Skeletal muscle changes in patients with obstructive sleep apnoea syndrome

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Summary

Previous studies have shown that chronic hypoxia leads to changes in skeletal muscle structure (fibre size and type) and activities of several bioenergetic enzymes. Whether this occurs also in conditions characterised by intermittent hypoxia, such as the obstructive sleep apnoea syndrome (OSAS), is unknown. To explore this possibility, we obtained a needle biopsy of the quadriceps femoris in 12 consecutive stable outpatients with severe OSAS (52 ± 9 year, apnoea–hypopnoea index 70 ± 14 h⁻¹) (x ± SD) and in six healthy volunteers (49 ± 8 year), where we quantified fibre type, size and protein content, as well as phosphofructokinase (PFK) and cytochrome oxidase (CytOx) activities. We found that fibre-type distribution was similar in patients and controls. In contrast, the diameter of type II fibres (74 ± 10 μm vs. 56 ± 11 μm, P < 0.05) and protein content (100 ± 14 vs. 88 ± 8 μg/mg) was higher in patients with OSAS. Likewise, we observed upregulation of CytOx (0.93 ± 0.38 vs. 0.40 ± 0.22 μkat/mg protein, P < 0.01) and PFK activities (5.35 ± 4.8 vs. 1.3 ± 1.3 μkat/mg protein, P < 0.05) in patients with OSAS. These results show that, paralleling which occurs in conditions characterised by continuous hypoxia, patients with OSAS (and intermittent hypoxia) also show structural and bioenergetic changes in their skeletal muscle.

KEYWORDS

OSAS; Cytochrome oxidase; Phosphofructokinase; Fibre-type morphometry

Introduction

Chronic exposure to hypoxia leads to changes in muscle structure (fibre size and type) and activity of several bioenergetic enzymes. These changes have been well characterised both in healthy subjects living at altitude¹,² and in patients with a variety of diseases characterised by chronic hypoxia, including chronic heart failure (CHF),³ peripheral arterial disease⁴ and chronic obstructive pulmonary disease (COPD).⁵⁻⁷ In these latter patients, for instance, there is atrophy (decreased fibre size),⁸ a shift towards type II fibres⁸ and increased activity of phosphofructokinase (PFK), the rate-limiting glycolitic enzyme⁵ and cytochrome oxidase (CytOx) the terminal enzyme of the mitochondrial electron transport chain.⁷

Whether this occurs also in clinical conditions characterised by intermittent (vs. chronic) exposure to hypoxia is unknown. Patients with obstructive sleep apnoea syndrome (OSAS) offer a naturally occurring example where to test this hypothesis because it is characterised by the repetition of
many episodes of arterial oxygen desaturation followed by a rapid normalisation of oxygen tension upon the restoration of ventilation.9,10 To date, the only "skeletal" muscle studied in patients with OSAS are those of the upper airway (the genioglossus11 and the musculus uvulae12). These previous studies have demonstrated that, compared to controls, patients with OSAS showed a shift towards type II fibres,12 higher glycolytic enzyme activities12 and more in vitro fatigability.11 However, because upper airway muscles in these patients are subjected to enormous mechanical changes during sleep, these changes cannot be directly attributed to hypoxia. Further, despite that these abnormalities tend to improve with treatment,11,12 given that the use of continuous positive airway pressure (CPAP) prevents both airway collapse and arterial desaturation, the pathogenic responsibilities of the increased mechanical load vs. tissue hypoxia are impossible to dissociate in vivo. To overcome this limitation, in the present study we sought to compare the structure (fibre-type, size and protein content) and activity (PFK and CytOx activity) of a limb muscle (quadriceps femoris) in patients with OSAS and in healthy subjects of similar age and exercise habits.

Methods

Population

We studied 12 consecutive stable outpatients with OSAS (recently diagnosed and without CPAP therapy) and six healthy volunteers of similar age and smoking history (Table 1). To avoid potential confounding factors, we studied only male sedentary subjects and we excluded patients with known neuromuscular disorders, cardiac failure, diabetes mellitus, alcoholism, COPD, daytime arterial hypoxemia (PaO2<75 mmHg) and/or those receiving treatment with oral steroids. In all patients, OSAS was diagnosed by standard polysomnography (Ultrasom Nicolette, Madison, WI) according to national and international guidelines.13,14 Control subjects were recruited from our respiratory unit. In them OSAS was excluded on the basis of the clinical history and sleep oximetry recording (Biochem International Incorporated, Waukesha, WI). All participants signed their informed consent after being fully aware of the nature, characteristics and risks of the study, which had been approved by the Ethical Review Board of our institution.

Table 1  Anthropometric and functional data (mean±SD) of the population studied.

<table>
<thead>
<tr>
<th></th>
<th>Patients with OSAS (n = 12)</th>
<th>Healthy subjects (n = 6)</th>
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<tbody>
<tr>
<td>Age (year)</td>
<td>52 ± 9</td>
<td>49 ± 8</td>
</tr>
<tr>
<td>Smoking history (pack-years)</td>
<td>15 ± 2</td>
<td>12 ± 5</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>35 ± 7</td>
<td>28 ± 4*</td>
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<tr>
<td>FEV₁ (% reference)</td>
<td>85 ± 11</td>
<td>104 ± 10*</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>84 ± 6</td>
<td>84 ± 5</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>79 ± 8</td>
<td>86 ± 6</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>42 ± 3</td>
<td>36 ± 2**</td>
</tr>
<tr>
<td>Apnoea-hypopnoea index (h⁻¹)</td>
<td>70 ± 14</td>
<td>NA</td>
</tr>
<tr>
<td>Mean nocturnal SaO₂ (%)</td>
<td>82 ± 12</td>
<td>97 ± 2**</td>
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NA: not available. FEV₁: forced expiratory volume in the 1st second; FVC: forced vital capacity; PaO₂ and PaCO₂: day-time values of the arterial partial pressure values of oxygen and carbon dioxide; SaO₂: arterial oxyhemoglobin saturation; *P<0.05,**P<0.01.

Lung function

Forced spirometry (GS W. Collins, USA) was obtained in all participants.15 Reference values were those of a Mediterranean population.16 Likewise, an arterial blood sample was obtained by radial artery puncture under local anaesthesia. This was immediately assayed for PaO₂, PaCO₂ and pH (IL BG3, Izasa, Spain).

Muscle biopsy

Muscle biopsy was obtained 1–2 weeks after polysomnography. A standard percutaneous needle biopsy technique was used to obtain several (~4) muscle samples from the lateral portion of muscle quadriceps femoris (at the mid-thigh level), under local anaesthesia, as previously reported in our laboratory.7 Enzyme activities (PFK and CytOx) were measured in fresh tissue. The remaining tissue sample was frozen immediately after the biopsy in isopentane (in liquid nitrogen) and stored at −80°C until analysis.

Measurement of enzymes activities

Fresh muscle samples were weighed (wet weight: 30–90 mg) and homogenised in 250 mM sucrose/1 mM HEPES/0.2 mM EDTA buffer (pH 7.0) in a Teflon/glass homogeniser, held in an ice bath.
Aliquots of this homogenate were assayed for total protein content\(^{17}\) and CytOx activity (E.C.1.9.3.1). The latter was determined at 37°C according to the protocol described by Wharton et al.,\(^{18}\) as previously described in our laboratory.\(^{2,19}\) The remaining homogenate was sonicated in an ice bath for 1 min, and then centrifuged at 2500 rpm, 4°C for 5 min. The pellet was discarded, and the supernatant was used for the determination of the activity of PFK (E.C.2.7.1.56),\(^{20}\) as well as soluble protein content.\(^{18}\) All enzymatic activities were measured in a Shimadzu spectrophotometer (Shimadzu, Nagoya, Japan) with continuos optical absorbance register at 37°C for 5 min. All reagents were obtained from Sigma Chemical Co (St. Louis, MO, USA).

**Muscle fibre-type analysis**

Muscle samples were cut 6\(\mu\)m thick in a cryostat (\(-20°C\)). Type I ("slow twitch") and II ("fast twitch") fibres were identified by the standard adenosine triphosphate (ATPase) (at 9.4 and 4.6 pH pre-incubation) and the standard reduced nicotinamide adenine dinucleotide tetrazolium (NADH-TR), as described previously.\(^{21,22}\) Morphometric evaluation was done using a semiautomatic system (Sigmascan, Jandel Scientific, Erkrath, Germany). Two independent observers quantified, in at least 100 fibres: (a) the percentage of type I and II fibres; and (b) the least diameter for each fibre type, which is minimally influenced by the cutting angle during the sampling process.\(^{21,22}\)

**Statistical analysis**

Results are shown as mean ± SD. The reproducibility of the morphometric measurements between the two observers was tested using the intraclass correlation coefficient.\(^{23}\) The Mann–Whitney test was used to compare values measured in patients and in controls. Potential relationships between variables of interest were evaluated using the Spearman coefficient. A \(P\) value lower than 0.05 was considered statistically significant.

**Results**

Table 1 shows the main anthropometric, smoking history (all individuals were ex-smokers), lung function and sleep variables determined in inpatients with OSAS and in healthy subjects. Age was similar in both groups. Patients were more obese than healthy controls. Spirometry and arterial blood gases were normal in both groups. All patients with OSAS had and apnoea–hypopnoea index (AHI) higher than 60 h\(^{-1}\) and showed frequent and profound episodes of arterial desaturation during sleep. As expected, mean nocturnal arterial oxygen saturation was lower (\(P<0.01\)) in patients with OSAS (82 ± 12%) than in controls (97 ± 2%).

Because morphometric measurements were highly concordant between the two observers (\(r = 0.92, P < 0.01\)) we used average values for the analysis. The percentage of fibre types was similar in patients and controls (Table 2). Yet, fibre size was higher in patients with OSAS, particularly that of type II fibres (Table 2). Likewise, total muscle protein content was higher in patients than in control subjects (Table 2). As shown in Fig. 1, patients with OSAS showed higher CytOx (0.93 ± 0.38 vs. 0.40 ± 0.22 \(\mu\)kat/mg protein, \(P<0.01\)) and PFK activities (5.35 ± 4.8 vs. 1.3 ± 1.3 \(\mu\)kat/mg protein, \(P<0.05\)) than healthy controls.

Neither the AHI nor the mean nocturnal SaO\(_2\) were significantly related to the morphometric parameters (percentage and size of fibres) or to the enzyme activities determined.

**Discussion**

This study shows that patients with OSAS present structural (increased diameter of type II fibres and increased protein content) and metabolic abnormalities (upregulation of CytOx and PFK activities) in their skeletal muscle.

**Previous studies**

To the best of our knowledge, no previous study has investigated the structure and bioenergetic
metabolism of skeletal (quadriceps femoris) muscle in patients with OSAS. Yet, some previous reports already suggested a potential alteration of muscle bioenergetics in OSAS. For instance, Williams and Moshenifar reported that oxygen uptake in these patients increases in parallel to cardiac output and suggested the presence of a pathological oxygen supply dependency in OSAS. 24 A later study by Vanuxem et al reported that patients with OSAS showed lower oxygen uptake and lower blood lactate concentration during peak exercise than healthy subjects (although this occurred at lower exercise intensity), and also suggested the presence of impaired muscle energy metabolism.25 However, muscle biopsies were not obtained in any of these two studies and, therefore, these hypotheses could not be tested directly. Likewise, none of them reported morphometric measurements in the skeletal muscle in these patients.

Morphometric analysis

The percentage of the different fibre types in patients with OSAS was not significantly different from that seen in healthy subjects. These results agree with several studies that have examined muscle fibre-type proportions following exposure to hypobaric hypoxia in healthy subjects.26,27 Generally, most of these studies have reported no adaptations in fibre-type proportions. These observations contrast with those reported in diseases characterised by chronic hypoxia, such as COPD28 or chronic heart failure,29 where increases in type II fibres have been described. Although this can be due to the different type of hypoxic insult suffered (chronic vs. intermittent), other factors cannot be excluded. For example, at variance with the patients with OSAS studied here with active life, patients with COPD or CHF often adopt an extreme sedentary life-style, which by its own can have a significant impact on skeletal muscle structure.30

The diameter of muscle fibres, particularly that of type II fibres (as well as their protein content), was increased in patients with OSAS compared with healthy subjects (Table 2). These observations contrast with those reported in COPD28 or CHF,29 where reduced fibre size (atrophy) have been described. Again we think this can be due to the different type of hypoxic insult suffered (chronic vs. intermittent) and deconditioning.30 On the other hand, it is possible that the increased body mass index (BMI) that characterises OSAS9 may have also contributed to the higher muscle fibre size and higher muscle protein content observed in the present study.31 In theory, another factor that can influence the increase in the size of type II fibres observed here could be the activity pattern of the patients. However, this is unlikely in our study because both groups of subjects had a similar level of physical activity.

Our results in limb muscle also contrast with those previously reported in upper airway muscles of patients with OSAS.11,12 In the latter muscles, there seems to be an increase in the percentage of type II fibres in OSAS. However, as discussed above, these upper airway muscles in OSAS are exposed to repetitive, short-lasted, maximal stress during sleep, which can contribute to explain the increase of type II fibres.11,12 In fact, the absence of such changes in limb skeletal muscle suggests that such mechanical stress, and not intermittent hypoxia, is the main mechanism explaining them.

Enzyme activities

We found that the activities of CytOx and PFK were higher in patients with OSAS than in healthy controls (Fig. 1). Because the activity of oxidative

Figure 1 Individual and mean (bars) values of CytOx and PFK activity in healthy subjects and in patients with OSAS.
enzymes correlates directly with oxygen consumption, the former observation is in keeping with the increased resting energy expenditure described in patients with OSAS. There are several mechanisms that can potentially explain the upregulation in CytOx activity. (1) Hypoxia: because a similar increase in CytOx activity has been described in other clinical conditions characterised by continuous tissue hypoxia, an obvious candidate is intermittent hypoxia due to OSAS. Under these circumstances, an increase in CytOx activity may help to sustain metabolic flux rates by increasing the blood to mitochondrion PO2 gradient. At variance with this possibility, however, we did not find a significant correlation between the AHI or mean nocturnal SaO2 and CytOx activity. Yet, it is possible that the narrow range of disease severity of the patients studied here (all of them with severe OSAS) may have limited the possibility of finding such relationship. It is also possible that other variables, such as the area under the SaO2 curve at night, or the number of episodes of desaturation may be better suited to estimate tissue oxygenation in these patients. (2) Obesity: despite that patients with OSAS were more obese (higher BMI) than healthy subjects (Table 1), we think that this is unlikely to explain our findings because obesity reduces (not enhances) energy expenditure, thus oxidative metabolism. Further, we did not find a significant correlation between CytOx activity and BMI (data not shown). (3) Level of physical activity: as we mentioned above, patients and controls have similar activity pattern and none of them participated in regular sport activities, so differences in CytOx activities cannot be explained by this factor. (4) Aging: oxidative metabolism decreases with age. Yet, patients and controls have similar age (Table 1) and therefore aging cannot explain our findings. (5) Smoking: several compounds of cigarette smoke such as carbon monoxide and nitric oxide can influence CytOx activity. However, none of the subjects included were a current smokers. (6) Drug effects: high doses of &beta;2-agonists can increase oxygen uptake (thus presumably, CytOx activity) and corticosteroids can induce myopathy. We exclude these effects because none of the subjects used them. And finally, (7) oxidative stress and systemic inflammation: both have been described in patients with OSAS and may therefore contribute to these muscle abnormalities. This possibility will have to be explored in future studies.

With respect to PFK activity our results agree with those reported in other situations of chronic hypoxia, such as COPD and high altitude living. Therefore, the increased PFK activity observed in our patients with OSAS can be explained by their intermittent exposure to hypoxia during sleep. This change can be viewed as an adaptive mechanism aimed at facilitating glycolitic oxidation under hypoxic conditions. These results, however, contrast with those of Series et al. who did not find significant differences in genioglossus of patients with OSAS compared with chronic snorers. However, in terms of the mechanical conditions of the genioglossus, snorers cannot be considered "healthy" controls. Further, as discussed above, the genioglossus in patients with OSAS is exposed to heavy mechanical forces during sleep that can clearly interfere with enzyme activities. Thus, our results suggest that intermittent hypoxia in OSAS upregulates PFK activity in limb muscles.

Our results differ from those reported at extreme high altitude in healthy subjects (> 5000 m), where a decrease in oxidative metabolism with minimal changes in glycolitic enzymes have been reported. However, they are in keeping with findings at moderately high altitude (3500–5000 m), where upregulation of these enzymes like that seen in our study, have been described. Also, studies of oxidative enzymes in peripheral muscle of hypoxic patients with COPD or peripheral arterial disease have also shown similar results. Finally, it has to be kept in mind that patients with OSAS are submitted to intermittent hypoxia whereas subjects at high altitude are exposed to continuous hypoxia, a factor that can also contribute to the observed differences.

Conclusions

Our study shows that skeletal muscle structure (increased muscle fibre size and protein content) and bioenergetics (upregulated CytOx and PFK activities) are abnormal in patients with OSAS. The functional implications of these observations, as well as their potential reversibility with CPAP treatment and the precise underlying mechanisms will have to be elucidated in future studies.

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References


