Original Article

OBSTRUCTIVE SLEEP APNEA PREDISPOSES TO NONALCOHOLIC FATTY LIVER DISEASE IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

Luciana Tock, MD[1]; Gláucia Carneiro, MD, PhD[1]; Sônia M. Togeiro, MD, PhD[2]; Helena Hachul, MD, PhD[2]; Andrea Z. Pereira, MD, PhD[1]; Sérgio Tufik, MD, PhD[2]; Maria T. Zanella, MD, PhD[1]

ABSTRACT

Objective: Some studies have shown a higher prevalence of nonalcoholic fatty liver disease (NAFLD) and obstructive sleep apnea (OSA) in patients with polycystic ovary syndrome (PCOS). The objective of this study was to assess NAFLD in PCOS women with and without OSA. A possible role of high serum androgen levels in the development of OSA in PCOS women was also investigated.

Methods: Biochemical, hormonal, and polysomnography parameters were determined in 38 premenopausal PCOS patients. NAFLD was evaluated by ultrasound. Testosterone was measured by an immunoassay.

Results: Serum androgen levels and the prevalence of NAFLD (83.3% vs. 26.9%; *P*<.001) were higher in patients with OSA than those without OSA. The mean apnea-hypopnea index (AHI) was higher in patients with NAFLD than in those without NAFLD (16.87 events [ev]/h vs. 1.57 ev/h; *P*<.002). On multivariate logistic regression, where body mass index ≥30 kg/m^2, homeostasis model assessment for insulin resistance ≥2.7, and OSA (AHI ≥5 ev/h) were independent variables, only OSA was an independent predictor of NAFLD (odds ratio [OR], 7.63; *P* = .044). Free testosterone levels ≥1.07 ng/dL were also independently associated with OSA (OR, 8.18; *P* = .023).

Conclusion: In PCOS women, the occurrence of OSA strongly predisposes them to development of NAFLD and a worse metabolic profile; hence, treatment of OSA might be beneficial for NAFLD. **(Endocr Pract. 2014;20:xxx-xxx)**

Abbreviations:
AHI = apnea-hypopnea index; **BMI** = body mass index; **ev** = events; **FFA** = free fatty acids; **GGT** = gamma glutamyltransferase; **HDL** = high-density lipoprotein; **HOMA-IR** = homeostasis model assessment for insulin resistance; **IR** = insulin resistance; **ISI** = insulin sensitivity index; **LDL** = low-density lipoprotein; **MS** = metabolic syndrome; **NAFLD** = nonalcoholic fatty liver disease; **NASH** = nonalcoholic steatohepatitis; **OGTT** = oral glucose tolerance test; **OSA** = obstructive sleep apnea; **PCOS** = polycystic ovary syndrome; **PSG** = polysomnography; **US** = ultrasound

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disease affecting women of reproductive age, with a prevalence of around 8% in women at this stage (1). Endocrine and metabolic diseases such as hyperandrogenism and insulin resistance (IR) are hallmarks of this syndrome. IR is present in 50 to 70% of PCOS women and plays a significant role in the physiopathology of PCOS as well as in the development of metabolic syndrome (MS). Compared to women without PCOS with a similar body mass index (BMI), patients with PCOS have a higher prevalence of both IR and MS (2-4). Obstructive sleep apnea (OSA) (5-7) and nonalcoholic fatty liver disease (NAFLD) (8-10) are also often associated with IR and MS. Other studies also show that the prevalence of OSA (11-13) and NAFLD (14-17) is high in PCOS women, and there is a higher prevalence of PCOS in women with NAFLD (18).

Submitted for publication November 6, 2012
Accepted for publication October 3, 2013
From the [1]Department of Medicine, Division of Endocrinology, Universidade Federal de São Paulo, São Paulo, Brazil, and [2]Department of Psycobiology, Sleep Disorders Center, Universidade Federal de São Paulo, São Paulo, Brazil.
Address correspondence to Dr. Luciana Tock, Rua Leandro Dupret 365, 04025-011, São Paulo/SP, Brazil. E-mail: lutock@hotmail.com.
Published as a Rapid Electronic Article in Press at http://www.endocrinepractice.org on November 18, 2013. DOI:10.4158/EP12366.OR
To purchase reprints of this article, please visit: www.aace.com/reprints.

OSA is a syndrome characterized by recurrent events of partial or total obstruction of the upper airway during sleep, leading to intermittent hypoxemia, which has obesity as the pillar of its physiopathology (5,11). Nevertheless, androgens appear to contribute to the aggravation of OSA. An association between this condition and male sex has been reported, and studies suggest that androgens influence sleep architecture, favoring the development of OSA (19-21). OSA contributes to an unfavorable metabolic profile, and there is evidence that it is an independent risk factor for IR (5,6,7,22).

NAFLD represents a broad spectrum of tissue changes, ranging from liver fatty infiltration to nonalcoholic steatohepatitis (NASH), with progression to cirrhosis in some cases (9). There is a clear association between NAFLD and MS components, regardless of obesity. However, abdominal (visceral) fat is an independent risk factor for the development of NAFLD (10,23), and the association between this disease and IR has been well demonstrated (8-10).

Studies suggest that OSA may both contribute to NAFLD development and accelerate its progression to NASH (24-26). However, there are no published data demonstrating a relationship between these two diseases in PCOS women. The primary objective of this study was to assess NAFLD in PCOS women with and without OSA. The secondary aim was to evaluate a possible role for hyperandrogenemia in the development of OSA in PCOS women.

METHODS

Population

Our study included 38 PCOS subjects ranging between 16 and 45 years in age. Subjects were recruited from the Endocrinology Division of the Federal University of São Paulo (UNIFESP), Brazil. The diagnosis of PCOS was based on the latest 2003 Rotterdam consensus (27), requiring the presence of at least two of the following features: (*1*) oligoamenorrhea or chronic anovulation, (*2*) clinical and/or biochemical hyperandrogenism, and (*3*) ultrasound (US) appearance of polycystic ovaries, after the exclusion of other known causes of hyperandrogenemia, such as congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing's syndrome. Exclusion criteria included the use of oral contraceptives, corticosteroids, antidiabetic or lipid-lowering drugs in the past 3 months, history of liver disease such as viral hepatitis B and C, hemochromatosis and autoimmune hepatitis, use of medications that alter liver enzymes, and daily ingestion of more than 20 g of ethanol. We also excluded patients with diabetes mellitus, untreated hypothyroidism, renal, hepatic, cardiac or pulmonary disease, and patients being treated for sleep apnea and any other condition that could influence the polysomnography (PSG) test, such as drugs (sympathomimetics, sympatholytics, and β-blockers), depression, and chronic diseases. The study was approved by the Ethics Committee of UNIFESP, and an informed written consent was obtained from all subjects.

Clinical, Anthropometric, and Biochemical Parameters

A questionnaire was used to document personal, medical, and drug history, regularity and length of menstrual cycles, and ovulation status. Signs of androgen excess (hirsutism, alopecia, acne) were noted in the physical examination. Hirsutism with a Ferriman-Gallwey score of 8 or above was considered as clinical evidence of androgen excess. Body weight (in kilograms), body height (in meters), waist and hip circumference (in centimeters) were measured. Waist circumference was taken as the narrowest measurement midway between the top of the iliac crest and the lower rib margin, whereas the hip circumference was taken as the widest measurement at the level of the greater trochanters. BMI was calculated from the ratio of the weight to height squared.

Blood specimens were obtained after a 12-hour overnight fast from all subjects in the early follicular phase of the menstrual cycle or after a period of amenorrhea of over 3 months for measurement of plasma glucose, total cholesterol, triglycerides, high-density lipoprotein (HDL), very-low-density lipoprotein, and serum ferritin levels as well as androgenic profile, liver function tests, and serology for hepatitis B and C. A standard 75-gram oral glucose tolerance test (OGTT) was performed, with measurements of fasting and 2-hour serum insulin for insulin resistance index calculations. Biochemical hyperandrogenemia was defined as elevated free testosterone, with levels ≥1.07 ng/dL, which is our laboratory's upper limit for normal women.

Sonographic Measurements

All subjects underwent US examination of the abdomen and pelvis by the same radiologist. A LOGIQ P5 (GE Healthcare, Wauwatosa, WI) with an 8 MHz transvaginal transducer and a 4 MHz convex transducer was used for US of the pelvis and abdomen, respectively. Hepatic steatosis was defined according to the criteria of Scatarige et al (28) and included increased hepatic echogenicity, posterior acoustic attenuation of the liver, and reduced echogenicity of the intrahepatic portal vein. Patients were classified according to the absence or presence of liver fat as well as the degree of fatty infiltration: grade 1 (mild steatosis), grade 2 (moderate steatosis), and grade 3 (severe steatosis).

Polysomnographic Parameters

A full-night PSG was performed at the sleep laboratory over the course of one night using a digital system (EMBLA® S7000, Embla Systems, Inc, Broomfield, CO). Trained technicians visually scored all of the PSG

data according to standardized criteria for investigating sleep (29). Electroencephalogram arousals and sleep-related respiratory events were scored in accordance with the criteria outlined in the American Academy of Sleep Medicine Manual for Scoring Sleep and Associated Events (30). The average number of episodes of apnea and hypopnea per hour of sleep (i.e., the apnea-hypopnea index [AHI]) was calculated. The AHI value was categorized as non-OSA (<5 events [ev]/h), mild OSA (5 to 14.9 ev/h), moderate OSA (15 to 30 ev/h), or severe OSA (>30 ev/h).

Laboratory Analyses

Plasma glucose, total cholesterol, triglyceride, HDL, aspartate aminotransferase, alanine aminotransferase (ALT), and gamma glutamyltransferase (GGT) levels were measured using an ADVIA 2400 Chemistry System (Siemens, Tarrytown, NY). Dehydroepiandrosterone and 17-hydroxyprogesterone levels were measured by enzyme-linked immunosorbent assays (Diagnostic Biochem Canada, Ontario, Canada and Labor Diagnostika Nord GmbH, Nordhorn, Germany, respectively). Total testosterone, dehydroepiandrosterone-sulfate, insulin, and ferritin levels were measured using a UniCelDxl 800 Immunoassay System (Beckman Coulter, Brea CA). The within-assay coefficient of variation for testosterone was 1.99%, and the between-assay coefficient was 4.22%. There are some limitations to measuring testosterone using a chemiluminescence immunoassay, but this was the only laboratory technique available. Androstenedione and sex hormone-binding globulin (SHBG) were measured using an IMMULITE 2000 Immunoassay System (Siemens). Low-density lipoprotein (LDL) levels were calculated using the Friedewald formula (31). Serum free testosterone and bioavailable testosterone were estimated by the formula previously validated by Vermeulen et al (32) (available at: http://www.issam.ch/freetesto.htm). IR was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) by dividing the product of fasting insulin (micro international units [μIU]/mL) and glucose (mmol/L) by 22.5, and IR was considered when the HOMA-IR was ≥2.7 (33). The insulin sensitivity index (ISI) for glycemia was calculated according to the formula developed by Belfiore et al (34): 2/ ([INSp × GLYp] +1), where INSp and GLYp are obtained by dividing the sum of plasma insulin (μUI/mL) and glycemia (mmol/L) (measured at 0 and 2 hours after oral glucose load) by the sum of the respective values of the normal population. Normal reference values were obtained from 35 normotensive individuals with normal BMI. All biochemical assays were performed at the Sleep Institute laboratory.

Statistical Analysis

Data are expressed as the mean (SD). Comparison of mean values between subjects with and without OSA and between subjects with and without NAFLD was performed using the Student's *t* test. Associations between variables were determined using the linear correlation test and Pearson chi-square test. Multivariate logistic regression analysis was used to identify the independent predictors of OSA and NAFLD among PCOS subjects. A *P*-value <.05 was considered significant. Statistical analyses were performed with the Statistical Package for Social Sciences for Windows, version 19.0 (SPSS Inc, Chicago, IL).

RESULTS

The clinical characteristics of all subjects are summarized in Table 1. Twelve patients (31.6%) with PCOS had OSA defined as an AHI ≥5 ev/h. Six (50%) of these patients had mild apnea, 2 (16.7%) had moderate apnea, and 4 (33.3%) had severe apnea. Seventeen (44.7%) of the PCOS subjects had NAFLD; 11 (64.7%) of these patients had grade 1 steatosis, 4 (23.5%) had grade 2 steatosis, and 2 (11.8%) had grade 3 steatosis. The prevalence of NAFLD was 83.3% in patients with OSA, compared with 26.9% in patients without OSA (*P*<.001).

As shown in Table 1, compared to subjects without OSA, those with this disturbance had higher BMI, waist circumference, waist to hip ratio, serum fasting insulin, 2-hour blood glucose levels during OGTT, HOMA-IR, serum ferritin, and GGT levels and had a more unfavorable lipid profile, with higher levels of total cholesterol, LDL-cholesterol, and triglycerides and a trend toward lower HDL-cholesterol. The ISI was lower in this group of women, and the androgen profile was also different, with higher levels of free testosterone associated with lower levels of SHBG. There was an association between the occurrence of hyperandrogenemia (free testosterone ≥1.07 ng/dL) and the presence of OSA, as 50% of patients with high free testosterone levels were also affected by OSA, compared with only 15% of those without hyperandrogenemia (*P* = .020). The AHI showed positive and significant correlation with anthropometric parameters (BMI: $r = 0.387$; $P = .016$; waist circumference: $r = 0.315$; $P = .054$; waist to hip ratio: $r = 0.329$; $P = .044$), fasting glucose levels ($r = 0.532$; $P = .001$), 2-hour blood glucose during OGTT ($r = 0.707$; $P<.0001$), fasting insulin ($r = .700$; $P<.0001$), HOMA-IR ($r = 0.753$; $P<.0001$), ferritin ($r = 0.627$; $P<.0001$), GGT ($r = 0.390$; $P = 0.016$), ALT ($r = 0.444$; $P = 0.005$), total cholesterol ($r = 0.403$; $P = 0.012$), LDL ($r = 0.413$; $P = 0.010$), and triglycerides ($r = 0.404$; $P = 0.012$). A significant inverse correlation was observed between the AHI and ISI ($r = –0.537$; $P = .001$). The correlations between AHI and levels of free and bioavailable testosterone were marginally significant ($r = 0.308$, $P = .060$ and $r = 0.319$, $P = .051$, respectively). The AHI was higher in patients with NAFLD than in patients without NAFLD (16.87 ev/h vs. 1.57 ev/h; *P*<.002). The correlation between hyperandrogenemia and NAFLD

Table 1
Clinical and Biochemical Characteristics of All Subjects

	All patients	Patients without OSA	Patients with OSA	*P*-value
n	38	26	12	
Age (years)	28.3 ± 6.8	28.4 ± 7.5	28.3 ± 5.0	.968
Number of patients with NAFLD (%)	17 (44.7%)	7 (26.9%)	10 (83.3%)	.001
– grade 1	11	5	6	
– grade 2/3	6	2	4	
Anthropometric measurements				
BMI (kg/m^2)	32.9 ± 7.7	30.67 ± 7.7	37.8 ± 4.8	.006
Waist circunference (cm)	103.2 ± 19.2	98.1 ± 19.9	114.4 ± 12.0	.013
Waist to hip ratio	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	.029
Hormonal profile				
Androstenedione (ng/mL)	3.1 ± 2.0	2.9 ± 1.9	3.4 ± 2.3	.431
DHEA (ng/mL)	10.2 ± 9.6	9.9 ± 9.5	10.8 ± 10.3	.792
DHEA-S (μg/dL)	155.9 ± 70.7	147.4 ± 63.1	174.4 ± 85.0	.280
Total testosterone (ng/dL)	62.7 ± 36.3	55.4 ± 31.3	78.6 ± 42.4	.066
SHBG (nmol/L)	30.5 ± 16.8	34.5 ± 18.1	21.7 ± 8.8	.027
Free testosterone (ng/dL)	1.3 ± 1.0	1.1 ± 0.8	1.9 ± 1.3	.014
Bioavailable testosterone (ng/dL)	28.4 ± 20.8	24.8 ± 17.6	36.1 ± 25.3	.119
Metabolic profile				
Fasting glucose (mg/dL)	94.3 ± 14.2	91.4 ± 7.9	100.6 ± 21.7	.061
2-h glucose during OGTT (mg/dL)	119.6 ± 43.8	108.2 ± 28.0	144.5 ± 60.7	.015
HOMA-IR (μIU/mL)	3.0 ± 2.3	2.3 ± 1.4	4.4 ± 3.2	.009
ISI	0.7 ± 0.3	0.8 ± 0.3	0.4 ± 0.2	.001
Ferritin (ng/mL)	54.8 ± 67.9	37.2 ± 25.5	93.1 ± 108.3	.016
AST (IU/L)	20.1 ± 4.0	19.6 ± 3.5	21.3 ± 4.9	.232
ALT (IU/L)	21.2 ± 8.9	19.7 ± 6.8	24.5 ± 12.0	.127
GGT (IU/L)	28.7 ± 15.0	23.9 ± 11.5	39.2 ± 16.8	.002
Total cholesterol (mg/dL)	182.6 ± 36.7	172.3 ± 35.8	205.0 ± 28.7	.009
LDL-cholesterol (mg/dL)	108.3 ± 30.4	98.9 ± 29.6	128.6 ± 21.6	.004
HDL-cholesterol (mg/dL)	52.0 ± 11.3	54.2 ± 12.4	47.1 ± 6.6	.071
Triglycerides (mg/dL)	108.0 ± 48.4	96.0 ± 42.0	133.8 ± 52.8	.023
Polysomnographic parameters				
AHI (events/h)	8.4 ± 16.1	1.3 ± 1.5	23.7 ± 22.3	<.001
Mean O_2 saturation (%)	96.1 ± 1.4	96.5 ± 1.3	95.2 ± 1.3	.008

Abbreviations: AHI = apnea-hypopnea index; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; DHEA = dehydroepiandrosterone; DHEA-S = dehydroepiandrosterone sulfate; GGT = gamma glutamyltransferase; HDL = high-density lipoprotein; HOMA-IR = homeostasis model assessment for insulin resistance; ISI = insulin sensitivity index; LDL = low-density lipoprotein; NAFLD = nonalcoholic fatty liver disease; OGTT = oral glucose tolerance test; OSA = obstructive sleep apnea; SHBG = sex hormone-binding globulin.

was not significant (data not shown). Furthermore, a linear relationship was noted between hyperandrogenemia and IR. For these reasons, testosterone was not incorporated into the regression analysis.

After adjusting for obesity (BMI ≥30 kg/m^2) in multivariate logistic regression, hyperandrogenemia (free testosterone ≥1.07 ng/dL) had a significant association with OSA in women with PCOS (Table 2). Free testosterone levels ≥1.07 ng/dL increased the risk of OSA in these women 8.2 fold.

In a subsequent multiple logistic regression analysis (Figure 1), with OSA (AHI ≥5), IR (HOMA-IR ≥2.7), and obesity (BMI ≥30kg/m^2) considered as independent variables and NAFLD as the dependent variable, only OSA was an independent predictor of the presence of NAFLD. The presence of OSA increased the chance of NAFLD 7.6 fold in woman with PCOS. This means that OSA is a predictor of NAFLD along with, but independent of, obesity and IR.

DISCUSSION

This study demonstrates that women with PCOS have high prevalence of OSA and NAFLD. The presence of these diseases is associated with marked IR and a worse metabolic profile in this group of patients. The association between PCOS and IR has been shown in several studies and seems to be responsible for many of the features of this syndrome as well as for the majority of metabolic and cardiovascular complications found in this group of women. Women with PCOS have a higher prevalence of both IR and MS than women with a similar BMI but without PCOS. Although this association is well established, the reason for a greater degree of IR in PCOS subjects continues to be investigated (2-4). Hyperandrogenism and genetic factors seem to be involved in this process. Obesity, which is commonly found in PCOS subjects, has a synergistic effect on the development of IR, especially when predominant in the abdominal region (4).

OSA was present in approximately one-third (31.6%) of PCOS subjects in our study and was closely associated with a higher degree of IR and a worse lipid profile. These results are consistent with previous studies demonstrating a higher prevalence of OSA in PCOS subjects than in healthy women and studies demonstrating an association between OSA and IR in these patients (11-13).

OSA seems to contribute to an unfavorable metabolic profile, and there is evidence that it is an independent risk factor for IR (5-7,22). The metabolic abnormalities found in these patients could be explained by recurrent episodes of upper airway obstruction and arousals that would cause a "stressful" situation, with nocturnal hyperstimulation of the sympathetic nervous system and hypothalamic-pituitary-adrenal axis, increasing the release of catecholamines and cortisol (both insulin counterregulatory). Added to this, chronic intermittent hypoxia may induce the production of inflammatory cytokines, increase oxidative stress, and decrease adiponectin, all of which contribute to an increase in IR (5-7,22,35).

In our study, most patients with OSA were also affected by NAFLD (83.3%), and the AHI was correlated with liver enzyme levels. Studies suggest that OSA may contribute to the development of NAFLD as well as to the acceleration of its progression to NASH (24-26). The initial insult would be fat accumulation in the liver due to IR triggered by OSA, leading to steatosis. NAFLD represents a broad spectrum of tissue changes, ranging from fatty liver infiltration to NASH and in some cases, progressing to cirrhosis (9). There is a clear association between NAFLD and MS components, in that abdominal (visceral) fat is an independent risk factor for the development of NAFLD (10,23). The increased flow of free fatty acids (FFAs) from visceral tissue adipocytes through the portal vein is one of the main pathogenic mechanisms of NAFLD. Reduced insulin sensitivity in these visceral adipocytes leads to increased FFA flow to the liver. This results in the accumulation of fat in the liver and contributes to hepatic IR (9). In addition to this altered FFA secretion, the adipose tissue participates in the development of NAFLD through altered secretion of adipokines, due at least in part to the accumulation of macrophages in the adipose tissue. It has been shown that hypoxemia in adipose tissue is associated with increased expression of proinflammatory adipokines such as interleukin-6 and leptin and with decreased expression of adiponectin (36), resulting in stimulation of lipolysis and reduction in FFA uptake in the adipocytes as a

Table 2
Multivariate Logistic Regression Analysis of the Predictors of OSA in PCOS Subjects

	Odds Ratio	95% Confidence Interval	*P*-value
Obesity (BMI ≥30 kg/m^2)	20.7	2.0-219.1	.012
Hyperandrogenemia	8.2	1.3-49.7	.023
Constant	0.001		.003

Abbreviations: BMI = body mass index; OSA = obstructive sleep apnea; PCOS = polycystic ovary syndrome.

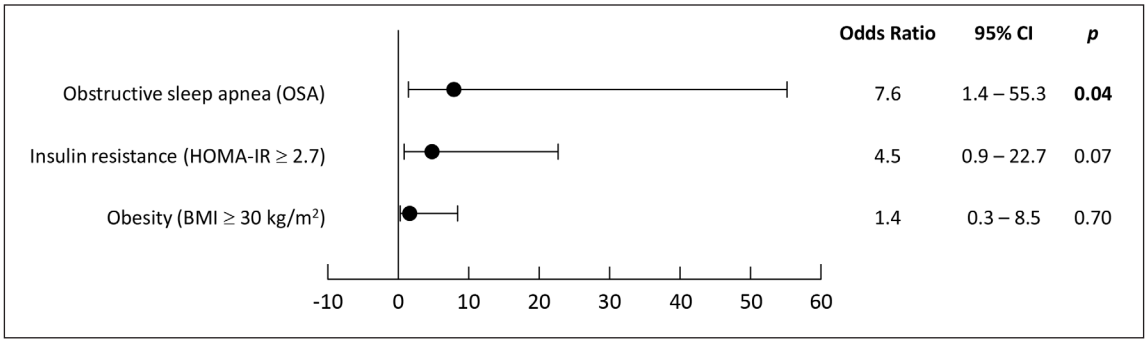

Fig. 1. Multivariate regression logistic analysis of nonalcoholic fatty liver disease predictors. *BMI* = body mass index; *CI* = confidence interval; *HOMA-IR* = homeostasis model assessment for insulin resistance; *OSA* = obstructive sleep apnea.

result of a direct inhibitory effect on fatty acid transporters (37). Repeated episodes of hypoxia can also deteriorate the function of the more susceptible hepatocytes, leading to inflammation and fibrosis (24-26). Experimental studies in mice have demonstrated that, in the liver, hypoxia contributes to the progression of NAFLD by upregulating the expression of lipogenic genes by downregulating genes involved in lipid metabolism and increasing IR (38). The deleterious effects of intermittent nocturnal hypoxia consequent to OSA on the liver and adipose tissue may explain the high prevalence of NAFLD observed in women with PCOS in our study, particularly in those more obese who had a greater amount of visceral fat. This is the first study showing a clear association between OSA and NAFLD in women with PCOS. We found that the presence of OSA increases the risk of a woman with PCOS being affected by NAFLD by 7.6 fold, independent of the degree of obesity and IR.

Hyperandrogenism—a common feature of PCOS—has also been implicated as a factor associated with the increased prevalence of OSA in these patients (11). Some studies suggest a role for androgens in sleep architecture and in the pathogenesis of OSA (19-21). In these studies, administration of exogenous testosterone in men and women induced hypoxia, increased upper airway collapse, changed the threshold for apnea, increased the AHI, and/or reduced the hours of sleep. These effects of testosterone should explain, at least in part, the higher prevalence of OSA in males than in females. In our study, 50% of the women with PCOS and hyperandrogenemia—defined as free testosterone ≥1.07 ng/dL—were also affected by OSA, compared with only 15% of those without high free testosterone levels. Actually, hyperandrogenemia was identified as an independent predictor of the presence of OSA in PCOS women. Some other authors have found a positive association between OSA and high serum androgen levels (11,39). Yang et al (39) showed that even in lean women with PCOS without diagnostic criteria for OSA, total testosterone levels are positively correlated with values of the AHI. However, some controversies exist, as Vgontzas et al (12) and Tasali et al (13) did not find similar results.

Other sex hormones may also have an influence on sleep. Studies in pregnant women and during the luteal menstrual phase demonstrate a stimulatory effect of progesterone on breathing, whereas an improvement in OSA was also observed after administration of synthetic progesterone (19,20). Thus, it is possible that prolonged exposure to an environment with low concentrations of progesterone, due to chronic anovulation in PCOS women, favors the development of OSA. The impact of estrogens on ventilation is not well established, however. It has been demonstrated that menopausal women receiving hormone replacement therapy show reductions in the AHI and in the degree of hypoxemia (19,20). Better results with combined replacement therapy (estradiol plus progesterone) than monotherapy have been reported (19,20).

Finally, we recognize some limitations of this study, including the small number of patients evaluated, the testosterone determination by immunoassay rather than mass spectrometry (considered the gold-standard method for this determination), and the diagnosis of NAFLD by US rather than liver biopsy.

CONCLUSION

Despite the above-mentioned limitations, our results indicate that in women with PCOS, the occurrence of OSA strongly predisposes them to development of both NAFLD and a worse metabolic profile; hence, treatment of OSA might be beneficial for NAFLD. Furthermore, high free testosterone levels in these patients may be a predisposing factor leading to OSA. Further studies are needed to evaluate the impact of OSA treatment on NAFLD in PCOS women.

DISCLOSURE

The authors have no multiplicity of interest to disclose.

ACKNOWLEDGMENT

We thank the Conselho Nacional de Pesquisa (CNPq) and Associação Fundo de Incentivo à Pesquisa (AFIP) for providing funding.

REFERENCES

1. **Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO.** The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab*. 2004;89:2745-2749.
2. **Schröder AK, Tauchert S, Ortmann O, Diedrich K, Weiss JM.** Insulin resistance in patients with polycystic ovary syndrome. *Ann Med*. 2004;36:426-439.
3. **Essah PA, Nestler JE.** The metabolic syndrome in polycystic ovary syndrome. *J Endocrinol Invest*. 2006;29: 270-280.
4. **Carmina E, Bucchieri S, Esposito A, et al.** Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance. *J Clin Endocrinol Metab*. 2007;92:2500-2505.
5. **Vgontzas AN, Papanicolaou DA, Bixler EO, et al.** Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. *J Clin Endocrinol Metab*. 2000;85:1151-1158.
6. **Ip MS, Lam B, Ng MM, Lam WK, Tsang KW, Lam KS.** Obstructive sleep apnea is independently associated with insulin resistance. *Am J Respir Crit Care Med*. 2002;165: 670-676.
7. **Punjabi NM, Shahar E, Redline S, Gottlieb DJ, Givelber R, Resnick HE.** Sleep-disordered breathing, glucose intolerance, and insulin resistance: The Sleep Heart Health Study. *Am J Epidemiol*. 2004;160:521-530.
8. **Marchesini G, Brizi M, Bianchi G, et al.** Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes*. 2001;50:1844-1850.
9. **Farrel GC, Larter CZ.** Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology.* 2006;43(2 suppl 1): S99-S111.
10. **Dâmaso AR, do Prado WL, de Piano A, et al.** Relationship between nonalcoholic fatty liver disease prevalence and visceral fat in obese adolescents. *Dig Liver Dis*. 2008;40:132-139.
11. **Fogel RB, Malhotra A, Pillar G, Pittman SD, Dunaif A, White DP.** Increased prevalence of obstructive sleep apnea syndrome in obese women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2001;86:1175-1180.
12. **Vgontzas AN, Legro RS, Bixler EO, Grayev A, Kales A, Chrousos GP.** Polycystic ovary syndrome is associated with obstructive sleep apnea and daytime sleepiness: role of insulin resistance. *J Clin Endocrinol Metab*. 2001;86: 517-520.
13. **Tasali E, Van Cauter E, Hoffman L, Ehrmann DA.** Impact of obstructive sleep apnea on insulin resistance and glucose tolerance in women with polycystic ovary syndrome. *J Endocrinol Metab*. 2008;93:3878-3884.
14. **Cerda C, Pérez-Ayuso RM, Riquelme A, et al.** Nonalcoholic fatty liver disease in women with polycystic ovary syndrome. *J Hepatol*. 2007;47:412-417.
15. **Gambarin-Gelwan M, Kinkhabwala SV, Schiano TD, Bodian C, Yeh HC, Futterweit W.** Prevalence of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. *Clin Gastroenterol Hepatol*. 2007;5: 496-501.
16. **Vassilatou E, Lafoyianni S, Vryonidou A, et al.** Increases androgen bioavailability is associated with non-alcoholic fatty liver disease in women with polycystic ovary syndrome. *Hum Reprod*. 2010;25:212-220.
17. **Ma RC, Liu KH, Lam PM, et al.** Sonographic measurement of mesenteric fat predicts presence of fatty liver among subjects with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2011;96:799-807.
18. **Brzozowska MM, Ostapowicz G, Weltman MD.** An Association between non-alcoholic fatty liver disease and polycystic ovarian syndrome. *J Gastroenterol Hepatol*. 2009;24:243-247.
19. **Behan M, Wenninger JM.** Sex steroidal hormones and respiratory control. *Respir Physiol Neurobiol*. 2008;164: 213-221.
20. **Andersen ML, Alvarenga TF, Mazaro-Costa R, Hachul HC, Tufik S.** The association of testosterone, sleep, and sexual function in men and women. *Brain Res*. 2011;1416: 80-104.
21. **Zhou XS, RowleyJA, Demirovic F, Diamond MP, Badr MS.** Effect of testosterone on the apneic threshold in women during NREM sleep. *J Appl Physiol*. 2003;94:101-107.
22. **Carneiro G, Ribeiro-Filho FF, Togeiro SM, Tufik S, Zanella MT.** Interactions between obstructive sleep apnea syndrome and insulin resistance. [Article in Portuguese] *Arq Bras Endocrinol Metabol*. 2007;51:1035-1040.
23. **Sabir N, Sermez Y, Kazil S, Zencir M.** Correlation of abdominal fat accumulation and liver steatosis: importance of ultrasonographic and anthropometric measurements. *Eur J Ultrasound*. 2001;14:121-128.
24. **Tanné F, Gagnadoux F, Chazouillères O, et al.** Chronic liver injury during obstructive sleep apnea. *Hepatology.* 2005;41:1290-1206.
25. **Mishra P, Nugent C, Afendy A, et al.** Apnoeic-hypopnoeic episodes during obstructive sleep apnoea are associated with histological nonalcoholic steatohepatitis. *Liver Int*. 2008;28:1080-1086.
26. **Aron-Wisnewsky J, Minville C, Tordjman J, et al.** Chronic intermittent hypoxia is a major trigger for non-alcoholic fatty liver disease in morbid obese. *J Hepatol*. 2012;56:225-233.
27. **The Rotterdam ESHRE/ASRM-Sponsored PCOC Consensus Workshop Group.** Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004;81:19-25.
28. **Scatarige JC, Scott WW, Donovan PJ, Siegelman SS, Sanders RC.** Fatty infiltration of the liver: ultrasonographic and computed tomographic correlation. *J Ultrasound Med*. 1984;3:9-14.
29. **Rechtschaffen A, Kales AA.** *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects*. Brain Information Service/ Brain Research Institute, UCLA, Los Angeles, California, 1968.
30. **Iber C, Acoli-Israel S, ChessonJr, Quans S.** *The AASM Manual for Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications*. American Academy of Sleep, 2007.
31. **Friedewald WT, Levy RI, Fredrickson DS.** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499-502.

32. **Vermeulen A, Verdonck L, Kaufman JM.** A critical evaluation of simple methods for estimation of free testosterone in serum. *J Clin Endocrinol Metab*.1999;84: 3666-3672.
33. **Geloneze B, Vasques ACJ, Stabe CF, et al.** HOMA1-IR and HOMA2-IR indexes in identifying insulin resistance and metabolic syndrome: Brazilian Metabolic Syndrome Study (BRAMS). *Arq Bras Endocrinol Metabol*. 2009;53: 281-287.
34. **Belfiore F, Iannello S, Volpicelli G.** Insulin sensitivity indices calculated from basal and OGTT-induced insulin, glucose, and FFA levels. *Mol Genet Metab*. 1998;63: 134-141.
35. **Carneiro G, Togeiro SM, Ribeiro-Filho FF, et al.** Continuous positive airway pressure therapy improves hypoadiponectinemia in severe obese men with obstructive sleep apnea without changes in insulin resistance. *Metab Syndr Relat Disord*. 2009;7:537-542.
36. **Wang B, Wood IS, Trayhurn P.** Dysregulation of the expression and secretion of inflammation-related adipokines by hypoxia in human adipocytes. *Pflugers Arch*. 2007;455:479-492.
37. **Yin J, Gao Z, He Q, Zhou D, Guo Z, Ye J.** Role of hypoxia in obesity-induced disorders of glucose and lipid metabolism in adipose tissue. *Am J Physiol Endocrinol Metab*. 2009;296:E333-E342.
38. **Piguet AC, Stroka D, Zimmermann A, Dufour JF.** Hypoxia aggravates non-alcoholic steatohepatitis in mice lacking hepatocellular PTEN. *Clin Sci (Lond)*. 2009;118: 401-410.
39. **Yang HP, Kang JH, Su HY, Tzeng CR, Liu WM, Huang SY.** Apnea-hypopnea index in nonobese women with polycystic ovary syndrome. *Int J Gynaecol Obstet*. 2009; 105:226-229.