index, 59±5 h⁻¹), and showed frequent and profound episodes of arterial desaturation (mean nocturnal SaO₂, 92.1±1.0%; mean lowest SaO₂ during sleep 61.9±4.9%). Eight patients were smokers, 3 referred systemic hypertension and 2 had suffered (presented) an acute myocardial infarction in the past. On average, patients used CPAP for 5.5±0.7 h·day⁻¹. Table 1 shows the main clinical and biological data of all the subjects studies. None of them changed significantly for the patients after treatment with CPAP.

Figure 1 shows the individual and mean thiobarbituric acid reactive substances TBARs (a) and lag phase values (b) determined in sample C in controls and in patients with OSAS. At diagnosis, patients with OSAS showed a higher TBARS concentration (28.1±2.8 versus 20.0±1.8 nmol MDA·mg⁻¹ LDL protein, p=0.02) and more susceptibility to LDL oxidation (shorter lag phase value) (83.8±3.4 versus 99.7±3.4 min, p=0.005) than controls. This latter measurement, however, corresponds only to a subset of patients (fig. 1b), as only those in whom the available volume of LDL ensured adequate reproducibility of the measurement were included. TBARS concentration and lag phase values were similar in smoker and nonsmoker patients. In keeping with a recent study [21], no significant correlation between TBARS or lag phase values and: age, BMI, apnoea-hypopnoea index, mean or lowest SaO₂ value at night was found. In contrast, the TBARS concentration was negatively related to the lag phase values (Rho -0.72, p=0.03). The abnormal TBARS and lag phase values determined in patients with

Table 1. – Mean±SEM values of several clinical and biochemical variables in patients with obstructive sleep apnoea syndrome (OSAS) (at diagnosis) and healthy subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients with OSAS</th>
<th>Healthy subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects n</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Age yrs</td>
<td>50±2*</td>
<td>44±2</td>
</tr>
<tr>
<td>BMI kg·m⁻²</td>
<td>31.7±1.2**</td>
<td>26.3±0.9</td>
</tr>
<tr>
<td>SBP mmHg</td>
<td>139±4*</td>
<td>123±4</td>
</tr>
<tr>
<td>DBP mmHg</td>
<td>82±3</td>
<td>77±3</td>
</tr>
<tr>
<td>Total cholesterol mmol·L⁻¹</td>
<td>5.5±0.2</td>
<td>5.2±0.2</td>
</tr>
<tr>
<td>Triglycerides mmol·L⁻¹</td>
<td>2.4±0.4*</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>LDL cholesterol mmol·L⁻¹</td>
<td>3.59±0.32</td>
<td>3.58±0.25</td>
</tr>
<tr>
<td>HDL cholesterol mmol·L⁻¹</td>
<td>1.26±0.12</td>
<td>1.31±0.12</td>
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</tbody>
</table>

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL: low density lipoprotein; HDL: high density lipoprotein. *: p<0.05; **: p<0.005.

Statistical analysis

Data is shown as the mean±SEM. The Student t-test (for paired or independent samples) and the one-way analysis of variance (ANOVA) were used to investigate the statistical significance of differences, as appropriate. Correlations between variables of interest were analysed using the Spearman rank test. A p-value lower than 0.05 was considered significant.
OSAS did not change significantly through the night (table 2). Effective treatment with CPAP during more than one year did not modify the increased TBARs value (27.9±3.2 nmol MDA·mg⁻¹ LDL protein, p=ns) but markedly decreased the oxidation susceptibility of LDL (124.9±8.5 min; p<0.001) (fig. 1).

Discussion

This study shows that abnormal lipid peroxidation occurs in patients with OSAS (fig. 1), and that it does not change appreciably through the night (table 2), and also chronic treatment with CPAP reduces the susceptibility of LDL to oxidation (fig. 1).

The mechanisms underlying the increased prevalence of cardiovascular diseases in OSAS [4, 5] are unknown [3, 6–8]. To gain further insight into these mechanisms, the basal level of lipid peroxidation (TBARs content) and the oxidation susceptibility of isolated LDL particles (lag phase) in patients with untreated OSAS [18] was determined. It was found that these patients had increased TBARs levels and shorter lag phase values than healthy controls (fig. 1). These measurements were significantly related and highly reproducible through the night (table 2). Further, these results could not be explained by the smoking status of the patient. Collectively, these results indicate that increased lipid peroxidation occurs in OSAS. This may provide a mechanistic explanation for the high prevalence of cardiovascular diseases reported in these patients [3–5]. In the keeping with this hypothesis, a recent publication by SARELAINEN et al. [21] demonstrated an increased concentration of autoantibodies against oxidized LDL in patients with OSAS. The results in this study, however, contradict a study by WALLI et al. [22] that has failed to identify abnormal lipid peroxidation in OSAS. Methodological differences between the study conducted by WALLI et al. [22] and the present study may probably explain this discrepancy. Finally, it was observed that chronic treatment with CPAP normalized the susceptibility of LDL particles to oxidation (fig. 1b), suggesting a potential beneficial effect of CPAP on excessive lipid peroxidation in OSAS. This was not the case in the study by WALLI et al. [22]. However, these authors assessed the effects of CPAP only after 1 night of treatment [22], while the patients in this study were treated for more than 1 yr.

This study has some potential limitations that deserve comment: 1) the small number of subjects included in the study limits the conclusions. These will have to be confirmed in a larger series of patients with OSAS. Also, the potential effects of abnormal lipid peroxidation in important end organs of these subjects, such as the heart or the brain, were not investigated. Future studies will also have to address this issue. 2) The controls were younger and had a lower BMI than patients with OSAS (table 1). However, differences were minor and probably of marginal biological significance. 3) In theory, obesity may have influenced the results independently of CPAP. The lack of TBARs changes after CPAP treatment may be explained by the absence of changes in BMI. However, the observation of less oxidation susceptibility of LDL after CPAP (fig. 1), in the absence of changes in BMI, suggests that obesity per se cannot explain the findings or at least not fully. 4) The fact that the effects of treatment with CPAP were not analysed in a randomized, controlled manner limit the strength of the conclusions. Finally, it is known that the oxidation of LDL is governed by three major factors: the amount of oxidizable substrate, the concentration of endogenous antioxidants, and the presence of oxidative stress [10, 23]. Given that the concentration of antioxidants in the patients was not

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**Table 2. Mean±SEM values of the thiobarbituric acid reactive substances (TBARs) and lag phase values determined in patients with obstructive sleep apnoea syndrome (OSAS) before, during and after sleep**

<table>
<thead>
<tr>
<th></th>
<th>Before sleep (~9 pm)</th>
<th>During sleep (~5 am)</th>
<th>After sleep (~9 am)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARs*</td>
<td>31.1±3.4</td>
<td>30.9±3.5</td>
<td>28.1±2.8</td>
</tr>
<tr>
<td>Lag phase min</td>
<td>89.9±4.6</td>
<td>96.6±3.3</td>
<td>83.9±3.4</td>
</tr>
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</table>

*nmol malondialdehyde·mg low density lipoprotein⁻¹. No significant difference between test times (ANOVA).
measured, its potential role in these patients remains speculative.

In summary, the results of this pilot study suggest that patients with obstructive sleep apnoea syndrome have abnormal lipid peroxidation and that chronic treatment with continuous positive airway pressure decreases the susceptibility of LDL to oxidation. These results may contribute towards explaining the relationship between obstructive sleep apnoea syndrome and cardiovascular disease. However, future studies will have to expand these results to a larger population, include measurements of antioxidants in plasma and investigate how this oxidative stress may impact important end organs of these patients.

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References