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LETTERS

Reduced plasma fetuin-A levels in patients with obstructive sleep apnoea

To the Editors:

Obstructive sleep apnoea syndrome (OSAS) has been increasingly linked to cardiovascular disease. fetuin-A is an inhibitor of vascular calcification and an anti-inflammatory cytokine. We tested the hypothesis that plasma levels of fetuin-A are decreased in patients with OSAS.

We studied 119 patients with OSAS and 35 controls. Participants were recruited and studied at the sleep unit at Hospital Universitari Son Espases, Palma de Mallorca, Spain. Serum levels of fetuin-A, glucose, triglycerides, cholesterol, high-density lipoprotein (HDL) cholesterol, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyltransferase (GGT) were determined.

Plasma fetuin-A levels were significantly lower in patients with OSAS than in controls (mean $\pm$SD 368 $\pm$ 66 *versus* 445 $\pm$ 53 ng·mL^{-1}, p=0.015). In multivariate analysis, fetuin-A levels were independently associated with OSAS (p=0.034). OSAS is associated with reduced levels of fetuin-A and fetuin-A could be one of the contributing factors for the development of cardiovascular complications in OSAS patients.

There is evidence that patients with OSAS have an increased risk for cardiovascular diseases [1]. The mechanisms underlying this association are unclear but candidate mechanisms include endothelial dysfunction, oxidative stress, systemic inflammation and metabolic dysregulation [2]. Vascular calcification has recently received much attention because of its relationship with cardiovascular disease [3, 4]. Chronic inflammation may promote vascular calcification and recent studies have demonstrated a relationship between vascular calcification and endothelial dysfunction [5, 6].

Fetuin-A is a circulating protein mostly synthesised in the liver [7, 8] that is known to be an important inhibitor of vascular calcification and a potent anti-inflammatory cytokine [8–10]. Fetuin-A is considered to be a mediator that links chronic inflammation to cardiovascular diseases [11]. However, previous studies investigating the role of fetuin-A in patients with cardiovascular disease have published contradictory results [12]. Furthermore, several studies have proposed that serum fetuin-A levels are associated with the presence of the metabolic syndrome, suggesting fetuin-A as a risk factor for this condition [13].

Therefore, the aim of this study was to determine serum levels of fetuin-A in patients with OSAS and to examine the associations between fetuin-A levels and a number of conventional cardiovascular and metabolic risk factors.

The study population included 154 subjects admitted to the Hospital Universitari Son Espases sleep unit from January 2010 to December 2010. They had all been referred to the sleep laboratory for snoring or suspected OSAS. The case or control status was defined by the apnoea–hypopnoea index (AHI) threshold of $\geqslant$10.

No participant suffered from any other chronic disease (chronic obstructive pulmonary disease, liver cirrhosis, thyroid dysfunction, rheumatoid arthritis, chronic renal failure and/or psychiatric disorders). The study was approved by the Hospital Universitari Son Espases Ethics Committee and all participants signed a consent form after being fully informed of the study goal and characteristics.

The diagnosis of OSAS was established by full polysomnography (E-Series; Compumedics Ltd, Abbotsford, Australia) that included recording of oronasal flow, thoracoabdominal movements, electrocardiography, submental and pretibial electromyography, electrooculography, electroencephalography and trancutaneous measurement of arterial oxygen saturation (Sa,O_2). Apnoea was defined by the absence of airflow for >10 s. Hypopnoea was defined as any airflow reduction that lasted >10 s and resulted in arousal or oxygen desaturation. We considered desaturation a decrease in Sa,O_2 >3%. The AHI was defined as the sum of the number of apnoeas plus hypopneas per hour of sleep. Excessive daytime sleepiness was quantified subjectively by the Epworth sleepiness scale. The occurrence of the metabolic syndrome was analysed according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) clinical criteria [14]. The diagnosis of cardiovascular disease (myocardial infarction, unstable angina, coronary bypass or coronary angioplasty, stroke or transient ischaemic attack) was recorded according to the clinical history.

After fasting overnight, venous blood samples were obtained between 08:00 h and 10:00 h. Blood was centrifuged and serum was immediately separated into aliquots and stored at -80°C until analysis. Glucose, triglycerides, total cholesterol, HDL cholesterol, creatinine, AST, ALT and GGT were determined using standard enzymatic methods on a Hitachi Modular analyser (Roche Diagnostics, Indianapolis, IN, USA). The plasma levels of fetuin-A were determined by ELISA using a commercial kit (BioVender laboratory Medicine, GmbH, Heidelberg, Germany). Measurements were always performed in duplicate and mean values were used for analysis.

Results are presented as percentages, median or mean $\pm$SD. Parametric (unpaired t-test) and nonparametric tests (Wilcoxon test) were performed to assess the statistical significance of differences between groups. Correlations between variables were explored using the Spearman-rank test. To determine the independent effect of sleep apnoea on fetuin-A levels we used stepwise multiple regression analyses with study group (OSAS

versus non-OSAS), AHI, arousal index, age, sex, body mass index (BMI), metabolic and cardiovascular risk factors as the independent variables and fetuin-A as the dependent variable. A p-value <0.05 was considered significant.

Characteristics of the study population are summarised in table 1. No differences were found for age, sex, BMI and waist circumference between patients with OSAS and controls. The prevalence of diabetes and metabolic syndrome was higher in the OSAS group than in the control group (p=0.006 and p=0.005).

Compared with controls subjects, OSAS patients showed abnormal plasma levels of glucose and GGT. Fetuin-A levels were significantly lower in patients with OSAS than in subjects without OSAS (368±66 *versus* 445±53 ng·mL^{-1}, p=0.015). Fetuin-A levels were significantly related to AHI (r= -0.226, p=0.006) and to the arousal index (r= -0.236, p=0.010). In multivariate analysis, study group and AHI were significantly associated with fetuin-A levels (p=0.034 and p=0.041, respectively).

We categorised all subjects into fetuin-A tertiles: 1) <332 ng·dL^{-1}, 2) 332–431 ng·dL^{-1}, and 3) >431 ng·dL^{-1}. Fetuin-A levels were inversely associated with the prevalence of OSAS: first tertile, 88%; second tertile, 81%; and third tertile, 60% (p=0.012). Patients with OSAS and a history of cardiovascular disease revealed a trend towards lower levels of fetuin-A compared with patients without prevalent cardiovascular disease (364±97 *versus* 402±159 ng·mL^{-1}, p=0.07). Fetuin-A levels did not differ between patients with and without diabetes (383±93 *versus* 387±147 ng·mL^{-1}, p=0.892) or metabolic syndrome (373±131 *versus* 394±147 ng·mL^{-1}, p=0.483).

TABLE 1 Baseline characteristics and sleep profiles in controls and obstructive sleep apnoea syndrome (OSAS) patients

	Controls	OSAS
Subjects n	35	119
Age yrs	47±13	47±12
Males	24 (70)	88 (74)
BMI kg·m^{-2}	28±6	28±4
Waist circumference cm	100±13	101±11
Hypertension	8 (23)	26 (22)
Diabetes	1 (3)	14 (12)*
Metabolic syndrome	4 (12)	41 (35)*
Cardiovascular disease	6 (17)	28 (24)
AHI events·h^{-1}	5.0±2.1	40.1±20*
Arousal index events·h^{-1}	22.2±4.5	46.9±18.3*
Mean S_{a,O_2} %	97±2.3	94±2.6*
Minimal S_{a,O_2} %	90±3.6	83.3±3
Glucose mg·dL^{-1}	94±4	103±22*
Triglycerides mg·dL^{-1}	124±51	137±64
Cholesterol mg·dL^{-1}	207±41	212±39
HDLc mg·dL^{-1}	56±15	55±16
Creatinine mg·dL^{-1}	0.88±0.2	0.96±0.3
AST U·L^{-1}	22±7	21±7
ALT U·L^{-1}	27±15	27±13
GGT U·L^{-1}	32±27	37±29*

Data are presented as mean±SD or n (%), unless otherwise stated. BMI: body mass index; AHI: apnoea–hypopnoea index; S_{a,O_2}: arterial oxygen saturation; HDLc: high-density lipoprotein cholesterol; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyltransferase. *: p<0.05.

This study shows that circulating fetuin-A levels are decreased in patients with OSAS and that they are associated with OSAS severity. These observations suggest that fetuin-A could be one of the contributing factors for the development of cardiovascular complications in OSAS patients.

The high cardiovascular mortality and morbidity rates in patients with OSAS are only partially explained by the high prevalence of traditional cardiovascular risk factors [2]. Accumulating evidence suggests that inflammatory balance and especially anti-inflammatory factors are determinants for the prognosis of atherosclerotic disease [15, 16]. However, recent studies have demonstrated that the prevalence and extent of vascular calcification is a strong predictor of cardiovascular events [17–19].

Fetuin-A deficiency is a potential missing link between a state of chronic inflammation and high incidence of cardiovascular events and mortality [20]. Fetuin-A is an anti-inflammatory mediator that participates in macrophage deactivation and inhibition of apoptosis of vascular smooth muscle cells [9]. In addition, fetuin-A complexes with calcium and phosphorus in the circulation and protects against vascular calcification [8, 9]. In this study, fetuin-A levels were significantly lower in OSAS patients than in subjects without OSAS. The mechanisms that support a relationship between OSAS and a decrease in the levels of fetuin-A are not known. We found a significant correlation between fetuin-A levels and AHI and arousal index suggesting that intermittent hypoxia and sleep fragmentation may influence the synthesis and the rate of fetuin-A release into the circulation. However, low fetuin-A levels in OSAS patients could be a consequence of the chronic inflammation that characterises OSAS. Patients with OSAS tend to be in a state of microinflammation in which downregulation of proteins such as fetuin-A may be expected. As chronic inflammation may contribute to fetuin-A depletion, it is plausible that serum fetuin-A levels may represent a useful marker for the prediction of clinical outcome in OSAS patients.

Functions and regulatory mechanisms of fetuin-A seem to differ according to the pathophysiological characteristics of the population studied. Fetuin-A was shown to act as a natural inhibitor of the insulin receptor tyrosine kinase in liver and skeletal muscle and different studies have suggested that high fetuin-A levels are associated with the presence or development of metabolic syndrome [13].

In this study, we controlled for most potential confounding factors and found no attenuation of the inverse association of fetuin-A with OSAS. Furthermore, patients with a history of cardiovascular disease showed lower levels of fetuin-A compared with patients without prevalent cardiovascular disease but differences just failed to reach the statistical level of significance. These observations suggest that the effects of fetuin-A may be of greater importance in the cardiovascular risk of these patients independent of metabolic factors.

Taken together our results suggest a mechanistic relationship between changes in fetuin A production or release and OSAS.

It is also possible that lower fetuin-A levels might reflect the presence of advanced atherosclerosis with calcification in patients with OSAS and could imply a novel link between OSAS and increased cardiovascular risk. Additional studies are needed to clarify the significance of these findings.

Several limitations should be considered. First, we did not evaluate biomarkers of inflammation so we could not investigate potential relationships between them and fetuin-A. Secondly, we did not measure the plasma levels of fetuin-A after continuous positive airway pressure and this may be a limitation in the assessment of the independent effects of OSAS on this marker. Thirdly, the number of subjects was limited, thus studies in larger populations are needed to confirm our data.

This study shows that plasma fetuin-A levels are decreased in OSAS. Future studies are needed to determine if low fetuin-A levels are related to the pathogenesis of cardiovascular risk in OSAS.

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