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ORIGINAL ARTICLE

The relation of serum 25-hydroxyvitamin-D levels with severity of obstructive sleep apnea and glucose metabolism abnormalities

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Abstract Obstructive sleep apnea (OSA) and 25-hydroxyvitamin-D_3 (25-OH-D) deficiency are two separate disorders associating with obesity, inflammation, and impaired glucose metabolism. We aimed to investigate the vitamin D status of OSA patients regarding to potential links between lower vitamin D levels and abnormal glucose metabolism, which is one of the main adverse outcomes of OSA. Study group is composed of 190 non-diabetic subjects who were suspected of having OSA. Subjects undergone polysomnography and were grouped due to apnea−hypopnea indices (AHI) as controls (AHI < 5, $n = 47$), mild OSA ($5 \leq$ AHI < 15, $n = 46$), moderate OSA ($15 \leq$ AHI < 30, $n = 47$), and severe OSA (AHI ≥ 30, $n = 50$). Serum 25-OH-D, HbA_1c, insulin levels were measured and 75-g oral glucose tolerance test was performed. Serum 25-OH-D level (ng/ml) of OSA patients were lower than control subjects (17.4 ± 6.9 vs. 19.9 ± 7.8), and decrement was parallel to severity of OSA; as 18.2 ± 6.4 ($5 \leq$ AHI < 15), 17.5 ± 7.4 ($15 \leq$ AHI < 30), and 16.3 ± 6.9 (AHI > 30), respectively ($P = 0.097$, $r = -0.13$). However, severe female OSA patients had significantly lower 25-OH-D levels (11.55 ng/ml), while control males had the highest mean value (21.7 ng/ml) ($P < 0.001$). Frequency of insulin resistance (IR) was 48%, prediabetes 41%, diabetes 16% in OSA patients. Mean 25-OH-D level of insulin resistant subjects (HOMA-IR ≥ 2.7, $n = 77$, AHI $= 35.5$) was lower than non-insulin resistant subjects (HOMA-IR < 2.7, $n = 113$, AHI $= 19.8$) as 16.18 ± 7.81 versus 19.2 ± 6.6, respectively ($P = 0.004$). 25-OH-D level of 91 non-diabetic subjects ($n = 91$, AHI $= 19.7$) was 19.5 ± 7.4, prediabetics ($n = 75$, AHI $= 28.7$) was 17.45 ± 6.9, and diabetics ($n = 24$, AHI $= 46.3$) was 13.8 ± 5.3 ($P = 0.02$). We showed that subjects with more severe OSA indices (AHI ≥ 15) tended to present lower vitamin D levels correlated to increased prevalence of IR, prediabetes, and diabetes. Vitamin D deficiency may play a role and/or worsen OSA's adverse outcomes on glucose metabolism. OSA patients may be considered for supplementation treatment which was shown to ameliorate abnormal glucose metabolism and inflammation.

Keywords Vitamin D · Obstructive sleep apnea · Prediabetes · Insulin resistance

Introduction

Obstructive sleep apnea (OSA) is a common disorder characterized with repetitive upper airway obstruction resulting in intermittent hypoxia [1]. Many clinical- and population-based studies emphasized the direct relation between increased incidence of impaired glucose metabolism in terms of insulin resistance (IR), type two diabetes mellitus (type 2 DM), and OSA. Although obesity is known to be an important risk factor both for OSA and abnormal glucose metabolism, most of the studies showed an independent association between these two entities [2]. There is growing evidence suggesting that low-grade systemic inflammation may serve as the mechanistic link between

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OSA and metabolic disorders including IR; however, still less is known about keys factors of causality [3].

Vitamin D insufficiency has long been suspected as a risk factor for glucose intolerance and diabetes. The serum concentration of 25-hydroxyvitamin-D_3 (25-OH-D), which also defines the nutritional status, was shown to be lower in obese and/or diabetic patients and in those who were at high risk for diabetes [4]. Adipose tissue, which is a well-known source of inflammatory cytokines, may be responsible for the decreased bioavailability and enhanced metabolic clearance of vitamin D in obese subjects [5]. On the other hand, the important roles of vitamin D in calcium homeostasis and generation of inflammatory response may explain the association between vitamin D deficiency and IR through beta cell dysfunction and impaired insulin secretion [4, 6]. Vitamin D supplementation was shown to improve insulin release and action in some studies; however, it is controversial [7]. Regarding to these mechanistic links, vitamin D deficiency may have a potential role in pathogenesis and/or metabolic outcomes of OSA in terms of inflammatory response and IR. In this cross-sectional study, we investigated the status of glucose metabolism (in terms of IR, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or existence of diabetes) and serum 25-OH-D levels of OSA patients in order to search for a correlation between severities of disease in terms of apnea−hypopnea index (AHI) and also compared to control subjects.

Materials and methods

One hundred ninety eligible subjects, who were suspected for having sleep disorders according to Epworth Sleepiness Scale (*by Murray W. Johns, Sleep 1991*; *14*:*540–5*), were recruited consecutively for polysomnography. The subjects who had previously diagnosed diabetes, serious or uncontrolled chronic diseases (malignancy, renal or hepatic failure, serious heart failure), or the ones who were on multi-drug medication for any reason had been excluded before. Weight and total fat mass (kg), total fat ratio (%), abdominal and visceral fat composition of the subjects were assessed by bioelectrical impedance analysis (BIA) by TBF-310GS^{TM} (Tanita Corporation, Tokyo, Japan) and height was recorded. All individuals participated in a detailed overnight study, and polysomnographic (Jaeger®, Hoechberg, Germany) records were analyzed by an expert using computer software (Profusion PSG 2). The diagnosis and severity of OSA were based on the definitions and cut-offs for AHI, recommended by the American Academy of Sleep Medicine. The study subjects were divided as control group (AHI < 5), mild OSA ($5 \leq$ AHI < 15), moderate OSA ($15 \leq$ AHI < 30), and severe OSA (AHI ≥ 30).

Serum samples were taken after an overnight fasting for routine biochemical investigation including renal and hepatic functions, serum electrolytes (calcium, phosphorus), and lipid profile. Fasting plasma insulin levels was quantified using a chemiluminescent enzyme-labeled immunometric assay (Immulite, Los Angeles, CA) with normal ranges of 2.6−24.9 μU/ml. IR was assessed from fasting glucose and insulin values using homeostasis model assessment (HOMA) calculations with the formula: {[fasting insulin (U/ml)] × [fasting glucose (mg/dl)}/405]. Subjects with HOMA-IR ≥ 2.7 were considered as "insulin resistant." The serum 25-OH-D concentration (ng/ml) was determined using a commercial RIA kit (Immuno-Biological Laboratories, Minneapolis, MN) with normal ranges of 11.1−42.9 ng/ml, and HbA_1c was measured by turbidimetric assay. Finally, 75-g oral glucose tolerance test (OGTT) was performed. The results were evaluated according to ADA criteria as DM, IFG, and IGT [8]. Subjects who had IFG and/or IGT were considered as "prediabetics". The study was performed during summer (June, 2010−September, 2010) and it complied with the declaration of Helsinki and was approved by the local research ethics committee. All subjects gave written informed consent.

All the statistical analyses were performed for each gender separately. Before the analysis, the data were checked whether they meet the assumption of normality for a *t* test or analysis of variance (ANOVA) in each column where the datum of subject's examined parameters included by Anderson–Darling test in software Minitab® 15.1. A total of 22 parameters were used for the analysis. Barlett's test performed for testing the homogeneity of variances. The Model I ANOVA for unequal sample sizes followed by the Tukey's multiple comparison tests. The Kruskal–Wallis test is used where the data is non-parametric. In the tables, data are expressed as means ± SD for the indicated number of observations. *P* value of <0.05 was considered statistically significant. A principal component analysis (PCA) was used for determining the variability of each parameter and their relationships on the meaningful principal components in software PAST®. Of the 22 parameters examined, six of them (TG, HDL, LDL, ALP, Ca, and P) excluded because of their outlier values which caused bias in the analysis. A canonical variate analysis (CVA) using the same 16 parameters performed after a priori grouping which were determined according to their polysomnography results in terms of AHI following a multivariate analysis of variance (MANOVA) with Bonferroni multiple comparisons test, and thereafter a variance–covariance matrix is drawn to understand the predictable relationship between the movement of 25-OH-D level and other parameters in software PAST®. The multilinear correlations values (*r*) were also calculated

and summarized in a table (not shown). The graphics was done with Microsoft Excel 2007.

Results

Study subjects were composed of 77 females and 113 males ($n = 190$) with a mean age of 49.7 ± 9.8 and 46.03 ± 10.8, respectively ($P = 0.003$). Control group involved 47 subjects (female/male; 19/28), where as OSA groups included 46 (18/28), 47 (19/28), 50 (21/29) patients for mild, moderate, and severe groups ($n = 143$), respectively. The characteristics of the OSA patients and control subjects, and comparison of some parameters in the study group are shown in Table 1. Mean serum 25-OH-D level of all study subjects was 17.99 ± 7.25 ng/ml. Although the difference between 25-OH-D levels of all four groups were statistically non-significant ($P = 0.09$), severe OSA patients had significantly lower 25-OH-D levels (16.31 ± 6.98 ng/ml) compared to control group (19.93 ± 7.81 ng/ml) ($P < 0.05$). Serum vitamin D levels were inversely correlated to AHI ($r = -0.13$). Mean body mass index of the study group was 30.67 ± 5.01 kg/m^2 (women: 33.24 kg/m^2 and men: 28.9 kg/m^2). Severe OSA subjects had significantly higher BMI values compared to control subjects (33.4 vs. 29.2 kg/m^2, $P = 0.001$). The body fat composition analyses showed that the increment in BMI was directly correlated to increments in abdominal fat and visceral fat proportions ($r = 0.75$ and $r = 0.4$, respectively); however, there was not such a correlation between BMI and waist−hip ratio. Serum 25-OH-D levels were inversely correlated to BMI ($r = -0.3$).

Mean fasting plasma glucose (FPG), fasting plasma insulin (FPI), HbA_1c (%), second-hour glucose levels of the 75-g OGTT (120 min glu mg/dl), and HOMA-IR values were all significantly different between study groups ($P < 0.05$), and each of these parameters correlated to the increment in AHI in OSA patients as: FPI ($r = 0.47$, $P < 0.001$), HOMA-IR ($r = 0.46$, $P < 0.001$), HbA_1c ($r = 0.38$, $P < 0.001$), FPG ($r = 0.21$, $P = 0.001$), and 120 min glucose levels ($r = 0.25$, $P = 0.003$). Control subjects presented significantly better glucose metabolism parameters compared to severe OSA patients in terms of HOMA-IR, HbA_1c, and 120 min glucose levels (2.1 vs. 4.3; 5.5 vs. 6.1%; 138.9 vs. 172.3 mg/dl, respectively, $P < 0.001$). IFG and/or IGT was found in 34% of control subjects (16/47) and in 41.2% of OSA patients (59/143). Twenty four subjects (12.6%) in the study population were

Table 1 Characteristic features and laboratory results of study subjects

Total	Control (AHI < 5)	Mild OSA (5 ≤ AHI < 15)	Moderate OSA (15 ≤ AHI < 30)	Severe OSA (AHI ≥ 30)	*P*
Number (*n*, female/male):	47 (19/28)	46 (18/28)	47 (19/28)	50 (21/29)	0.994
Age	42.79 ± 9.55	47.78 ± 10.35	49.79 ± 10.62	49.66 ± 10.38	0.003
BMI	29.24 ± 4.93	29.03 ± 4.12	30.76 ± 5.09	33.41 ± 4.64	0.001
Waist/hip ratio (cm)	1.04 ± 0.06	1.00 ± 0.06	1.00 ± 0.06	1.01 ± 0.06	0.016*
Total fat ratio (%)[a]	28.5 ± 9.3	27.9 ± 7.9	29.9 ± 9.1	33.1 ± 7.6	0.014
Visceral fat rate[a]	14.12 ± 3.79	14.62 ± 4.38	15.53 ± 4.97	17.72 ± 4.67	0.001
Abdominal fat rate[a]	36.87 ± 9.56	37.87 ± 8.52	39.25 ± 8.95	42.73 ± 7.33	0.006
FPG	91.77 ± 12.73	93.83 ± 12.73	99.85 ± 17.81	104.12 ± 21.99	0.001
FPI	9.25 ± 2.99	10.86 ± 4.53	12.43 ± 7.31	16.60 ± 10.25	<0.001
HbA_1c	5.52 ± 0.33	5.52 ± 0.35	5.74 ± 0.52	6.07 ± 0.77	<0.001*
120 min glucose[b]	138.91 ± 33.30	148.04 ± 35.96	148.11 ± 40.94	172.26 ± 62.20	0.003
HOMA-IR[c]	2.14 ± 0.73	2.54 ± 1.13	3.18 ± 2.11	4.30 ± 2.82	<0.001*
Non-diabetics (*n*, %)	30 (15.8)	24 (12.6)	21 (11)	16 (8.4)	
Type 2 DM (*n*, %)	1 (0.6)	2 (1.1)	7 (3.7)	14 (7.4)	<0.05
IFG ± IGT (*n*, %)	16 (8.4)	20 (10.5)	19 (10)	20 (10.5)	
25-OH-D	19.93 ± 7.81	18.29 ± 6.48	17.55 ± 7.42	16.31 ± 6.98	0.097

AHI Apnea−hypopnea index, *BMI* body mass index (kg/m^2)

[a] Measured by BIA instrument (Tanita^{TM}), Fasting plasma glucose (FPG): 70−110 mg/dl; Fasting plasma insulin (FPI): 2.6–24.9 U/ml; HbA1c: 4.5–6.1%

[b] Second hour glucose level (mg/dl) of 75-g glucose tolerance test

[c] HOMA-IR:{[fasting insulin (U/ml)] × [fasting glucose (mg/dl)}/405, IFG and IGT: Impaired fasting glucose (>100 mg/dl) and impaired glucose tolerance (120 min glucose > 140 mg/dl); 25-Hydroxy vitamin D (25-OH-D): 11.1−42.9 ng/ml

* Indicate the Kruskal–Wallis test, whereas the others indicates ANOVA (*P* value of <0.05 was considered statistically significant

diagnosed with type-2 diabetes, 21 (%87.5) of whom were moderate−severe OSA patients. While 68% of severe OSA patients were diabetics or prediabetics, 63.8% of control subjects had none of these entities. On the other hand, 25-OH-D levels also inversely correlated to HbA_1c ($r = -0.22$), HOMA-IR ($r = -0.21$), 120 min glucose ($r = -0.2$), FPI ($r = -0.16$), and FPG ($r = -0.16$). Mean serum 25-OH-D level of non-diabetic/non-prediabetic subjects was 19.56 ± 7.47 ng/ml ($n = 91$, AHI = 19.7 ± 21.4), whereas prediabetics was 17.45 ± 6.9 ng/ml ($n = 75$, AHI = 28.7 ± 24.1) and diabetics was 13.82 ± 5.33 ng/ml ($n = 24$, AHI = 46.3 ± 28.9) ($P < 0.05$). Similarly among prediabetic subjects, severe OSA patients had lower mean 25-OH-D levels (16.9 ± 7.4 ng/ml) than controls subjects (18.12 ± 6.8 ng/ml) although statistically no significant. In PCA, the first two components explained 61.54% of the total variance. The first PC has the maximum loadings which were calculated for second hour glucose (glu120), fasting glucose (glu0), HbA_1c as 0.98, 0.63, and 0.62, respectively, whereas the vitamin D level as −0.24 which separates the control group from the rest gradually (Fig. 1). The CVA—not illustrated—also supported the same clustering schema performed for women and men separately (Wilk's lambda 0.73, $F = 5.09$, $P < 0.05$ for women and Wilk's lambda 0.20, $F = 4.12$, $P < 0.05$ for men).

IR (HOMA-IR ≥ 2.70) was detected in 48.3% of OSA patients and in 17% of control subjects (Table 2). Most of the insulin resistant women subjects (91.2%; 34/37) were OSA patients and 70.6% of whom had AHI ≥15. On the other hand, 84.2% of women had not IR in the control group. The ratio of male insulin resistant subjects was also significantly higher in OSA patients (87.5%; 35/40), while 82.1% of control group had HOMA <2.70 ($P < 0.001$). HOMA-IR was significantly correlated with abdominal fat proportions in women and with BMI in men ($P < 0.001$). Mean serum 25-OH-D level of insulin resistant subjects (HOMA-IR ≥ 2.70, $n = 77$, AHI = 35.5 ± 28.4) was lower than non-insulin resistant subjects (HOMA-IR <2.70, $n = 113$, AHI = 19.8 ± 23.2), as 16.18 ± 7.81 vs. 19.22 ± 6.6 ng/ml, respectively ($P = 0.04$). However, the difference was more significant between insulin resistant and non-insulin resistant women (12.26 ± 6.43 vs. 15.94 ± 6.99) compared to men (21.02 ± 5.66 vs. 19.82 ± 7.26) ($P < 0.05$).

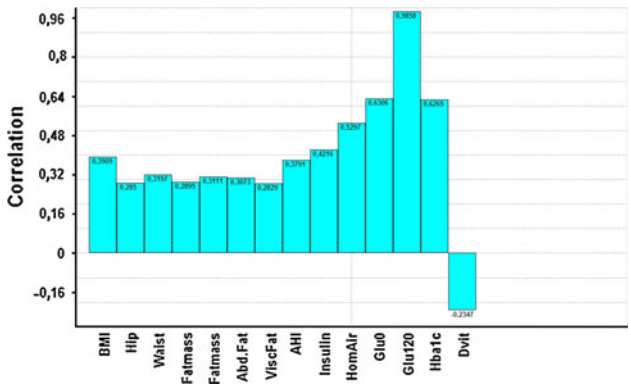

Fig. 1 The correlation of serum vitamin D levels and all other parameters in study subjects after performing the PCA

Comparisons of the parameters according to genders were shown in Table 3. Mean serum 25-OH-D level of women subjects was 14.17 ng/ml and men's was 20.6 ng/ml ($P < 0.001$). While severe female OSA patients had the lowest mean 25-OH-D level (11.55 ng/ml), control males had the highest average (21.7 ng/ml) ($P < 0.001$). There was no statistically significant difference between mean age and 25-OH-D levels among male subjects in the study groups; however, BMI, HOMA-IR, and HbA_1c levels were inversely correlated to AHI ($r = 0.6$, 0.43, and 0.42, respectively, $P < 0.05$). On the other hand, there was significant difference between age, vitamin D status, and glucose metabolism indices of women according to AHI groups ($P < 0.05$), but not between the BMI and visceral fat proportions. Fifty three percent of women subjects diagnosed with type-2 DM or prediabetes and 61.9% of these patients had AHI ≥ 15. On the other side, 51.3% of men diagnosed with type-2 DM or prediabetes, 65.5% of whom had AHI ≥ 15. Women subjects with AHI ≥ 30 had the lowest vitamin D levels (11.5 ng/ml) and the worst glucose metabolism indices ($HbA1_c$, HOMA-IR, 120 min glu) compared to control and mild OSA groups ($P < 0.001$). The relation of serum 25-OH-D levels with AHI, HOMA-IR, $HbA1_c$, and post-load glucose levels according to genders was shown in Fig. 2.

Discussion

OSA refers to a constellation of metabolic disorders, in particular IR and diabetes (DM). We found that ∼22% of moderate−severe OSA patients had previously undiagnosed type-2 DM independent of age and BMI. Similarly, we showed that fasting and post-load glucose levels rose dramatically parallel to AHI, and mean HbA_1c levels also confirmed the prediabetic state especially in moderate−severe OSA groups (5.74 and 6.07%, respectively). In a large clinical study, Meslier and colleagues [9] revealed that type-2 DM was present in 30% of OSA patients, while fasting and post-load second hour blood glucose levels increased and insulin sensitivity decreased with rising severity of OSA. On the other hand, IR in OSA patients had been elaborated in many studies [9–11]. In our study, HOMA-IR values were shown to increase in mild−severe OSA groups and OSA patients had abnormal glucose metabolisms commensurate with severity of OSA.

Table 2 Comparisons of IR patients (HOMA-IR ≥ 2.70) with non-insulin resistant subjects

	Women		Men	
	HOMA < 2.70	HOMA ≥ 2.70	HOMA < 2.70	HOMA ≥ 2.70
Number (*n*)				
AHI < 5	16	3	23	5
5 ≤ AHI < 15	8	10	21	7
15 ≤ AHI < 30	11	8	14	14
AHI ≥ 30	5	16	15	14
Age	49.05 ± 9.49	50.51 ± 10.25	45.93 ± 10.36	46.20 ± 11.68
BMI (kg/m^2)	31.47 ± 4.28**	35.16 ± 5.61**	27.67 ± 3.15***	31.16 ± 4.32***
Waist/hip ratio (cm)	1.00 ± 0.07	1.02 ± 0.07	0.97 ± 0.06	0.99 ± 0.06
Visceral fat rate[a]	12.36 ± 2.48**	14.35 ± 4.17**	15.79 ± 4.55**	18.90 ± 4.70**
Abdominal fat rate[a]	44.91 ± 5.90***	49.64 ± 3.93***	32.40 ± 6.14**	36.46 ± 5.34**
25-OH-D (ng/ml)	15.94 ± 6.99*	12.26 ± 6.43*	21.02 ± 5.66	19.82 ± 7.26

HOMA-IR: {[fasting insulin (U/ml)] × [fasting glucose (mg/dl)}/405, *AHI* Apnea–hypopnea index

[a] Measured by BIA instrument (Tanita^{TM})

* $0.01 < P \leq 0.05$, ** $P = 0.01$, *** $P < 0.001$

However, causality between OSA and abnormal glucose metabolism is controversial but general convictions assembled around the frame of obesity. Obesity is the most well-known common risk factor for OSA, severity of OSA and IR separately. Obese individuals are at about threefold higher risk for OSA and severity of OSA changes about 3% for every 1% change in body weight [1, 2]. In most studies, BMI was used as the index of obesity; however, an independent association was found between OSA and altered glucose metabolism in BMI matched subjects [9, 11, 12]. BMI may not be an adequate measure of body fat distribution thus researches head toward the relation between visceral adiposity with OSA. Vgontzas and colleagues [13] showed that BMI correlated significantly with subcutaneous fat but not with visceral fat. However, visceral fat, but not subcutaneous fat, was significantly correlated with indices of sleep apnea. In our study, we evaluated BMI, waist–hip ratio, and also total body fat, abdominal fat (as subcutaneous fat), and visceral fat proportions of subjects according to each gender by bioelectrical impedance (BIA) method, which has been validated previously against a four-compartment model and was comparable to other techniques including conventional tetrapolar impedance, skinfold thickness, BMI-based formulas, and computed tomography [14]. While BMI correlated gradually with total fat mass (kg), abdominal fat, WHR, and visceral fat proportions in women, respectively, no significant correlation was found with WHR in men. On the other hand, AHI was significantly correlated with visceral fat in both genders especially in moderate–severe OSA groups. These results may suggest that severity of OSA is more related with visceral fat than BMI and WHR in terms of obesity, especially in men. Visceral fat is a metabolically active tissue producing large amounts of proinflammatory cytokines, which are able to alter insulin signaling by inactivating its receptors and may impair insulin secretion and/or function via increasing free fatty acids and inducing beta cell apoptosis [15, 16]. Consequently, visceral fat accumulation may be one of the missing links between IR and OSA particularly in moderate–severe patients. Although in our study population, severe OSA patients had significantly higher BMI values, those patients' visceral fat proportions were not significantly different. However, according to evidence, it can be suggested to choose sample population with BMI and body fat composition matched subjects.

The most well-known role of vitamin D is the regulation of calcium absorption and bone metabolism; however, there is growing evidence about its possible roles in the pathogenesis of inflammation, IR, and diabetes [4, 5]. Hypovitaminosis D is a prevalent disorder in some countries, such as Turkey [17, 18]. Despite appropriate geographic situation for sufficient sunshine, vitamin D deficiency is common especially among women from Turkey, which may probably due to dressing habits [18]. Meanwhile, we collected the serum samples in summer (June–September, 2010) in order to exclude seasonal variations in our study. Women with severe OSA had the lowest vitamin D levels, most of whom were also diabetic, prediabetic, or insulin resistant. Many studies show that vitamin D insufficiency inversely associates with the presence of prediabetes and type-2 DM although the relationship is not consistent [19]. Data from one of the largest cross-sectional studies showed that 25-OH-D concentrations were inversely correlated with diabetes prevalence based on fasting glucose and IR (HOMA-IR) [20]. In our study, we found HOMA-IR, HbA_1c, FPG, and second hour

Table 3 Comparisons of male and female study subjects

	Control AHI < 5	Mild OSA 5 ≤ AHI < 15	Moderate OSA 15 ≤ AHI < 30	Severe OSA AHI ≥ 30	Total	*P* value
Men						
Number	28	28	28	29	113	
Age	41.61 ± 9.23	47.93 ± 11.83	47.00 ± 10.78	47.52 ± 10.50	46.03 ± 10.79	0.094
BMI	26.84 ± 2.95	27.55 ± 2.89	28.87 ± 3.83	32.25 ± 3.81	28.91 ± 3.96	**<0.001**
Visceral fat ratio	15.07 ± 4.04	15.89 ± 4.87	16.42 ± 4.46	20.05 ± 4.50	16.89 ± 4.82	**<0.001**
FPG	92.57 ± 13.81	94.36 ± 13.67	98.32 ± 17.83	99.10 ± 15.80	96.12 ± 15.41	0.323
FPI	9.34 ± 2.74	9.95 ± 3.97	12.47 ± 7.56	15.77 ± 10.73	11.93 ± 7.39	**0.003**
HbA1c	5.49 ± 0.33	5.45 ± 0.29	5.66 ± 0.44	5.96 ± 0.65	5.64 ± 0.49	**0.006***
120 min glucose[a]	139.07 ± 34.13	142.61 ± 28.62	145.61 ± 43.85	170.10 ± 59.30	149.53 ± 44.42	**0.032**
HOMA-IR[b]	2.14 ± 0.66	2.36 ± 1.12	3.12 ± 2.10	3.89 ± 2.85	2.89 ± 2.00	**0.023***
Non-diabetics (*n*, %)	19 (34.5)	14 (25.4)	13 (23.5)	9 (16.4)	55 (48.7)	0.032
Type 2 DM (*n*, %)	0 (0)	1 (9.1)	3 (27.3)	7 (63.6)	11 (9.7)	0.023
IFG ± IGT (*n*, %)	9 (19.2)	10 (21.3)	13 (27.6)	15 (31.9)	47 (41.6)	0.04
25-OH-D	21.70 ± 7.00	19.45 ± 5.72	21.52 ± 6.36	19.75 ± 5.93	20.60 ± 6.27	0.415*
Women						
Number	19	18	19	21	77	
Age	44.53 ± 10.00	47.56 ± 7.83	53.89 ± 9.18	52.62 ± 9.68	49.75 ± 9.82	**0.007**
BMI	32.77 ± 5.20	31.34 ± 4.74	33.55 ± 5.52	35.02 ± 5.27	33.24 ± 5.27	0.176
Visceral fat ratio	12.71 ± 2.95	12.64 ± 2.48	14.21 ± 5.48	14.50 ± 2.52	13.56 ± 3.61	0.237
FPG	90.58 ± 11.21	93.00 ± 11.45	102.11 ± 18.02	111.05 ± 27.35	99.57 ± 20.03	**0.003**
FPI	9.05 ± 3.39	12.26 ± 5.10	12.37 ± 7.13	17.76 ± 9.68	13.00 ± 7.46	**0.002**
HbA1c	5.56 ± 0.33	5.63 ± 0.42	5.86 ± 0.62	6.23 ± 0.90	5.83 ± 0.66	**0.031***
120 min glucose[a]	138.68 ± 32.95	156.50 ± 44.70	151.79 ± 37.09	175.20 ± 67.40	156.05 ± 49.07	**0.023**
HOMA-IR[b]	2.15 ± 0.83	2.81 ± 1.12	3.26 ± 2.20	4.86 ± 2.75	3.32 ± 2.15	**<0.001***
Non-diabetics (*n*, %)	11 (30.6)	10 (27.8)	8 (22.2)	7 (19.4)	36 (46.8)	0.03
Type 2 DM (*n*, %)	1 (7.7)	1 (7.7)	4 (30.7)	7 (53.9)	13 (16.8)	0.01
IFG ± IGT (*n*, %)	7 (25)	7 (25)	7 (25)	7 (25)	28 (36.4)	0.947
25-OH-D	17.33 ± 839	16.49 ± 7.31	11.71 ± 4.41	11.55 ± 5.40	14.17 ± 6.93	**0.01***

AHI Apnea−hypopnea index, *BMI* Body mass index kg/m2, visceral fat measured by BIA instrument (Tanita™), Fasting plasma glucose (FPG): 70−110 mg/dl, Fasting plasma insulin (FPI): 2.6−24.9 U/ml, HbA1c: 4.5−6.1%

[a] Second hour glucose level (mg/dl) of 75-g glucose tolerance test

[b] HOMA-IR:{[fasting insulin (U/ml)] × [fasting glucose (mg/dl)}/405, IFG and IGT: Impaired fasting glucose (> 100 mg/dl) and impaired glucose tolerance (120 min glucose > 140 mg/dl)

* Indicate the Kruskal–Wallis test, whereas the others indicates ANOVA (*P* value of <0.05 was considered statistically significant)

glucose levels correlated with the highest versus lowest 25-OH-D concentrations (Table 1). The mechanistic role of vitamin D in glycemic control was studied in several studies. Pancreatic beta cells were showed to express VDR (vitamin D receptor) and 1-alpha hydroxylase enzyme which may mediate the direct effects of vitamin D [21]. Insulin release is a calcium-dependent process and hypovitaminosis D may alter the balance between extra and intra cellular calcium compartments resulting in beta-cell secretory dysfunction and impaired glucose stimulated insulin release, especially in response to oral glucose load [22]. Besides, impaired insulin signal transduction resulted in decreased glucose transporter-4 activity may conclude peripheral IR in vitamin D insufficiency [22, 23]. Another possible link of vitamin D to IR is its role in inflammatory system. It has been showed that active vitamin D inhibited mononuclear and T lymphocyte proliferation by decreasing the production of inflammatory cytokines (IL-1β, IL-2, IL-6, IFN-γ, and TNF-α) and low-circulating levels of 25-OH-D result in inhibition of VDR-dependent immune response [24, 25]. Vitamin D may improve insulin sensitivity and promote cytokine induced beta cell apoptosis by directly modulating the generation and effects of these cytokines [21, 26–29]. These proinflammatory cytokines involved in IR are also involved in OSA pathogenesis. The association of OSA with low-grade systemic inflammation

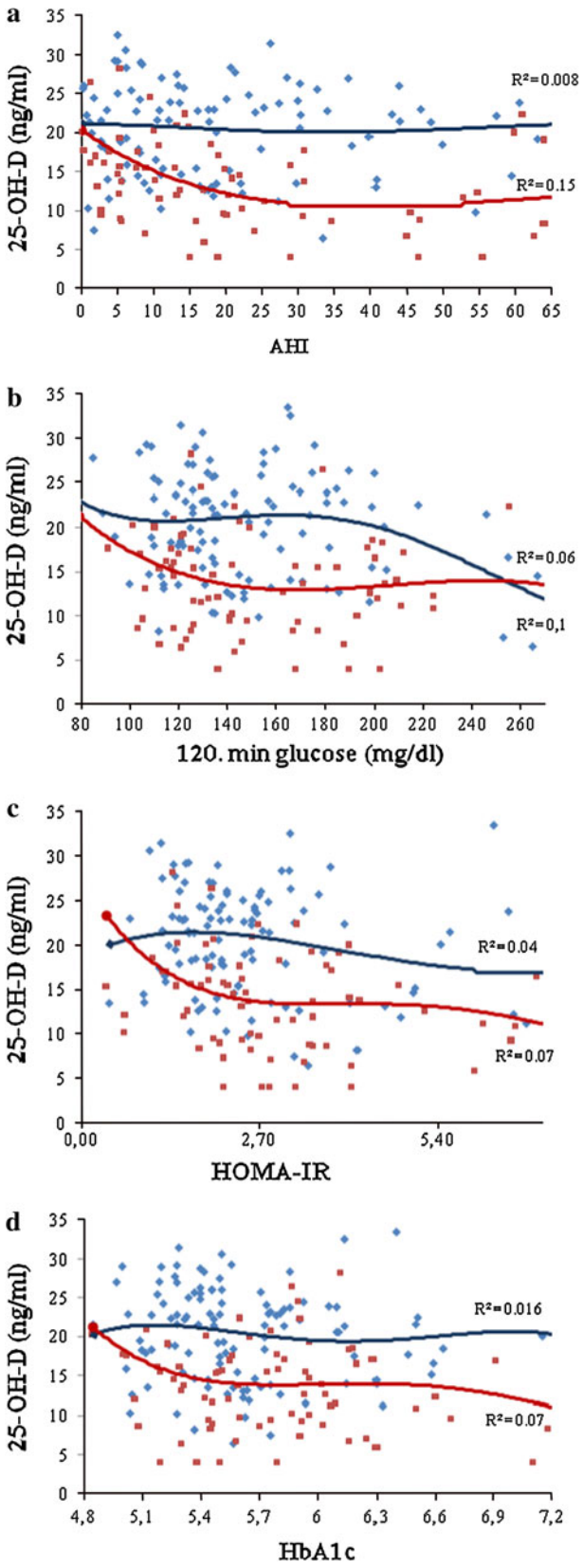

Fig. 2 The relation of serum 25-OH-D levels with **a** apnea−hypopnea index, **b** HbA1c, **c** second hour plasma glucose levels during 75-g OGTT, and **d** IR (HOMA-IR)

is well acknowledged; however, the underlying mechanisms have not been well understood. Number of studies has reported a significant elevation of TNF and IL-6 levels in OSA patients or an effect of AHI on cytokine levels [2, 30, 31]. Based on current evidence; intermittent hypoxia and reoxygenation episodes in OSA may result in the generation of reactive oxygen species which can up-regulate inflammatory pathways [3, 32].

Obesity is postulated to relate with vitamin D deficiency. Obese individuals were shown to have lower plasma 25-OH-D concentrations [5, 33]. It was suggested that bioavailability of vitamin D decreases from cutaneous/dietary sources in obese individuals because of its deposition in fat compartment [34]. However, diabetic/insulin resistant subjects presented lower vitamin D levels compared to age−BMI matched non-diabetic subjects [4, 19, 20, 22]. In our study, severity of OSA in terms of AHI associated directly with BMI, body fat proportions, HbA1c, and HOMA-IR, while inversely associated with vitamin D levels. The severity of OSA was shown to be associated with adverse metabolic outcomes (glucose metabolism) even in age−BMI matched subjects [2, 33]. Mechanistically hypovitaminosis D may aggravate the grade of systemic inflammation as well as IR in moderate−severe OSA patients, who are under risk for vitamin D deficiency maybe because of their increased adiposity. Vitamin D supplementation was shown to ameliorate IR in separate studies. Schleithoff et al. [35] administered 2,000 IU/day vitamin D_3 to their study subjects and after 9 months serum levels of anti-inflammatory IL-10 increased by 43% and pro-inflammatory TNF-α suppressed. Recent data suggested that vitamin D supplementation at prediabetic stage may be of benefit in delaying the progression to clinical type-2 DM [7, 26]. According to our findings, "prediabetes" are more common among OSA patients which may be another reason for their evaluation about vitamin D status.

As a result, OSA patients, especially older post-menopausal women with severe OSA, can be considered for vitamin D supplementation treatment which may be of benefit in reducing the grade of inflammation, improving glucose intolerance and providing cardiovascular risk reduction. However, further investigation is needed to clarify the mechanistic links, especially in terms of inflammatory pathways.

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Conflict of interest The authors declare that they have no conflict of interest.

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