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Usefulness of Fetuin-A to Predict Risk for Cardiovascular Disease among Patients with Obstructive Sleep Apnea

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Abstract

Patients with obstructive sleep apnea (OSA) are at increased risk for cardiovascular diseases (CVD). Fetuin-A, a novel hepatokine, has been associated with the metabolic syndrome (MetS), insulin resistance, and type 2 diabetes (T2DM), all of which are highly prevalent in OSA and associated with increased CVD risk. The goal of the study was to determine whether fetuin-A could be involved in the pathogenesis of CVD risk in OSA, via relationships of fetuin-A with MetS components and/or insulin resistance. Overweight/obese, non-diabetic volunteers (n=120) were diagnosed with OSA by in-laboratory nocturnal polysomnography. Steady-state plasma glucose (SSPG) concentrations derived during the insulin suppression test were used to quantify insulin-mediated glucose uptake; higher SSPG concentrations indicated greater insulin resistance. Fasting plasma fetuin-A and lipoprotein concentrations were measured. Whereas neither the prevalence of MetS nor the number of MetS components was associated with tertiles of fetuin-A concentrations, the lipoprotein components of MetS, triglycerides and high density lipoprotein cholesterol (HDL-C), increased (p <0.01) and decreased (p <0.05), respectively, across fetuin-A tertiles. Additionally, comprehensive lipoprotein analysis revealed that very low density lipoprotein (VLDL) particles and its subfractions (VLDL₁₊₂ and VLDL₃) were increased across fetuin-A tertiles. On the other hand, neither insulin resistance nor sleep measurements related to OSA were found to be modified by fetuin-A concentrations. In conclusion, abnormalities of lipoprotein metabolism, but not MetS or insulin resistance *per se*, may represent a mechanism by which fetuin-A contributes to increased CVD risk in OSA.

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Keywords

fetuin-A; cardiovascular disease risk; metabolic syndrome; insulin resistance; obstructive sleep apnea; lipoprotein concentrations

Introduction

There is substantial evidence that patients with obstructive sleep apnea (OSA) are at increased risk for cardiovascular diseases (CVD).¹ Pathophysiologic abnormalities in OSA including oxidative stress, inflammation, endothelial dysfunction, dyslipidemia, and insulin resistance have been implicated in this pathway, but the relative contribution of these putative mechanisms in mediating CVD risk is not clear. Fetuin-A, a circulating glycoprotein secreted by the liver, may have anti-atherosclerotic properties via its ability to inhibit ectopic calcification.² Fetuin-A levels have been found to be inversely associated with coronary artery calcification severity in individuals without clinically apparent CVD,³ although the direction of association of fetuin-A with CVD⁴⁻⁶ outcomes or mortality has been variable across different populations. At the same time, elevations in fetuin-A have also been linked to the metabolic syndrome (MetS),⁷ insulin resistance,⁸⁻¹¹ and type 2 diabetes (T2DM),^{11, 12} all of which are highly prevalent in OSA¹³ and associated with increased CVD risk. The goal of the study was to evaluate whether fetuin-A could be involved in the pathogenesis of CVD risk among patients with OSA, and whether the mechanisms could be attributed to the relationship of fetuin-A with MetS and/or insulin resistance in OSA. To pursue this goal we studied 120 overweight/obese individuals who had been diagnosed with OSA but without known histories of CVD or T2DM, and examined the relationships of fetuin-A with individual components of MetS, comprehensive lipid/lipoprotein profiling, and insulin-mediated glucose disposal.

Methods

Eligibility for the study included men and women who were overweight/obese (25.0 – 40.0 kg/m²) and aged 30–70 years old. Recruitment occurred between July 2010 and July 2014 through local print, online, and radio advertisements and at the Stanford Sleep Medicine Center.

Individuals were excluded if they had a fasting glucose $\geq$ 126 mg/dL, history of T2DM or use of antidiabetic medications, CVD, kidney or liver diseases, or received treatment for OSA. The study protocol was approved by the Stanford Administrative Panels for the Protection of Human Subjects. Informed consent was obtained from all participants.

Full-night polysomnography (PSG) was performed at the Stanford Sleep Medicine Center according to standard procedures.¹⁴ In addition to characteristic symptoms, a diagnosis of OSA was made by having an apnea-hypopnea index (AHI) $\geq$ 5 events/hour. Manual scoring of all PSGs was conducted in accordance to the American Academy of Sleep Medicine Manual (2007).¹⁴ OSA severity was defined as follows: mild OSA: AHI 5–14.9 events/hr; moderate: 15–30 events/hr; and severe: $>$ 30 events/hr. Additional measurements included minimum (minO₂) and mean oxygen (meanO₂) saturation, and oxygen desaturation index

(ODI, number of events/hour in which 3% decline in oxygen saturation occurs from baseline).

After a 12 hour fast, individuals were admitted to the Stanford Clinical and Translational Research Center. Individuals underwent the modified insulin suppression test¹⁵ to quantify insulin-mediated glucose disposal. Participants received intravenous octreotide (0.27 $\mu\text{g}/\text{m}^2/\text{min}$), insulin (32 $\text{mU}/\text{m}^2/\text{min}$), and glucose (267 $\text{mg}/\text{m}^2/\text{min}$) over 3 hours. During the final 30 minutes blood was drawn every 10 minutes for measurements of plasma insulin and glucose. These 4 values were averaged to obtain steady-state plasma insulin (SSPI) and steady-state plasma glucose (SSPG) concentrations for each individual. Since SSPI is similar for all individuals, the SSPG provides a direct measure of the ability of insulin to mediate disposal of the infused glucose load. Thus, higher SSPG concentrations indicate greater insulin resistance. SSPG is highly correlated with measures derived from the hyperinsulinemic euglycemic clamp ($r = -0.87$).^{15, 16}

Fasting plasma samples were frozen at -80°C until measurements were performed. Plasma fetuin-A levels were quantified by a human fetuin-A sandwich enzyme-linked immunosorbent assay (ELISA) kit (Epitope Diagnostics, San Diego, CA) using 2 polyclonal antibodies that bind to different epitopes of human fetuin-A. Intra-assay precision is 4.8–5.5% and inter-assay precision is 5.7–6.8%. Lipoprotein analysis was performed by VAP methodology (Atherotech, Inc) as described previously.¹⁷

MetS was defined using the “harmonious” criteria,¹⁸ of which 3 of the 5 following components must be met: fasting plasma glucose ≥ 100 mg/dL (5.56 mmol/L); systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg ; triglycerides ≥ 150 mg/dL (1.70 mmol/L); high density lipoprotein cholesterol (HDL-C) < 40 mg/dL (1.30 mmol/L) in men or < 50 mg/dL (1.04 mmol/L) in women. Criteria for abdominal obesity (for individuals in the United States) included waist circumference (WC) ≥ 88 cm in women or ≥ 102 cm in men.¹⁸

Statistical analyses were performed using IBM SPSS Statistics 22.0 (Armonk, NY). Fetuin-A and lipoprotein concentrations that were not normally distributed were log-transformed prior to analysis. Individuals were categorized into tertiles of fetuin-A. A generalized linear model (ANCOVA) to adjust for age, sex, race, and BMI was used to assess for significant associations between groups. P-values were adjusted for multiple pairwise comparisons using sequential Bonferroni adjustment. Linear trend of lipoprotein concentrations across tertiles of fetuin-A were estimated by ANOVA. Chi-squared tests were performed to compare MetS prevalence, number of MetS components, and LDL density patterns across tertiles of fetuin. $P < 0.05$ indicated statistical significance, and all hypothesis testing was two-tailed.

Results

Baseline characteristics of the study population are shown in Table 1. Almost two-thirds of the cohort was male. They were middle-aged, obese, and had impaired fasting glucose. A substantial proportion met criteria for MetS (43%). They ranged 6-fold in degree of insulin

sensitivity (SSPG 51 to 309 mg/dL). On average they had severe OSA (AHI 34.0 events/hr), but were distributed across all categories of OSA severity. Fetuin-A levels did not differ between individuals with or without MetS (0.425 ± 0.10 vs. 0.419 ± 0.10 g/L, $p=0.69$).

Table 2 evaluates the trend of anthropometric and MetS components across tertiles of fetuin-A. Neither the proportion of individuals with MetS ($p=0.53$) nor mean number of MetS components ($p=0.58$) was significantly different across groups. Of the individual MetS components, triglycerides increased and HDL-C declined linearly across fetuin-A tertiles ($p < 0.05$); no differences were detected in the other MetS components.

To explore the relationship between lipoprotein particles and fetuin-A further, comprehensive lipoprotein analysis was performed (Table 3). Triglycerides, very low density lipoprotein (VLDL), and VLDL subfractions (VLDL₁₊₂ and VLDL₃) increased across fetuin-A tertiles, indicating a worse lipoprotein profile with higher fetuin-A concentrations. Pairwise comparisons demonstrated that the significant associations for triglycerides, VLDL, VLDL₁₊₂, and VLDL₃ were between the upper vs. lowest tertile of fetuin-A. There was also a linear trend for decreased HDL-C and its subfraction HDL₂ across fetuin-A tertiles. Finally, LDL density patterns A, A/B, and B were compared across fetuin-A tertiles, and no differences were detected ($p=0.30$).

The relation of fetuin-A with insulin mediated-glucose disposal (SSPG) is displayed in Figure 1. SSPG concentrations did not differ by tertiles of fetuin-A levels ($p=0.50$). Furthermore, a significant correlation between fetuin-A and SSPG was not observed ($r=0.03$, $p=0.79$).

Finally, associations between fetuin-A concentrations and sleep parameters were examined. Severity of OSA (AHI) was not different across fetuin-A tertiles ($p=0.79$). There were no statistically significant correlations between fetuin-A and AHI ($r=-0.08$, $p=0.37$), minO₂ ($r=0.01$, $p=0.88$), meanO₂ ($r=0.03$, $p=0.73$), or ODI (-0.11 , $p=0.22$).

Discussion

The purpose of this study was to determine whether fetuin-A could be implicated in mechanisms for CVD risk in patients with OSA, via relations with MetS and/or insulin resistance. While fetuin-A was not associated with MetS in OSA patients *per se*, the individual lipoprotein components of MetS, triglycerides and HDL-C, increased and decreased, respectively, across increasing fetuin-A tertiles. Expanded evaluation of lipoproteins extended these findings to include associations with VLDL particles and its subclasses, suggesting that dyslipidemia may be a mechanism by which fetuin-A contributes to increased CVD risk in OSA. On the other hand, neither insulin resistance nor OSA severity was found to be modified by fetuin-A.

Previous publications have reported associations of fetuin-A with increased prevalence of MetS in general populations that included individuals with T2DM^{19–21} and/or CVD.^{7, 21} To our knowledge, the present study is the first to examine relations between fetuin-A and MetS in patients with OSA. Neither the presence of nor the number of MetS components was associated with fetuin-A in our experimental cohort. Why our findings differed from others

is not clear, but the population characteristics may account for this disparity. Patients with T2DM or CVD were excluded in our cohort, perhaps indicative of a healthier population (despite having OSA) from a cardio-metabolic standpoint. That said, the prevalence of MetS in our cohort was similar^{20, 21} to or greater⁷ than that of other cohorts. It is also possible that the presence of OSA may have had an impact, although none of the sleep variables had clear associations with fetuin-A.

Focusing specifically on the association of fetuin-A with individual MetS components, the relationship between fetuin-A and lipid parameters has appeared to be most consistent. Indeed, only triglycerides and HDL-C among the MetS criteria remained significantly associated with fetuin-A after multivariable adjustment among older patients with CVD.⁷ Other studies found the link between triglycerides (rather than HDL-C) and fetuin-A to be more robust, which is supported by our findings and reinforced by our data in VLDL particles. Of clinical relevance is that increased triglyceride concentrations and VLDL particles and decreased HDL-C is predictive of increased atherogenicity.²² Thus, it could be argued that elevated fetuin-A concentrations contribute to the increased risk of CVD in subjects with OSA via mechanisms related to dyslipidemia. Earlier studies showed that fetuin-A can bind lipids forming a lipoprotein-like particle, serve as a carrier protein of free fatty acids, and induce cholesterol efflux from cells.²³ Via its inhibitory effects on the insulin receptor²⁴, fetuin-A may also contribute to impaired insulin inhibition of lipolysis, leading to increased free fatty acid release and hepatic VLDL production. The role of fetuin-A in lipid metabolism deserves further investigation.

However, this potentially adverse effect of fetuin-A on CVD must be placed within the context of conflicting evidence as to the direction of the relationship between fetuin-A and CVD risk. Consistent with our data, Weikert et al.⁶ described an association between elevated fetuin-A concentrations and heart attack and stroke. Higher fetuin-A also correlated with greater degree of carotid artery stiffness in healthy individuals, independent of CVD risk factors including lipids.²⁵ By contrast, no association was observed between fetuin-A and heart disease in women,⁵ although in the presence of high C-reactive protein levels, higher fetuin-A was associated with less heart disease risk. Lower fetuin-A also conferred greater CVD mortality in older adults without diabetes, but reduced risk of CVD death in those with diabetes.⁴ Furthermore, low plasma fetuin-A concentrations have been associated with increased carotid artery stiffness and intima-media thickness in OSA.²⁶ Thus, in patients with OSA there is evidence that *high* fetuin-A concentrations might modulate CVD risk via an atherogenic lipoprotein profile as in the present study, as well as evidence that *low* fetuin-A concentrations might increase CVD risk via an adverse effect on the vasculature. Recent attention has also been brought to measurement differences detected across commercially available fetuin-A assays,²⁷ which may complicate the interpretation of fetuin-A and CVD risk further. It is obvious that considerable uncertainty remains concerning the role of plasma fetuin-A in the pathogenesis of CVD.

Worthy of discussion is the lack of a detectable relationship between plasma fetuin-A and SSPG. Although we are unaware of any information concerning plasma fetuin-A and insulin resistance in OSA, there is data supporting an association between elevated fetuin-A and insulin resistance in diabetic and non-diabetic persons.^{8–10} It should be pointed out that we

used a specific method to quantify insulin-mediated glucose disposal, whereas we identified only one dataset cited in 2 publications^{11, 28} where an association between fetuin-A concentrations and insulin resistance was elucidated with a similar approach and in which an association was detected. In most instances, homeostatic model assessment was used as a surrogate estimate of insulin resistance (HOMA-IR),^{8–10} and the correlation between HOMA-IR and fetuin-A was modest ($r \sim 0.2$). One explanation is that while there may be a modest association between fetuin-A and insulin action in the population-at-large, one cannot be discerned in a population characterized by an increased prevalence of insulin resistance, i.e., patients with OSA.¹³ In support of this notion is the demonstration of a modest but significant association between fetuin-A and HOMA-IR in nondiabetic individuals ($r=0.197$, $p=0.014$), but not in patients with T2DM ($r=0.01$, $p=0.91$).⁹ Thus, our data suggest that differences in fetuin-A are unlikely to play a significant role in modulating insulin resistance or increased T2DM risk in patients with OSA.

Finally, fetuin-A was not significantly correlated with AHI or with any other sleep variables. These results differ from 2 studies that showed that higher fetuin-A concentrations were correlated with lower AHI in patients with OSA.^{26, 29} Fetuin-A concentrations levels were also found to be modestly lower (by 13 to 17%) in patients with as compared to without OSA.^{26, 29} Nonetheless, the association, if any, between plasma fetuin-A concentrations and AHI appears to be modest in magnitude, making it unlikely that differences in plasma fetuin-A concentrations play a major role in modulation of clinical severity of OSA.

The strength of our study is the use of specific and quantitative measurements of both sleep (polysomnography) and insulin action (insulin suppression test) in 120 individuals with OSA. A weakness is that this is a cross-sectional study, and we can only evaluate risk of T2DM and CVD, not outcome. In conclusion, what seems to be most apparent from our findings, and in consideration of other relevant publications, is that fetuin-A is associated with adverse changes in lipoprotein metabolism that may account for increased CVD risk in patients with OSA. Further investigation in this area could help to clarify the pathophysiological importance of plasma fetuin-A concentrations in mediating CVD outcomes, particularly given the disparate views presented as to the link between fetuin-A concentrations and CVD in general populations.

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Figure 1.
Relationship of steady-state plasma glucose (SSPG) concentrations with fetuin tertiles.

Table 1

Characteristics of the study population (n = 120)

Variable	Mean	Range
Age (years)	50 ± 10	30 – 68
Men	77 (64%)	
White/Asian/Hispanic or Latino/Black	75/ 25/ 15/ 5	
Body Mass Index (kg/m ²)	30.6 ± 3.0	25.7 – 37.9
Waist circumference (cm)	103 ± 10	87 – 131
Metabolic syndrome	52 (43%)	
Fasting plasma glucose (mg/dL)	102 ± 9	82 – 123
Steady-state plasma glucose (mg/dL)	170 ± 65	51 – 309
Systolic blood pressure (mmHg)	125 ± 13	99 – 166
Diastolic blood pressure (mmHg)	79 ± 8	60 – 98
Apnea-hypopnea index (events/hour)	34.0 ± 24	5.2 – 111.8
Mild/ moderate/ severe OSA	31 (26%)/ 35 (29%) / 54 (45%)	
Fetuin-A (gram/L)	0.42 ± 0.10	0.26 – 0.91

Data are expressed as mean ± S.D. unless otherwise indicated.

Table 2
Comparison of anthropometric and metabolic syndrome components across fetuin-A tertiles in patients with obstructive sleep apnea

Variable	Fetuin Tertile			P value	P value for trend
	1 (<0.370 g/L)	2 (0.370-0.446 g/L)	3 (> 0.446 g/L)		
	(n=40)	(n=40)	(n=40)		
Age (years)	52 ± 10	48 ± 11	50 ± 9	0.31	0.33
Men	22 (55%)	27 (68%)	28 (70%)	0.33	N/A
White/Asian/Hispanic/Black	26/ 3/ 7/ 4	22/ 14/ 3/ 1	27/ 8/ 5/ 0	0.023	N/A
Body Mass Index (kg/m ²)	30.8 ± 3	30.1 ± 2.8	31.0 ± 3	0.43	0.74
Metabolic syndrome	15 (38%)	20 (50%)	17 (43%)	0.53	N/A
Metabolic syndrome components	2.25 ± 1.1	2.50 ± 1.2	2.40 ± 1.3	0.58	N/A
Waist circumference (cm)	103 ± 10	102 ± 9	104 ± 11	0.72	0.72
Fasting plasma glucose (mg/dL)	101 ± 9	102 ± 10	101 ± 9	0.75	0.80
HDL-C (mg/dL)	54 ± 17	47 ± 16	46 ± 12	0.046	0.02
Triglycerides (mg/dL)	114 ± 60	147 ± 58	198 ± 328	0.004	0.002
Systolic blood pressure (mmHg)	126 ± 15	124 ± 10	124 ± 14	0.84	0.61
Diastolic blood pressure (mmHg)	80 ± 8	79 ± 6	80 ± 9	0.99	1.0

Data are expressed as mean ± SD unless otherwise specified.

Table 3

Comparison of lipoprotein measurements across tertiles of fetuin-A concentrations

Variable	Fetuin Tertile			P value ^d	P value for trend ^b
	1 (<0.370 g/L)	2 (0.370 – 0.446g/L)	3 (> 0.446 g/L)		
Total cholesterol (mg/dL)	193 ± 47	195 ± 42	186 ± 36	0.61	0.42
Total LDL (mg/dL)	117 ± 39	121 ± 35	112 ± 31	0.57	0.55
LDL ₁ (mg/dL)	16.3 ± 7	17.8 ± 8	16 ± 7	0.37	0.94
LDL ₂ (mg/dL)	27 ± 18	20 ± 16	20 ± 13	0.25	0.20
LDL ₃ (mg/dL)	42 ± 20	44 ± 17	39 ± 16	0.41	0.55
LDL ₄ (mg/dL)	11.3 ± 9	16.8 ± 13	14.7 ± 14	0.09	0.21
IDL (mg/dL)	13.4 ± 6	16.3 ± 6	15.3 ± 7	0.07	0.18
Total VLDL (mg/dL)	23 ± 11	27 ± 9	28 ± 10 ^c	0.01	0.003
VLDL ₁₊₂ (mg/dL)	9.8 ± 6	12.2 ± 5	12.8 ± 6 ^c	0.01	0.002
VLDL ₃ (mg/dL)	12.8 ± 5	14.8 ± 4	14.7 ± 4 ^d	0.02	0.015
Triglycerides (mg/dL)	114 ± 60	147 ± 58	198 ± 328 ^c	0.008	0.002
Total HDL (mg/dL)	54 ± 17	47 ± 16	46 ± 12	0.16	0.024
HDL ₂ (mg/dL)	14 ± 8	11 ± 7	11 ± 5	0.15	0.026
HDL ₃ (mg/dL)	39 ± 11	36 ± 9	35 ± 8	0.97	0.42
Lp(a) (mg/dL)	7.3 ± 4	6.1 ± 5	6.5 ± 4	0.39	0.59
Apolipoprotein B (mg/dL)	93 ± 24	100 ± 22	95 ± 22	0.46	0.66
Apolipoprotein A1 (mg/dL)	153 ± 26	144 ± 25	143 ± 20	0.42	0.09
Apolipoprotein B/Apolipoprotein A1 ratio	0.62 ± 0.2	0.71 ± 0.2	0.68 ± 0.2	0.22	0.18

Data are expressed as mean ± SD unless otherwise specified.

^a P-value reflects differences among tertiles after adjustment for age, sex, race, and BMI.^b P-value for test of linear trend across tertiles.^c P< 0.01 for tertile 3 vs tertile 1.^d P<0.05 for tertile 3 vs tertile 1.