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1 **Transferrin saturation is independently associated with the severity of obstructive sleep**  
2 **apnea syndrome and hypoxia among obese subjects**

3

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26 **ABSTRACT**

27 Introduction and aims: Obstructive sleep apnea syndrome (OSAS) is a frequent complication  
28 of obesity. Intermittent chronic hypoxia which frequently results from OSAS could modulate  
29 the systemic control of iron metabolism and alter serum iron parameters, especially among  
30 obese patients. Aims: to evaluate whether serum parameters of iron bioavailability and storage  
31 (primary), as well as age, waist circumference, arterial hypertension and tobacco use  
32 (secondary) are associated with OSAS severity and/or hypoxia. Methods: design: a single-  
33 center retrospective study with prospective data collection; inclusion criteria: consecutive  
34 patients referred for initial assessment for obesity underwent nocturnal respiratory polygraphy  
35 and iron status serum assessment within a 3-month period. The adjusted analyzes were  
36 performed using ANOVA and reported as adjusted means and 95% confidence interval (95%  
37 CI). Results: 13 men and 56 women were included. OSAS prevalence: 72% (n=50). Ferritin  
38 (mean±SD, 260±276 vs. 111±89 µg/l, p=0.01) and transferrin saturation (31±10 vs. 24±9%,  
39 p=0.002) were significantly higher in case of moderate/severe OSAS than in absent/mild  
40 OSAS, independently from gender and tobacco use. Serum iron (19.4 µg/l [CI95%, 16.5-22.3]  
41 vs. 16.2 µg/l ([14.1-18.2], p=0.056) and transferrin saturation (31.5 % [26.3-36.7]) vs. 25.3%  
42 [21.6-29.1], p=0.043) were higher when time under oxygen saturation <90% was >15%. Age  
43 (mean±SD, 51±11 vs. 41±12 yr, p=0.001), waist circumference (136±18 vs. 123±12 cm,  
44 p=0.003), arterial hypertension (59% (n=13/22) vs. 23% (n=11/47), p=0.004) and tobacco use  
45 (64% (n= 14/22) vs. 32% (n= 15/47), p=0.01) were significantly greater in moderate/severe  
46 OSAS than in absent/mild OSAS. Conclusions. Transferrin saturation was associated with  
47 OSAS severity and time under hypoxia. This suggests a relationship between OSAS-induced  
48 hypoxia and iron metabolism among obese patients.

49

50 **Keywords:** obesity; metabolic syndrome; iron; obesity surgery.

## 51 INTRODUCTION

52 Obstructive sleep apnea syndrome (OSAS) is defined by recurrent episodes of apnea or  
53 hypopnea during sleep that result from complete or partial upper airway collapse. It leads to  
54 chronic intermittent hypoxia (1). OSAS affects about 4% of the general population (2), but its  
55 prevalence can reach 30 to 50% among obese patients (3). Identifying and treating OSAS  
56 among obese patients is essential, because OSAS facilitates the onset of metabolic and  
57 cardiovascular diseases (4), and favors weight gain and aggravation of obesity (5–7).

58 Iron metabolism is finely tuned. Serum iron is mainly provided by macrophages during the  
59 erythrophagocytic process that enables iron recycling and by enterocytes in the course of  
60 digestive absorption of iron from nutrients. Serum concentration of iron and transferrin iron  
61 saturation levels are indicators of the iron that is bioavailable for cells. Hepcidin controls iron  
62 homeostasis (8,9). Hepcidin is a peptide mainly synthesized by hepatocytes and secreted into  
63 the plasma. It modulates the expression of ferroportin, the only known iron exporter from  
64 cells towards plasma. An abnormal decrease in hepcidin levels in plasma increases the serum  
65 iron concentration and transferrin saturation, exposing the patient to iron overload, which, in  
66 turn, increases serum levels of ferritin, the protein involved in the storage of excess iron  
67 (9,10). An abnormal increase in hepcidin levels decreases transferrin saturation and exposes  
68 the subject to iron deficiency (8). Hepcidin expression is increased by iron storage and  
69 inflammation (8,9). Conversely, hepcidin expression is decreased by anemia and hypoxia,  
70 through mechanisms that could involve erythropoietin, hypoxia inducible factor (11,12) and  
71 erythroferrone (13) which is synthesized by erythroblasts during erythropoiesis (14). In case  
72 of hypoxia, the down-regulation of hepcidin expression becomes a priority, irrespective of  
73 body iron levels (15); this leads to iron mobilization and an increase in transferrin saturation,  
74 in order to provide adequate amounts of iron for the erythropoiesis process, which is activated  
75 to compensate for the hypoxia.

76 OSAS has been reported to be associated with polycythemia (16) and increased hematocrit  
77 levels (17). In addition, erythropoietin plasma concentrations are higher among patients with  
78 OSAS (18). Therefore, we hypothesize that among obese patients with OSAS, an increase in  
79 iron transferrin saturation could be a marker for the severity of OSAS and hypoxia. The aims  
80 of our study were to evaluate whether serum parameters of iron bioavailability and storage  
81 (primary), and age, waist circumference, arterial hypertension and tobacco use (secondary)  
82 are associated with OSAS severity and/or hypoxia.

83

## 84 **PATIENTS AND METHODS**

### 85 **Study design and population**

86 This was a single-center retrospective study based on prospective data collection. The 69  
87 patients were consecutively recruited from June 1<sup>st</sup>, 2016 to April 23, 2017 in the Nutrition  
88 Unit of the University Hospital of Rennes (CHU Rennes), Rennes, France. The study included  
89 all patients eligible for obesity surgery according to the French recommendations, i.e. body  
90 mass index (BMI)  $\geq 40$  or BMI  $\geq 35$  kg/m<sup>2</sup> with obesity complications (cardiovascular diseases  
91 including arterial hypertension, OSAS, type 2 diabetes, disabling osteoarticular disease, non-  
92 alcoholic steatohepatitis). They underwent a systematic nocturnal respiratory polygraphy to  
93 screen for OSAS as part of the initial assessment. The exclusion criteria were: previous  
94 diagnosis of OSAS, time between the nocturnal respiratory polygraphy and the plasma iron  
95 status assessment  $>3$  months, history of obesity surgery (except gastric banding), ongoing iron  
96 supplementation, diseases known to be associated with iron metabolism dysregulation  
97 (genetic hemochromatosis, active chronic inflammatory disease), and pregnancy. Since this  
98 retrospective study was based on routine practice, informed consent was not required by  
99 French law.

100

101 **Obesity-related diseases and therapy**

102 Patients were considered to have type 2 diabetes in the following cases: fasting blood glucose  
103  $\geq 7.0$  mmol/l, prior history of diabetes, oral antidiabetic drug use and/or insulin therapy.

104 Fasting hyperglycemia was defined as fasting blood glucose  $\geq 5.6$  mmol/l. Arterial  
105 hypertension was defined as systolic (SBP) or diastolic (DBP) blood pressures  $\geq 140$  or  $\geq 90$   
106 mmHg, respectively, or if patients were under antihypertensive therapy. Metabolic syndrome  
107 was defined according to the International Diabetes Foundation 2005 criteria (4). History of  
108 tobacco use, respiratory diseases (chronic obstructive pulmonary disease, asthma), or chronic  
109 inflammatory diseases (systemic, rheumatic or gastrointestinal) were reported. Drugs that can  
110 interfere with iron metabolism, such as proton pump inhibitors (PPIs), nonsteroidal anti-  
111 inflammatory drugs (NSAIDs), aspirin, or that affect sleep, such as psychotropes  
112 (antidepressants, benzodiazepines, hypnotics), were identified.

113

114 **Anthropometric data**

115 Weight, height, body mass index, and waist and hip circumferences were measured in all  
116 subjects the day of the nocturnal respiratory polygraphy. After one night fasting, body  
117 composition was measured at 8 am using bioimpedance analysis (Bodystat 1500, Bodystat Ltd,  
118 Isle of Man, UK). Patients were advised to restrict themselves to routine physical activity the  
119 day before. After skin swabbing with 70% ethanol, four adhesive electrodes (3M Red Dot T,  
120 3M Health Care, Borken, Germany) were placed on the dorsal side of the left hand, left wrist,  
121 left foot, and left ankle while the patient was supine with palms facing inwards. An electrical  
122 current of 0.8 mAmp was produced at 50 KHz for five seconds by the bioimpedance analyser.  
123 The following data was collected: fat mass and fat-free mass (% of weight and kg), and total  
124 body water (%).

125

126 **Nocturnal respiratory polygraphy**

127 Nocturnal respiratory polygraphy was performed in all patients using the CID-Lx polygraph  
128 (CIDELEC, Angers, France). All graphs obtained during sleeping time were analyzed by a  
129 senior pneumologist specialized in sleep disorders (BD). Apnea was defined by a complete  
130 cessation of nasal-oral airflow for at least 10 seconds. The apnea-hypopnea index (AHI) was  
131 defined as the number of apneas or hypopneas per hour of sleep. OSAS was defined as AHI  
132  $\geq 5$ /hour. The severity of OSAS was defined as mild if  $5 \leq \text{AHI} \leq 15$ , moderate if  $15 < \text{AHI} \leq 30$ , or  
133 severe if  $\text{AHI} > 30$ . Hypoxia was defined as the percentage of time with arterial oxygen  
134 saturation  $< 90\%$  at 15% of sleeping time or more. The lowest oxygen saturation value was  
135 recorded.

136  
137 **Iron serum parameters and blood analyses**

138 Measurements of serum iron parameters were part of the systematic fasting blood analyses;  
139 the measurement methods are shown in brackets: blood cell count (impedance flow  
140 cytometry), prothrombin time (chronometry), glucose (hexokinase colorimetric assay),  
141 glycated hemoglobin (in mmol/mol hemoglobin according to the recommendations of the  
142 International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and in %  
143 according to the National Glycohemoglobin Standardisation Program), albumin (bromocresol  
144 green colorimetric assay), transthyretin (immunoturbidimetry), C-reactive protein (CRP)  
145 (latex enhanced immunoturbidimetry), alanine aminotransferase (ALT), aspartate  
146 aminotransferase (ALT),  $\gamma$ -glutamyl-transpeptidase ( $\gamma$ GT) (all three by spectrophotometry  
147 according to the recommendations of the IFCC), triglycerides, total and HDL-cholesterol  
148 (colorimetry), and LDL-cholesterol (calculated from the Friedewald formula). Plasma iron  
149 parameters were assessed on heparinized plasma. Serum iron concentrations were determined  
150 by the colorimetric method (ferrozine assay), transferrin concentration, by

151 immunoturbidimetry, and ferritin by electrochemiluminescence (Cobas 8000 Roche). The  
152 transferrin saturation coefficient (TSC), and total transferrin binding capacity (TIBC) were  
153 calculated. The biological analyses were all performed in the Department of Biochemistry at  
154 CHU Rennes.

155

### 156 **Study endpoints**

157 The primary endpoint was the severity of OSAS, as defined by the AHI severity categories  
158 described above in the 'Nocturnal respiratory polygraphy' section. The secondary endpoints  
159 were the percentage of time under hypoxia, i.e. oxygen saturation <90%, and the lowest  
160 oxygen saturation value, serum iron parameters, anthropometric data, body composition,  
161 obesity-related diseases, age, tobacco use, and the metabolic syndrome parameters (waist  
162 circumference, arterial pressure, plasma glucose, triglycerides, and HDL-cholesterol). The  
163 numbers of patients in each category are shown in Tables 2, 3 & 4.

164

### 165 **Statistical analysis**

166 Quantitative variables are described as numbers (percentage) and means  $\pm$  standard deviation  
167 (SD). The groups were compared using Student's test (2 groups) or ANOVA tests (>2 groups)  
168 when the distribution was normal, or using Mann-Whitney or Wilcoxon (two groups) non-  
169 parametric tests otherwise. For qualitative variables, the numbers and percentage are  
170 presented for each category. The groups were compared using  $\chi^2$  parametric or Fisher non-  
171 parametric tests (theoretical number <5). The adjusted analyses were performed using  
172 ANOVA and presented as adjusted means and 95% confidence interval (95% CI). The  
173 significance threshold was 5% for all analyses. The analyses were carried out on SAS  
174 software, version 9.4.

175



## 176 RESULTS

### 177 Patients' enrollment and characteristics

178 The study flow chart is shown in **Figure 1**. Among the 69 patients included, 13 were men  
179 (19%) and 56 were women (81%). The patient characteristics are summarized in **Table 1**.

180 Among the eight patients with type 2 diabetes, five were treated with metformin, five with  
181 glucagon-like peptide (GLP)-1 analog, and two with insulin. Their mean glycated hemoglobin  
182 was 6.97% (range 6.30-8.40).

183

### 184 OSAS prevalence and severity

185 The overall prevalence of OSAS was 72% (n=50/69), including 40% (n=28) with mild OSAS,  
186 13% (n=9) with moderate OSAS, and 19% (n=13) with severe OSAS (**Table 1**). The mean  
187 oxygen saturation was 91.6±3.0%. Twenty-seven percent (n=19) of the patients had minimum  
188 oxygen saturation ≤75%, and 32% (n=22) had oxygen saturation below 90% for more than  
189 15% of the recording time (**Table 1**).

190

### 191 Relationships between iron parameters, OSAS severity, and hypoxia

192 Bio-clinical data according to the severity of OSAS is presented in **Table 2**. Serum iron  
193 concentration, transferrin saturation, and ferritin were significantly higher in the  
194 moderate/severe OSAS group than in the absent/mild OSAS group. After excluding the  
195 patients on PPIs, NSAIDs, aspirin, or psychotropes (n=27), the differences regarding serum  
196 iron parameters remained statistically significant (data not shown). Plasma CRP and  
197 hemoglobin were not statistically different. Transferrin saturation was associated with both  
198 the severity of OSAS (evaluated according to AHI) (**Table 3**) and with time with oxygen  
199 saturation <90% (**Table 4**), independently from gender, tobacco use, age, arterial  
200 hypertension, waist circumference, blood glucose and triglycerides.  $\gamma$ GT levels were

201 independently associated with the severity of OSAS (**Table 3**). Iron and CRP concentrations  
202 were higher when time under oxygen saturation <90% was >15% (**Table 4**). Transaminases,  
203 prothrombin time, transferrin and albumin levels were not statistically related to OSAS  
204 severity nor to time in hypoxia (**Tables 3 and 4**).

205

#### 206 **Other variables associated with OSAS and its severity**

207 As expected, patients with moderate/severe OSAS had more frequent and more marked  
208 hypoxia and more time with oxygen saturation <90% (**Table 2**). Patients with  
209 moderate/severe OSAS were significantly older, had larger waist circumference, more  
210 frequently presented arterial hypertension, and had higher fasting blood glucose and  
211 triglyceride levels. They were also more often smokers than patients with absent or mild  
212 OSAS. The group with moderate/severe OSAS was more likely to be on anti-hypertensive  
213 drugs than the absent/mild OSAS group (11 (50%) vs. 10 (21%); p=0.016). There was no  
214 difference in the use of anti-diabetic treatment, NSAIDs, PPIs, psychotropes, statins or  
215 fibrates between the absent/mild OSAS and moderate/severe OSAS groups.

216

#### 217 **DISCUSSION**

218 In a group of obese patients eligible for obesity surgery, the severity of OSAS, defined by  
219 AHI and the time in hypoxia – i.e. peripheral oxygen saturation <90% - were both  
220 independently associated with an increase in transferrin iron saturation, regardless of gender,  
221 tobacco use, biological inflammation, metabolic syndrome parameters, or drug therapy.  
222 Serum iron levels were also related to time in hypoxia, whereas the serum transferrin  
223 concentration was not, suggesting that the increase in serum transferrin saturation is mostly  
224 related to an increase in serum iron.

225 The relationship between iron metabolism, metabolic syndrome, obesity and insulin resistance  
226 is well known. Whether iron metabolism disturbances participate in metabolic syndrome or  
227 are one of its consequence remains debated (19). Our study suggests that hypoxia secondary  
228 to OSAS could have an important role in the relationship between iron metabolism, and  
229 metabolic syndrome/overweight/obesity.

230 Among patients with OSAS, obesity and/or insulin resistance, sub-clinical inflammation  
231 could lead to a decrease in both serum iron and transferrin saturation levels through IL6 and  
232 an increase in serum hepcidin (20), the key regulator of iron metabolism. This hypothesis is  
233 encouraged by the finding of increased iron storage, plasma ferritin and liver iron content  
234 together with increased hepcidin levels in hemosiderosis patients (21). Among severely  
235 obese patients, a lower transferrin saturation level is associated with an increase in hepcidin  
236 expression in adipose tissue macrophages (22).

237 Our data show that both serum iron and transferrin saturation levels are increased alongside a  
238 slight increase in CRP in moderate/severe OSAS, confirming an inflammatory context for  
239 these patients. It is also possible that hypoxia could have counteracted the expected decrease  
240 in transferrin saturation related to inflammation and subsequent increase in hepcidin level.

241 Indeed, hypoxia is a well-known repressor of hepatic hepcidin expression (23). Hypoxia could  
242 reduce the transcription of the *Hepcidin AntiMicrobial Peptide (HAMP)* gene that encodes for  
243 hepcidin by inducing Hypoxia inducible factor 2 (HIF-2) (24,25).

244 Unfortunately, because of the retrospective study design, hepcidin serum levels were not  
245 measured. However, our study results could point to a hypothetical role of hepcidin in the way  
246 in which hypoxia and inflammation act on iron metabolism among obese patients with OSAS.

247 A decrease in hepcidin plasma levels favored by hypoxia could in turn favor the increase in  
248 transferrin saturation. The level of hepcidin expression is controlled by numerous factors,  
249 especially at transcriptional level (26). Physiopathological events occurring during OSAS can

250 generate at least two antagonist signals that could modulate hepcidin transcription:  
251 inflammation and hypoxia. Inflammation increases hepcidin expression through the  
252 IL6/STAT3 signaling pathway, thus limiting iron release from macrophages and enterocytes  
253 and reducing transferrin saturation (26-29).

254 Our data shows that among patients with OSAS registering more than 15% of the time with  
255 oxygen saturation under 90%, there is a significant increase in transferrin saturation,  
256 alongside an increase in CRP. This finding supports the hypothesis that the degree of  
257 hypoxemia could counteract the effect of inflammation, favoring iron release from cells.  
258 Hypoxia could blunt the stimulating effect of inflammation on hepcidin expression (**Figure**  
259 **2**). Abakay et al reported a decrease in serum iron and an increase in hepcidin level among  
260 OSAS patients compared to non-OSAS patients (30). They also showed that AHI was  
261 negatively correlated with serum iron levels and positively with hepcidin levels. Among  
262 patients with the highest hepcidin levels, two clusters in the data distribution were observed.  
263 However, the authors did not analyze the relationship between hypoxia and iron  
264 parameters/hepcidin. Among Chinese patients with and without OSAS, Liu et al reported a  
265 slight increase in hepcidin serum levels among patients with severe OSAS (31). Unfortunately  
266 Liu et al did not assess serum iron metabolism parameters or inflammatory status, so that the  
267 relationships between inflammatory parameters and AHI cannot be studied, nor can the  
268 impact of time in hypoxia and maximum hypoxemia.

269 Our hypothesis that hypoxia could explain the increase in transferrin saturation in OSAS  
270 requires confirmation. The precedence of hypoxia over other regulators of iron metabolism  
271 has been previously reported among patients with anemia secondary to chronic diseases (32)  
272 and among beta-thalassemic patients (33,34). In these extreme conditions, hypoxia takes  
273 precedence over the effect of active inflammation or the increase in iron storage, resulting in  
274 promotion rather than inhibition of iron release from macrophages and enterocytes and

275 increase in transferrin saturation. In addition, hypoxia could modulate the expression of iron  
276 metabolism genes, thus contributing to an increase in transferrin saturation. In enterocytes,  
277 HIF-2 can increase the transcriptional expressions of the *Duodenal Cytochrome b (DCYTb)*  
278 and *Divalent Metal Transporter 1 (DMT1)* genes encoding respectively for a reductase and a  
279 transporter of iron involved in non-heminic iron uptake from the digestive lumen (35,36). The  
280 transcriptional expression of the *SLC40A1* gene encoding for the ferroportin protein, the iron  
281 exporter from enterocytes toward plasma, is also increased by HIF-2.

282 Except for  $\gamma$ GT, the markers of liver diseases did not differ between absent/mild and  
283 moderate/severe OSAS groups. Thus, cytolysis or hepatic insufficiency are not likely to be  
284 involved in the increases in serum iron and transferrin saturation.

285 After adjustment on potential confounding factors, we found no difference in serum ferritin  
286 concentrations according to the severity of OSAS or hypoxia. This is consistent with previous  
287 studies (38). Despite the statistically significant relationship between transferrin saturation  
288 level and severity of OSAS, serum transferrin saturation remained within the normal values.

289 However, as found in metabolic syndrome patients (39), we cannot rule out that, among  
290 patients with severe OSAS, the increase in transferrin saturation could favor the appearance in  
291 plasma of small amounts of non-transferrin-bound iron, an abnormal molecular form of iron  
292 rapidly taken up by the liver, pancreas and heart, which could participate in the cell damage  
293 related to oxidative stress (40). During OSAS it has been reported that its severity, defined by  
294 AHI, was not associated with systemic oxidative stress levels, but the minimum oxygenation  
295 desaturation level was associated with a decrease in antioxidant potential (41). This suggests  
296 an involvement of anti-oxidant defenses in condition of hypoxia during OSAS.

297 Our study has several limitations. The most important is that, due to the retrospective design,  
298 we did not measure serum levels of hepcidin, the key regulator of iron metabolism. Measuring  
299 hepcidin among our obese patients with OSAS could have helped us to understand the

300 mechanisms of the changes observed in iron parameters in relation to hypoxia and  
301 inflammation. A prospective study is clearly needed. Also because of the retrospective design,  
302 we did not assess antioxidant potential or non-transferrin-bound iron. Our hypothesis that  
303 hypoxemia could explain the increase in transferrin saturation in OSAS needs to be  
304 confirmed. Finally, we were not able to assess whether excessive alcohol intake could have  
305 played a role in  $\gamma$ GT increase, as well as in the increase in serum ferritin (37). Indeed  
306 excessive alcohol intake is known to be associated with the severity of OSAS.  
307 In conclusion, our data shows that transferrin iron saturation is independently associated with  
308 OSAS severity and the time in hypoxia. More studies are needed to evaluate the mechanisms  
309 involved in transferrin iron saturation increase among obese patients with OSAS, and their  
310 role in the natural course of OSAS. In view of our findings, transferrin iron saturation in  
311 plasma merits exploration as a longitudinal marker of the severity of OSAS or hypoxia among  
312 obese patients.

313

#### 314 **Statement of authorship**

315 All authors have made substantial contributions: NLE, OL and RT conceived, and designed  
316 the study, analysed, and interpreted the data, and drafted the article; NLE, RT, and BD  
317 collected the data; CR analysed the data; all authors have approved the final version.

318

#### 319 **Conflict of interest statement**

320 The authors have no conflict of interest related to this article to declare.

321

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325

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427 **Table 1 – Clinical and demographic characteristics of obese patients with obstructive**  
 428 **sleep apnea syndrome (n=69).**  
 429

<b>Variables</b>	
Women / Men – n (%)	56 (81) / 13 (19)
Age (yr)	44.3 ± 12.6
Gastric banding history - n (%)	4 (6)
Tobacco use - n (%)	29 (42)
Smoking cessation (N= 28)* - n (%)	13 (46)
OSAS - n (%)	50 (72)
Mild	28 (40)
Moderate	9 (13)
Severe	13 (19)
Minimum oxygen saturation ≤ 75%	19 (27)
Time with oxygen saturation < 90% >15%	22 (32)
<b>Anthropometric data</b>	
Body mass index	42.8 ± 6.3
Body composition	
Fat-free mass (%)	52.6 ± 6.5
Fat mass (%)	47.4 ± 6.5
Total body water (%)	39.5 ± 4.7
Fat mass index (kg/m <sup>2</sup> )	20.4 ± 5.3
Waist circumference (cm)	126.8 ± 15.2
Waist/Hip ratio	1.0 ± 0.1
<b>Comorbidities – n (%)</b>	
Type 2 diabetes	8 (12)
Arterial hypertension	24 (35)

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Metabolic syndrome	44 (64)
Asthma	13 (19)
COPD	2 (3)
Chronic inflammatory diseases	4 (6)

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430 COPD, chronic obstructive pulmonary disease.

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431 **Table 2 – Univariate analysis of factors associated with obstructive sleep apnea**  
 432 **syndrome and its severity (n=69).**  
 433

<b>Variables</b>	<b>Absent/mild OSAS N=47</b>	<b>Moderate/severe N=22</b>	<b>P</b>
Women / Men - n (%)	41 (87) / 6 (13)	15 (68) / 7 (32)	0.096
Age (yr) – mean±SD	41.0 ± 12.0	51.5 ± 11.1	0.001
Tobacco use - n (%)	15 (32)	14 (64)	0.013
Smoking cessation (N= 28)-n(%) (%)	4 (27)	9 (69)	0.024
<b><u>Nocturnal polygraphy</u> – mean±SD</b>			
Minimum oxygen saturation (%)	83.5 ± 9.1	71.0 ± 10.5	<0.0001
% time oxygen saturation <90%	11.6 ± 25.6	38.3 ± 30.4	<0.0001
Oxygen saturation decrease (%)	4.1 ± 1.1	7.9 ± 2.7	<0.0001
Oxygen saturation <90% / hour (n)	9.5 ± 5.5	51.2 ± 27.3	<0.0001
<b><u>Anthropometric data</u> – mean±SD</b>			
Body mass index (kg/m <sup>2</sup> )	41.6 ± 4.6	45.6 ± 8.3	0.044
Waist circumference (cm)	122.6 ± 11.6	135.6 ± 18.3	0.003
Total body water (%)	39.0 ± 3.6	40.5 ± 6.3	0.297
Fat free mass (kg)	59.3 ± 10.3	65.7 ± 13.0	0.031
Fat free mass (%)	52.8 ± 5.4	52.1 ± 8.6	0.749
Fat mass (kg)	54.0 ± 9.9	62.1 ± 20.6	0.089
Fat mass (%)	47.2 ± 5.4	47.9 ± 8.6	0.642
Fat mass index (kg/m <sup>2</sup> )	19.6 ± 3.6	22.3 ± 7.5	0.335
<b><u>Comorbidities</u> – n (%)</b>			
Type 2 diabetes	4 (8.5)	4 (18)	0.255
Arterial hypertension	11 (23)	13 (59)	0.004
Metabolic syndrome	28 (60)	16 (73)	0.289
Asthma	8 (17)	5 (23)	0.742
COPD	1 (2)	1 (4.5)	0.539
<b><u>Blood parameters</u> – mean±SD</b>			
Iron (µmol/l)	15.2 ± 4.8	18.7 ± 5.6	0.021
Transferrin (g/l)	2.6 ± 0.4	2.4 ± 0.4	0.211
Transferrine saturation (%)	24.2 ± 8.8	31.5 ± 9.6	0.002
Ferritin (µg/l)	111.5 ± 88.9	260.5 ± 276.3	0.011
Hemoglobin (g/dl)	13.4 ± 1.2	13.5 ± 1.0	0.593
Hematocrit (%)	40.6 ± 3.2	40.8 ± 3.0	0.787
CRP (mg/l)	8.3 ± 5.6	9.5 ± 7.5	0.728
ALAT (IU/l)	28.8 ± 14.1	38.9 ± 20.5	0.029
ASAT (IU/l)	24.5 ± 8.6	28.1 ± 11.8	0.233
γGT (IU/l)	31.6 ± 14.1	74.8 ± 101.7	0.030
Prothrombin time (%)	99.4 ± 12.8	98.8 ± 11.7	0.342
Albumin (g/l)	41.9 ± 2.7	42.5 ± 3.3	0.412
Glucose (mmol/l)	5.5 ± 1.0	5.9 ± 1.0	0.016
Triglycerides (mmol/l)	1.3 ± 0.5	1.7 ± 0.8	0.007
Total cholesterol (mmol/l)	4.8 ± 0.8	5.3 ± 1.5	0.127
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.1 ± 0.3	0.430
LDL cholesterol (mmol/l)	3.0 ± 0.7	3.2 ± 1.4	0.501

434

ALAT, alanine amino transferase; ASAT, aspartate amino transferase; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein;  $\gamma$ GT, gamma-glutamyl-transpeptidase.

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**Table 3 - Serum iron parameters independently associated with the severity of OSAS (n=69).**

Variables	Absent OSAS (n=19)		Mild (n=28)		Moderate (n=9)		Severe (n=13)		P value
Transferrin saturation (%)	26.3	[21.1-31.6]	23.7	[19.4-28.1]	29.3	[22.3-36.2]	34.5	[27.7-41.3]	<b>0.048</b>
Ferritin (µg/l)	167.7	[113.4-247.9]	128.0	[90.2-178.7]	248.5	[143.6-430.0]	214.8	[131.3-351.3]	0.107
Iron (µmol/l)	17.5	[14.6-20.5]	15.2	[12.8-17.7]	17.8	[13.9-21.7]	20.2	[16.4-24.0]	0.097
Transferrin (g/l)	2.7	[2.5-2.9]	2.6	[2.4-2.8]	2.5	[2.2-2.8]	2.4	[2.1-2.6]	0.362
CRP (mg/m)	6.2	[4.5-8.5]	5.9	[4.4-7.9]	6.8	[4.4-10.6]	10.8	[7.2-16.2]	0.100
ASAT (IU/l)	25.7	[21.8-30.4]	26.6	[23.1-30.6]	29.9	[23.7-37.7]	29.3	[23.9-36.1]	0.640
ALAT (IU/l)	32.9	[26.5-40.9]	31.8	[26.3-38.5]	42.1	[31.0-57.3]	41.2	[31.5-54.1]	0.218
γGT (IU/l)	38.6	[29.4-50.8]	44.8	[35.5-56.6]	55.1	[37.8-80.5]	80.9	[58.6-111.8]	<b>0.013</b>
Prothrombin time (%)	100.3	[93.1-107.6]	96.8	[90.6-103.1]	102.1	[92.2-111.9]	97.9	[88.9-106.9]	0.713
Albumin (g/l)	43.2	[41.7-44.6]	42.3	[41.0-43.5]	42.9	[40.9-44.9]	44.6	[42.8-46.4]	0.169

Comparisons of different parameters per AHI group adjusted for gender, tobacco use, age, arterial hypertension, waist circumference, blood glucose and triglycerides. Adjusted mean (95% confidence interval); ANOVA test. ALAT, alanine amino transferase; ASAT, aspartate amino transferase; CRP, C-reactive protein; γGT, gamma-glutamyl-transpeptidase.



**Table 4 - Serum iron parameters independently associated with the percentage of time with oxygen saturation under 90% (n=69).**

Variables	Oxygen saturation<90% ≤ 15% of time (n=47)		Oxygen saturation<90% > 15 % of time (n=22)		P value
Transferrin saturation (%)	25.3	[21.6-29.1]	31.5	[26.3-36.7]	<b>0.043</b>
Ferritin (µg/l)	165.2	[126.8-215.3]	202.0	[129.5-315.3]	0.407
Iron (µmol/l)	16.2	[14.1-18.2]	19.4	[16.5-22.3]	0.056
Transferrin (g/l)	2.58	[2.4-2.7]	2.6	[2.4-2.8]	0.861
CRP (mg/m)	6.1	[4.8-7.7]	9.0	[6.6-12.4]	<b>0.044</b>
ASAT (IU/l)	26.1	[23.2-29.3]	29.8	[25.3-35.0]	0.179
ALAT (IU/l)	34.5	[29.4-40.5]	37.3	[29.6-46.9]	0.574
γGT (IU/l)	51.2	[41.2-63.8]	55.0	[41.8-72.5]	0.689
Prothrombin time (%)	99.4	[94.4-104.5]	97.9	[91.0-104.7]	0.713
Albumin (g/l)	42.9	[41.9-44.0]	43.5	[42.1-44.9]	0.529

Comparisons of biological parameters by percentage of time under hypoxia (oxygen saturation <90%) adjusted for gender, tobacco use, age, arterial hypertension, waist circumference, blood glucose and triglycerides. Adjusted mean (95% confidence interval); ANOVA test. ALAT, alanine amino transferase; ASAT, aspartate amino transferase; CRP, C-reactive protein; DBP, diastolic blood pressure; γGT, gamma-glutamyl-transpeptidase.

## Figure legends

**Figure 1 – Study flow chart.**

**Figure 2 – Diagram representing the potential mechanisms that could modulate systemic iron metabolism and parameters among obese patients with severe OSAS.**

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Figure 1

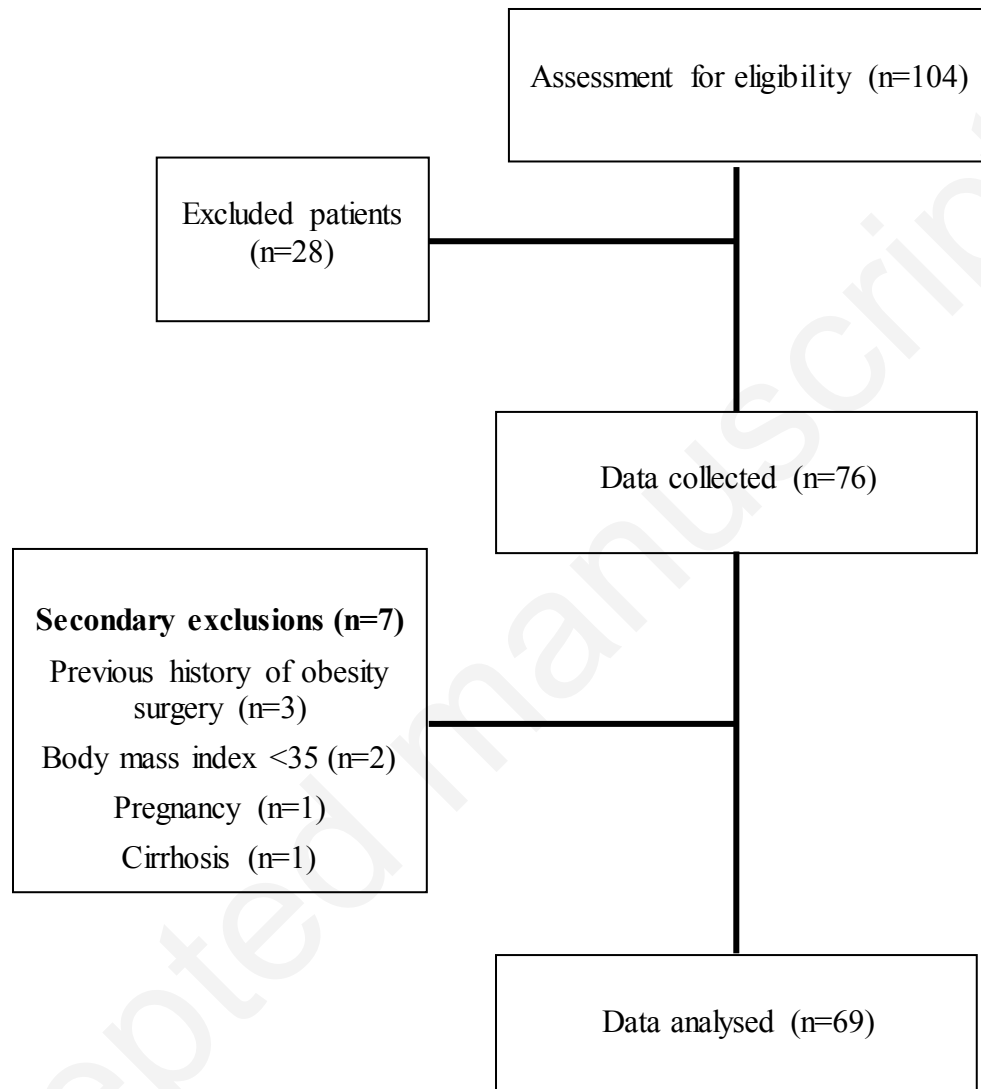


Figure 2

