

CIRCULATING IRISIN LEVELS IN NEWLY DIAGNOSED OBSTRUCTIVE SLEEP APNEA PATIENTS

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ABSTRACT

INTRODUCTION: Obstructive sleep apnea syndrome (OSAS) is commonly associated with obesity, insulin resistance, metabolic syndrome, hypertension, and coronary artery disease. Irisin is a newly identified myokine and its serum concentration was found to be correlated with cardiac troponin and creatin kinase-MB in acute myocardial infarction patients. Furthermore, irisin levels were positively associated with endothelium-dependent vasodilation in type 2 diabetic patients.

AIM: In this study, we aimed to investigate serum irisin level in the newly diagnosed OSAS patients.

MATERIALS AND METHODS: After obtaining ethical approval, 32 OSAS patients were included. All patients gave written informed consent. Diagnosis of OSAS was verified by an overnight polysomnography (PSG) and made by an apnea hypopnea index equal to or higher than 5. Venous blood samples were collected in the morning between 08.00 – 10.00 after PSG (n=25) or after one-night CPAP treatment (n=7). Serum irisin concentrations were studied by ELISA.

RESULTS AND CONCLUSION: Serum irisin concentrations were significantly higher in newly diagnosed OSAS group than in OSAS group after one night of CPAP treatment (199.7±42.4 vs 159.7±18.3 ng/mL respectively; p<0.01). These results suggest that increased serum irisin levels can be reduced by CPAP treatment and elevated serum irisin levels may be due to increased respiratory muscle activity and body temperature.

Keywords: sleep apnea, plasma irisin, polisomnography, CPAP treatment, myokines, ELISA

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INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a prevalent systemic disease characterized by repetitive episodes of breathing pauses due to upper airway obstruction during sleep (1). Prevalence of OSAS in population-based studies varies between 3% to 7% and 2% to 5% in adult men and women, respectively (2). OSAS is commonly associated with obesity, insulin resistance, metabolic syndrome, hypertension, coronary artery disease and myocardial

al infarction (3). Although the cardiovascular consequences of OSAS represent the biggest health burden of the disease, the mechanisms which link obstructive sleep apnea (OSA) to cardiovascular disorders have not been completely understood. Current body of knowledge suggests that oxidative stress and systemic inflammation mediate endothelial dysfunction and eventually development of cardiovascular disease in OSAS (3). Adipocyte dysfunction and altered adipokine secretion profile (including leptin increase and adiponectin decrease) may also contribute to the association of OSAS and heart disease (4). Recently, we demonstrated higher arginase activity and lower nitric oxide levels in patients with OSAS than in healthy controls (5). Furthermore, arginase activity was correlated with the presence of cardiovascular disease in patients with OSAS (5). Another potential candidate responsible for the endothelial dysfunction and development of cardiovascular disease in OSAS is irisin.

Irisin is a newly identified myokine secreted from muscle tissue and its serum concentration was found to be correlated with cardiac troponin and creatin kinase-MB in acute myocardial infarction patients (6). Several recent studies demonstrated an association between irisin and endothelial function (7-9). Lower levels of irisin were found to be independently associated with endothelial dysfunction in non-hypertensive, non-diabetic obese subjects (9). Circulating irisin levels were positively associated with endothelium-dependent arterial dilation in type 2 diabetic patients (7). Furthermore, irisin had protective effects on endothelium and improved endothelial function in an animal model of type 2 diabetes (8). These data collectively suggest that irisin may have a role in the pathogenesis of endothelial dysfunction and development of cardiovascular disorders in patients with obstructive sleep apnea syndrome. However, there are only limited data on circulating irisin levels in OSAS patients.

AIM

In this preliminary study, we investigated circulating irisin levels in the newly diagnosed OSAS patients. Furthermore, we assessed the effects of one-night CPAP treatment on serum irisin levels.

MATERIALS AND METHODS

Study Group

The study protocol was approved by local ethics committee and all procedures performed were in accordance with the ethical standards of the institutional and/or national research regulations. The study population consisted of 32 patients with OSAS (mean age, 47.7±12.6 year). Diagnosis of OSAS was verified by whole night computerized polysomnography. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters. All of the patients had complaints of snoring, witnessed apnea, daytime sleepiness, and frequent night awakenings. An apnea hypopnea index (AHI) higher than 5 was accepted as the diagnostic marker of OSAS. Fasting blood samples were obtained in the morning after the sleep study.

Polysomnography

Full-night polysomnography (PSG) was performed by a computerized system (Compumedics 44E Series, Compumedics Australia) and a software (Profusion PSG2, Compumedics, Australia). Twenty-channel polysomnography included electroencephalography (F3A2, F4A1, C4A2, C3A1, O2A1, O1A2), left and right ocular movements, submental electromyography, a nasal cannula to record nasal pressure and a thermistor to monitor oronasal airflow, respiratory effort by thoraco-abdominal belts, finger pulse oximetry, a neck microphone to record snoring, left and right anterior tibialis movement sensors, electrocardiography and body posture. PSG recordings were continued for at least 6 hours. Apnea was defined as a complete cessation of airflow lasting for at least 10 seconds. Hypopnea was defined as a decrease in airflow, if it was associated with either arousal (defined as the appearance for 3 seconds of an alpha rhythm on EEG channels or an increase in the submental EMG signal) or oxygen desaturation of at least 3%. AHI was calculated by dividing the total number of apnea and hypopnea events by the total sleep time. The minimal oxygen saturation was determined as the lowest saturation value associated with a respiratory event.

CPAP Treatment

Overnight continuous positive airway pressure was applied in automatic titration mode under PSG in the sleep laboratory (S8 Autoset Spirit™ II,

ResMed, Australia). Auto-titrating mode automatically adjusts pressure in response to snore, flow limitation and obstructive sleep apneas. All patients used a nasal mask. Minimum and maximum pressure limits were set at 4 cmH₂O and 20 cmH₂O, respectively. Seven patients underwent one-night positive airway pressure treatment during polysomnographic recording.

Blood Sampling

Peripheral venous blood samples were obtained in the morning after the diagnostic study night or after one night of CPAP treatment. The blood samples, centrifuged at 4,000 x g for 10 min, and serum samples were aliquoted and stored at -20°C for analysis.

Biochemical Measurements

Serum irisin measurements were performed by enzyme linked immunosorbent assay (ELISA). Commercial kit (Irisin Competitive ELISA Kit, Cat. No. AG-45A-0046EK-KI01, Adipogen, Switzerland) was used for irisin measurement based on leaflet in-box instructions. Intra- and inter-assay coefficient of variation were <8% and <10%, respectively. Sample absorbance of ELISA reader was set at 450 nm.

Statistical Analysis

All data were given as means and standard deviations unless otherwise indicated. Normal distribution of variables was tested by One Sample Kolmogorov Smirnov test. Comparison of mean values obtained pre- and post-exercise measurements were performed by paired samples t-test for parametric variables, and Wilcoxon signed ranks test for non-parametric variables. Between group comparisons were made by t-test for parametric variables and by Mann Whitney U test for non-parametric variables. The analysis of covariance test was used to test the difference between the mean percent changes in both gender groups. A p value lower than 0.05 was accepted as statistically significant.

RESULTS

General characteristics of the OSAS and CPAP groups are given in Table 1. OSAS and CPAP groups were comparable in terms of age, weight, height and body mass index. Male-to-female ratio was 6/1 and 17/8 in CPAP and OSAS groups respectively. There was no significant gender difference between the two groups (Chi-Square $p>0.05$). Polysomnography

showed that apnea hypopnea index was significantly lower and minimum oxygen saturation was significantly higher in CPAP group than in OSAS group (Table 1). All patients in CPAP group had moderate-to-severe OSAS based on apnea hypopnea index. The mean apnea-hypopnea index reduced from 50.7 ± 27.3 to 3.3 ± 7.2 h⁻¹ with one night of CPAP treatment. The

Table 1. General characteristics, polysomnography and serum irisin results of obstructive sleep apnea and continuous positive airway pressure (CPAP) patients

	OSAS group N=26	CPAP group N=7
Age, yr	45.8±11.6	51.1±13.3
Height, m	1.68±0.09	1.69±0.07
Weight, kg	94.0±23.2	90.1±12.3
BMI, kg/m ²	33.0±7.8	31.7±5.8
AHI, hour ⁻¹	34.2±30.7	3.3±7.2***
MinO ₂ , %	80.9±10.1	91.1±6.0**
REM, %	17.2±7.7	16.4±5.5
N1, %	5.6±5.3	4.0±3.2
N2, %	54.8±17.0	41.2±12.3*
N3, %	22.3±12.8	38.3±11.0**
Irisin, ng/mL	199.7±42.4	159.7±18.3**

Abbreviations: OSAS, obstructive sleep apnea syndrome; CPAP, continuous positive airway pressure; BMI, body mass index; AHI, apnea hypopnea index; REM, rapid eye movement; N1, stage 1 NREM sleep; N2, stage 2 NREM sleep; N3, stage 3 NREM sleep.

* $p<0.05$; ** $p<0.01$; *** $p<0.001$ (Mann-Whitney U test)

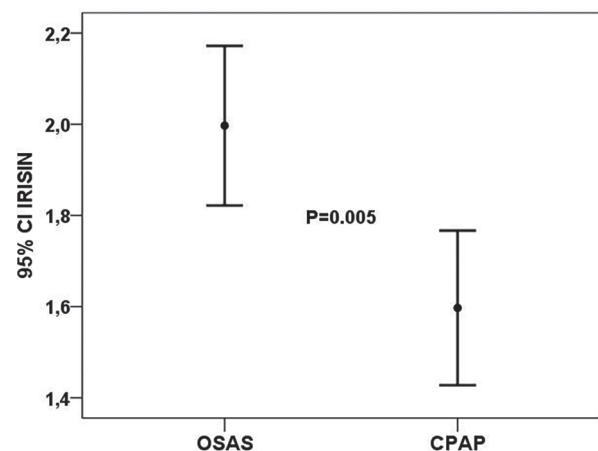


Fig. 1. Comparison of serum irisin concentrations of patients with obstructive sleep apnea syndrome (OSAS) and OSAS patients after one night of continuous positive airway pressure (CPAP) treatment

mean serum irisin level was significantly higher in OSAS group (Fig. 1).

DISCUSSION

The main finding of this study is that there are higher serum irisin levels in newly diagnosed OSAS patients than OSAS patients with one night of CPAP treatment. There may be several possible explanations for higher irisin levels in OSAS. One explanation is the increased inspiratory effort in patients with sleep disordered breathing (10). Obstructive events during sleep resume with an increase in respiratory muscle activity. As irisin is secreted from muscles, increased activity of respiratory muscles during nocturnal obstructive events in these patients may contribute to elevated serum irisin levels. Unfortunately, no previous study correlated irisin alterations to respiratory effort in OSAS patients. Another explanation is body temperature-induced irisin secretion. Both serum and saliva irisin concentrations have been shown to increase after a 45-minute Turkish bath with an ambient temperature of $47\pm 3^{\circ}\text{C}$ (11). Patients with OSAS commonly report nocturnal sweating and hyperhidrosis which may be a surrogate marker of increased body temperature. On the contrary, some other studies suggested irisin as a cold-activated thermogenic factor (12). In fact, cold exposure is known to drive browning of white adipose tissue (13) which is the original function of irisin (14). In addition, we have already known that nocturnal hypoxia may influence adaptive thermogenesis in apneic patients (15). Thus, alterations in circulating irisin concentration of OSAS patients would be related to the regulation of body temperature in these patients.

Li et al. (16) reported decreased serum irisin concentrations in patients with OSAS. They suggested that irisin may be involved in the mechanism of OSAS development and progression through its anti-inflammatory effects (16), which means first irisin levels decrease and then OSAS develops. On the contrary, we found elevated serum irisin levels in OSAS which reduced after one night of CPAP treatment. Our findings suggested that altered circulating irisin level was a result rather than a cause in OSAS.

Elevated serum irisin levels may have some pathophysiologic and clinical implications. Increased irisin levels were found to be in positive correlation with insulin resistance (17, 18) and metabolic

syndrome independent from obesity (17). They were also associated with vascular atherosclerosis in non-diabetic adult subjects (18). Previous studies showed an association between OSAS and high frequency of glucose metabolism disorders and insulin resistance (19). Furthermore, 8 weeks of CPAP treatment increased insulin secretion capacity in patients with moderate-to-severe OSAS (20). These studies suggest that elevated irisin levels may, at least in part, have a role in development of metabolic syndrome, insulin resistance, and atherosclerosis in OSAS. Curiously, irisin has some potential to be a marker of metabolic disorder in patients with sleep apnea.

This study has several limitations. First, a control group without OSAS is lacking. Adding such group would enable us to compare between OSAS and control subjects. Second, we did not evaluate the cardiovascular comorbidities in this patient group. Analysis of such subsets would increase the level of difference in irisin concentrations. And finally, the exercise history of these patients was not evaluated. Regular exercise versus sedentary lifestyle may show differential impact on irisin levels.

CONCLUSION

Here for the first time, we report elevated serum irisin levels in patients with OSAS which reduced following a night under CPAP treatment. Elevated serum irisin levels may be due to increased respiratory muscle activity and body temperature.

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Compliance with ethical standards

Conflict of interest: All authors declare that they have no affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

REFERENCES

1. American Academy of Sleep Medicine. International classification of sleep disorders, 3rd ed. Darien, IL: American Academy of Sleep Medicine; 2014. p. 53-62.
2. Punjabi NM. The epidemiology of adult obstructive sleep apnea. *Proc Am Thorac Soc.* 2008;5(2):136-43. doi: 10.1513/pats.200709-155MG.
3. Kent BD, Ryan S, McNicholas WT. Obstructive sleep apnea and inflammation: relationship to cardiovascular co-morbidity. *Respir Physiol Neurobiol.* 2011;178(3):475-81. doi: 10.1016/j.resp.2011.03.015.
4. Ozturk G, Yaprak M, Gurel EE, Ural K, Tuncer B, Ozturk L. Adipobiology of obstructive sleep apnea syndrome. *Adipobiology.* 2014;6:23-9.
5. Yüksel M, Okur HK, Pelin Z, Ogunc AV, Ozturk L. Arginase activity and nitric oxide levels in patients with obstructive sleep apnea syndrome. *Clinics(Sao Paulo).* 2014;69(4):247-52. doi: 10.6061/clinics/2014(04)05.
6. Aydin S, Aydin S, Kobat MA, Kalayci M, Eren MN, Yilmaz M, et al. Decreased saliva/serum irisin concentrations in the acute myocardial infarction promising for being a new candidate biomarker for diagnosis of this pathology. *Peptides.* 2014;56:141-5. doi: 10.1016/j.peptides.2014.04.002.
7. Xiang L, Xiang G, Yue L, Zhang J, Zhao L. Circulating irisin levels are positively associated with endothelium-dependent vasodilation in newly diagnosed type 2 diabetic patients without clinical angiopathy. *Atherosclerosis.* 2014;235(2):328-33. doi: 10.1016/j.atherosclerosis.2014.04.036.
8. Zhu D, Wang H, Zhang J, Zhang X, Xin C, Zhang F, et al. Irisin improves endothelial function in type 2 diabetes through reducing oxidative/nitrative stresses. *J Mol Cell Cardiol.* 2015;87:138-47. doi: 10.1016/j.yjmcc.2015.07.015.
9. Hou N, Han F, Sun X. The relationship between circulating irisin levels and endothelial function in lean and obese subjects. *Clin Endocrinol.* 2015;83(3):339-43. doi: 10.1111/cen.12658.
10. Pelin Z, Karadeniz D, Ozturk L, Gozukirmizi E, Kaynak H. The role of mean inspiratory effort on daytime sleepiness. *Eur Respir J.* 2003;21(4):688-94. DOI: 10.1183/09031936.03.00298903.
11. Aydin S, Aydin S, Kuloglu T, Yilmaz M, Kalayci M, Sahin I, et al. Alterations of irisin concentrations in saliva and serum of obese and normal-weight subjects, before and after 45 min of a Turkish bath or running. *Peptides.* 2013;50:13-8. doi: 10.1016/j.peptides.2013.09.011.
12. Calton EK, Soares MJ, James AP, Woodman RJ. The potential role of irisin in the thermoregulatory responses to mild cold exposure in adults. *Am J Human Biol.* 2013;28(5):699-704. doi: 10.1002/ajhb.22853.
13. Lim S, Honek J, Xue Y, Seki T, Cao Z, Andersson P, Yang X, et al. Cold-induced activation of brown adipose tissue and adipose angiogenesis in mice. *Nat Protocol.* 2012;7(3):606-15. doi: 10.1038/nprot.2012.013.
14. Hofmann T, Elbelt U, Stengel A. Irisin as a muscle-derived hormone stimulating thermogenesis – a critical update. *Peptides.* 2014;54:89-100. doi: 10.1016/j.peptides.2014.01.016.
15. Hins J, Series F, Almeras N, Tremblay A. Relationship between severity of nocturnal desaturation and adaptive thermogenesis: preliminary data of apneic patients tested in a whole-body indirect calorimetry chamber. *Int J Obesity.* 2006;30(3):574-7. doi: 10.1038/sj.ijo.0803159.
16. Li Y, Li X, Sun D, Cai S. Association of serum irisin concentrations with the presence and severity of obstructive sleep apnea syndrome. *J Clin Lab Anal.* 2016;31(5):1-5. doi:10.1002/jcla.22077.
17. Park KH, Zaichenko L, Brinkoetter M, Thakkar B, Sahin-Efe A, Joung KE, et al. Circulating irisin in relation to insulin resistance and the metabolic syndrome. *J Clin Endocrinol Metab.* 2013;98(12):4899-907. doi: 10.1210/jc.2013-2373.
18. Sesti G, Andreozzi F, Fiorentino TV, Mannino GC, Sciacqua A, Marini MA, et al. High circulating irisin levels are associated with insulin resistance and vascular atherosclerosis in a cohort of nondiabetic adult subjects. *Acta Diabetol.* 2014;51(5):705-13. doi: 10.1007/s00592-014-0576-0.
19. Bulcun E, Ekici M, Ekici A. Disorders of glucose metabolism and insulin resistance in patients with obstructive sleep apnoea syndrome. *Int J Clin Pract.* 2012;66(1):91-7. doi: 10.1111/j.1742-1241.2011.02795.x.
20. Cuhadaroglu C, Utkusavas A, Ozturk L, Salman S, Ece T. Effects of nasal CPAP treatment on insulin resistance, lipid profile, and plasma leptin in sleep apnea. *Lung.* 2009;187(2):75-81. doi: 10.1007/s00408-008-9131-5.