RESEARCH LETTER

Testosterone deficiency in men with obstructive sleep apnea syndrome: is hepcidin a player in the game?

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Introduction Obstructive sleep apnea syndrome (OSAS) is characterized by repetitive collapsing of the upper respiratory tract during sleep.[1] One of the complications of this chronic systemic inflammatory state is hypogonadism.[2] In response to inflammation, the serum hepcidin level rises. Testosterone probably exerts an inhibitory effect on hepcidin levels, and even overrides iron- and inflammation-mediated mechanisms in a chronic inflammatory state.[3] On the other hand, a disrupted iron balance may influence the gonadal axis. Patients with severe iron overload, such as those with hemochromatosis, often suffer from hypogonadotropic hypogonadism. In chronic inflammatory conditions, such as OSAS or diabetes, the hepcidin level is elevated. Eventually, this conservative adaptation mechanism leads to increased iron storage, and might be responsible for low testosterone levels via the same mechanism as that observed in hemochromatosis.[4] This study aimed to assess whether hypogonadism in patients with OSAS could be associated with iron metabolism.

Patients and methods **Patients** This was a cross-sectional study including consecutive male patients aged over 50 years admitted to the outpatient sleep department between January 2016 and January 2018 because of symptoms suggesting OSAS. A total of 92 patients were enrolled, all of whom were assessed with the Epworth Sleepiness Scale (ESS). Those who scored 10 points or more underwent polysomnography. The flowchart depicting the recruitment process of the study is shown in Supplementary material, *Figure S1*. The diagnostic criteria for OSAS were apnea / hypopnea index (AHI) greater than or equal to 5 and symptoms suggesting OSAS, or AHI greater than or equal to 15, irrespectively of the symptoms.[5] The patients with total testosterone levels below 12 mmol/l and symptoms of hypogonadism were diagnosed as hypogonadal according to the European Academy of Andrology (EAA) guidelines.[6] The study population was divided into 3 groups, including 1) 45 eugonadal patients with OSAS, 2) 24 hypogonadal patients with OSAS, and 3) a control group of 23 eugonadal patients with the ESS score below 10 or normal polysomnography results. The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Bioethics Committee of Poznan University of Medical Sciences (641/15). Informed consent was obtained from all individuals involved in the study.

In each group, we analyzed the prevalence of comorbidities, such as cardiovascular disease (CVD), hypertension, hyperlipidemia, obesity, prediabetes, and diabetes.

Laboratory analysis Fasting blood samples were collected for the assessment of iron metabolism markers: hepcidin, human soluble transferrin receptor (sTfR), ferritin, iron, and total iron-binding capacity (TIBC). We evaluated the hormonal function by measuring testosterone and luteinizing hormone (LH) levels. To assess metabolic complications, the levels of insulin, aminotransferases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), fasting glucose, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TGs) were measured. Additionally, we assessed the complete blood count (CBC), as well as the levels of C-reactive protein (CRP), creatinine, and thyroid-stimulating hormone (TSH). The hepcidin level was measured with the Hepcidin 25 (bioactive) high-sensitivity enzyme-linked immunoassay

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(HS ELISA; DRG Instruments GmbH, Marburg, Germany). The levels of sTfR and ferritin were measured with an ELISA Assay Kit (DRG Instruments GmbH). Iron, TIBC, testosterone, LH, insulin, ALT, AST, fasting glucose, TC, LDL-C, HDL-C, TG, CRP, creatinine, and TSH levels were assessed via the electrochemiluminescence method using a Cobas 8000 analyzer (Roche Diagnostics, Rotkreuz, Switzerland). CBC was evaluated with an automated flow cytometer Sysmex-XN 1000 (Sysmex Europe GmbH, Bornbarch, Germany).

Polysomnography An experienced sleep technician performed full polysomnography at the Sleep Laboratory in the Department of Pulmonology, Allergology, and Respiratory Oncology, Poznan University of Medical Sciences, using EMBLA S4000 – Remlogic, Somnologica Studio 5.0; Natus 2009 (Embla, Broomfield, Colorado, United States). The examination lasted from 10 PM to 6 AM. We monitored and documented abdominal and thoracic movements, snoring sounds, positions during sleep, airflow through the nose and mouth (thermistor, nasal cannula), hemoglobin oxygen saturation (finger pulse oximetry), as well as electrocardiogram, electrooculogram, electromyogram, and electroencephalogram. Apnea was characterized by an at least 90% reduction of the airflow, while hypopnea, an at least 30% reduction lasting for more than 10 seconds and a drop in oxygen saturation greater than 4%. AHI was defined as the average number of apneas and hypopneas per 1 hour of sleep.[5]

Statistical analysis Statistical analysis was performed with the MedCalc Statistical Software, version 20.015 (MedCalc Software Ltd., Ostend, Belgium). Normality of data distribution was analyzed by the D'Agostino–Pearson test. In multiple comparisons, the Kruskal–Wallis test or analysis of variance was used. The Conover test was performed for post hoc analysis. The Spearman rank correlation coefficient was calculated to find the relationships between testosterone and insulin, body mass index (BMI), and hepcidin. Frequency tables were used to compare categorical variables between the eugonadal and hypogonadal patients with OSAS. A *P* value below 0.05 was considered statistically significant.

Results The clinical characteristics and comparisons of the analyzed groups are presented in TABLE 1. The Spearman rank correlation coefficient indicated a negative correlation between the testosterone level and BMI (R = –0.3; *P* = 0.004) (Supplementary material, *Figure S2*), insulin level (R = –0.361; *P* = 0.004) (Supplementary material, *Figure S3*), and hepcidin level (R = –0.235; *P* = 0.03) (Supplementary material, *Figure S4*) in the whole population. There was no significant difference between the prevalence of CVD (*P* = 0.5), hyperlipidemia (*P* = 0.6), obesity (*P* = 0.47), prediabetes (*P* = 0.95), and diabetes (*P* = 0.44) between the eugonadal and hypogonadal patients with OSAS. Hypertension was more common in the eugonadal individuals with OSAS than the hypogonadal ones (*P* = 0.046).

Discussion To the best of our knowledge, this is the first study investigating the association between testosterone levels and iron balance in the patients with OSAS. Every third patient diagnosed with OSAS demonstrated hypogonadism. We identified a few vital factors that may influence the testosterone level in OSAS.

We observed an inverse correlation between the hepcidin concentration and testosterone levels. Although the hepcidin level was higher in the control group than in the patients with OSAS, the lowest hepcidin level was detected in the patients with the highest testosterone levels. Testosterone plays an essential role in erythropoiesis, and it was even shown to override iron regulation in chronic inflammatory disease. Additionally, testosterone replacement was found to reduce inflammatory reactions.[7,8] On the other hand, previous studies postulated that iron balance disruptions might impact the regulation of the pituitary-gonadal axis in men.[4] The human body lacks the mechanism to remove redundant iron.[3] Testosterone, by inhibiting hepcidin, increases the amount of bioavailable iron.[7] In the state of iron abundance, a drop in the testosterone level might constitute a negative feedback mechanism.[4] In our study, iron concentration was the highest in the healthy control group, which confirms this hypothesis. There were no significant differences in ferritin, TIBC, sTfR, and hemoglobin levels between the patients with OSAS and the controls.

Another vital factor inversely associated with testosterone levels in our study was BMI. Obesity was shown to be widespread among the patients with OSAS. It is the most important factor predicting low testosterone levels in men. Elevated BMI could be a primary determinant of the gonadal function in OSAS patients.[9] Adipose tissue was desricbed as a functioning endocrine organ releasing cytokines, including interleukin-6. This cytokine could stimulate the hepatic hepcidin production. The hepcidin level was shown to rise along with the increase in total body fat, but it was also dependent on fat distribution.[10] In our study, the hepcidin level was higher in the participants with lower BMI, contrary to a previous study.[10] Possibly, the hepcidin concentration might be linked to fat distribution, and not only to BMI.

We demonstrated that insulin could adversely affect the testosterone concentration in OSAS. Additionally, the insulin level was the highest in the patients with OSAS and hypogonadism. Data from other studies support these results, showing that OSAS impairs insulin sensitivity and glucose tolerance.[11] While hypogonadism was previously associated with insulin resistance,[12] Dhindsa et al[8] have shown tha testosteron administration increases insulin sensitivity and reduces inflammation.[8] On the other hand, insulin

TABLE 1 Clinical and biochemical characteristics of the study population

Parameter	Eugonadal OSAS (n = 45)	Hypogonadal OSAS (n = 24)	Control group (n = 23)	*P* value
Testosterone, nmol/l	14 (12.37–18.92)[a]	8.2 (6.1–9.1)[a,b]	12.9 (11.82–15.8)[b]	<0.001
LH, mU/ml	5.3 (3.57–7.67)	4.65 (3.25–4.65)	5.1 (3.77–6.52)	0.44
CRP, mg/l	1.3 (0.7–2.82)	2.55 (1.15–4)[a]	1.1 (0.6–2.27)[a]	0.04
BMI, kg/m^2	30.49 (27.91–33.79)[a]	30.85 (28.66–35.23)[b]	27.97[a,b] (26.42–30.85)	0.01
Insulin, µU/ml	13.38 (8.33–20.08)[a]	20.1 (13.79–24.98)[a,b]	13.46 (9.49–16)[b]	0.02
Fasting glucose, mmol/l	5.94 (5.42–6.61)[a,b]	6.19 (5.72–7.14)[a,c]	5.47 (5.19–6.03)[b,c]	0.003
Hemoglobin, mmol/l	9.12 (8.87–9.63)	9.15 (8.69–9.37)	9.25 (9.01–9.74)	0.22
RBC, × 10^6/µl	4.83 (4.62–5.13)	4.82 (4.67–5.04)	4.9 (4.77–5.13)	0.22
Hepcidin, ng/ml	13.9 (9.08–19.51)	18.02 (10.9–26.62)	20.13 (12.31–23.35)	0.05
Iron, µmol/l	17.55 (14.51–22.52)[a]	17.19 (13.52–21.94)[b]	21.31 (17.01–26.68)[a,b]	0.04
sTfR, µg/ml	0.71 (0.65–0.89)	0.74 (0.62–0.88)	0.88 (0.65–1.02)	0.12
Ferritin, ng/ml	149 (110.75–224)	174 (121.5–240)	191 (126.5–273.75)	0.59
TIBC, µg/dl	299.8 (41.21)	317.5 (55.27)	296 (28.57)	0.17
Creatinine, µmol/l	83.1 (73.37–90.17)	86.63 (80–94.14)	78.68 (69.39–85.52)	0.12
ALT, U/l	27 (21–39.25)	26.5 (19.5–36)	24 (19.25–32.5)	0.42
AST, U/l	23 (19–26.25)	21.5 (17–27.5)	21 (19–24)	0.4
Total cholesterol, mmol/l	4.61 (3.82–5.49)	4.79 (4.1–5.58)	4.95 (4.43–5.92)	0.18
HDL-C, mmol/l	1.19 (0.97–1.32)	1.19 (0.95–1.35)	1.32 (1.17–1.63)	0.08
LDL-C, mmol/l	2.58 (1.82–3.4)	2.57 (1.93–3.77)	3.29 (2.38–4.23)	0.01
Triglycerides, mmol/l	2.85 (2.18–4.05)	3.73 (2.81–5.24)	2.95 (2.44–3.51)	0.11
TSH, µU/ml	1.24 (0.88–1.83)	1.43 (0.9–2.1)	1.22 (0.91–1.84)	0.71
Age, y	61 (5.69)	62 (6.64)	63 (9.16)	0.48

Data are presented as median (interquartile range) or mean (SD).

P values <0.05 were considered significant. The significant difference was found between groups marked by the same letter (a, b, c).

SI conversion factors: to convert LH to IU/l, multiply by 1.0; CRP to nmol/l, by 9.524; insulin to pmol/l, by 6.945; ferritin to pmol/l, by 2.247; TIBC to µmol/l, by 0.179; ALT and AST to µkat/l, by 0.0167.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; OSAS, obstructive sleep apnea syndrome; RBC, red blood cell count; sTfR, souble transferrin receptor; TSH, thyroid-stimulating hormone; TIBC, total iron-binding capacity

resistance was previously associated with a higher risk of decreased testosterone levels.[13] Hyperinsulinemia was shown to directly promote and induce the synthesis of hepcidin,[14] which is elevated in chronic inflammatory states, such as OSAS.

Lastly, we analyzed comorbidities. The prevalence of CVD, hyperlipidemia, obesity, prediabetes, and diabetes did not differ between the eugonadal and hypogonadal patients with OSAS. Hypertension was more common in the patients with OSAS with normal testosterone levels. In contrast, in recently published studies, male hypogonadism was linked with and even predicted metabolic disturbances.[15] Our results may be influenced by the presence of OSAS and a limited number of included patients.

The main limitations of the present study are the small sample size, a single measurement of androgen levels, and the lack of free testosterone assessment. There is no clear cutoff value for low testosterone levels in men to diagnose hypogonadism. We chose a testosterone value of 12 nmol/l, as it was previously associated with higher incidence of symptoms in hypogonadal patients.[6] Of note, our study did prove a causative relationship between iron metabolism and hypogonadism—these entities might overlap.

To conclude, we observed a high prevalence of hypogonadism in a population of Polish men with OSAS. Routine testosterone measurement might be beneficial for these patients. Multiple factors, including BMI, as well as hepcidin and insulin levels, influenced the testosterone level in OSAS. The exact mechanism through which the iron balance disruptions might influence testosterone levels remains to be elucidated.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

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