

## Relationship between serum levels of fetuin-A with apo-A1, apo-B100, body composition and insulin resistance in patients with type 2 diabetes

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### Abstract

**Background:** Some results exist on fetuin-A as marker for vascular disease in type 2 diabetes. We examined the relationship between serum fetuin-A with some factors, in patients with type 2 diabetes mellitus (T2DM).

**Methods:** From October 2012 to June 2013, a total of 131 T2DM patients were recruited and evaluated for various parameters including HOMA-IR, Apo-A1, Apo-B100, body fat percentage and waist circumference. Serum fetuin-A levels were measured by enzyme-linked immunosorbent assay (ELISA), and Serum glucose with a Cobas MIRA analyzer by enzymatic method. Apo-B100 and apo-A1 were measured by immunoturbidimetry with a Cobas MIRA analyzer. HOMA-IR was calculated by the following formula: [fasting insulin (uIU/mL) × fasting blood glucose (mmol/L)]/22.5.

**Results:** The mean levels of HOMA-IR were significantly increased progressively across fetuin-A tertiles (p for trend=0.04) in women but not men. Fetuin-A had just a significant positive correlation with Apo-A1 (r=0.22, p=0.02).

**Conclusion:** This present study showed that levels of serum fetuin-A are significantly associated with insulin resistance in women with T2DM.

**Keywords:** Fetuin-A, Apo-A1, Apo-B100, Body Fat Percentage, Insulin Resistance, Type 2 Diabetes.

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### Introduction

Type 2 diabetes Mellitus (T2DM) represents a major global public health hazard and, together with obesity, constitutes an important contributor to the expected decline in life expectancy (1). The pathophysiology of type 2 diabetes is multifaceted: In addition to impaired insulin secretion from cells, reduced insulin sensitivity was found to play a major role in the pathogenesis of

the disease (2).

According to a recent study in Tehran (3) the prevalence of DM is much greater than that in industrialized countries (4) (14% versus 2%) and about one-third of the patients with diabetes in Tehran (3) and half of those in Iran (5) are unaware of their illness.

Diabetic dyslipidemia, which is called atherogenic dyslipidemia, is a cluster of

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lipoprotein abnormalities characterized by elevated triglycerides (TGs), reduced high-density lipoprotein-cholesterol (HDL-C), and small, dense low-density lipoprotein (LDL) particle. This abnormal lipid profile commonly presents in type 2 diabetes (6). Type 2 diabetes is characterized through increasing in insulin resistance, plasma triglyceride, apoB, homocysteine and decreasing in apoA-I and HDL-C. ApoB and apoA-I are more important predictors for preventing from cardiovascular diseases compared to the LDL-C and HDL-C, respectively (7).

Apolipoprotein B (apo B) was significantly higher in diabetic compared with non-diabetic people and also apo B is superior to LDL cholesterol as a marker of atherogenic risk is adequately clear (8).

ApoA-I is more important than the HDL particle cholesterol content for pathways that render HDL. Anti-atherogenic, including ATP binding cassette A-mediated cellular cholesterol efflux, Lecithin-cholesterol acyltransferase-mediated maturation of HDL particles, and several antioxidative processes (9).

Numerous circulating proteins have been shown to be elaborate in the regulation of insulin sensitivity such as adiponectin (10), retinol binding protein 4 (11), and fetuin-A (previous name for the human protein 2-Heremans-Schmid glycoprotein, AHSG) (12).

Fetuin-A is an endogenous inhibitor of the insulin-stimulated insulin receptor tyrosine kinase (13). Fetuin-A is a 60 kDa glycoprotein produced exclusively by the liver and secreted into serum in relatively high concentrations in humans (14). This parameter is known to inhibit ectopic calcium deposition and protect from vascular calcification. Also, fetuin-A is shown to act as an endogenous inhibitor of the insulin receptor tyrosine kinase in liver and skeletal muscle, resulting in insulin resistance (IR) in these target tissues. In several epidemiological studies, higher serum fetuin-A levels are associated with IR, metabolic syndrome (MS) and type 2 diabetes mellitus

(T2DM) (14-15).

Studies in humans have proved that circulating fetuin-A levels are positively associated with fat accumulation in the liver, insulin resistance, and the metabolic syndrome (14-16). Recently, 2 independent prospective cohort studies have shown that fetuin-A is positively associated with risk of type 2 diabetes mellitus. Besides induction of insulin resistance, recent data suggest that fetuin-A is involved in subclinical inflammation (17-18).

Higher Fetuin-A concentrations were associated with gain of visceral adipose tissue, a major component of the metabolic syndrome (19). In addition, obese people exhibited elevated Fetuin-A levels, which decreased significantly during exercise- and diet-induced weight loss (20).

In the present study, we examined the relationship between fasting serum fetuin-A levels with IR, Apo-A1, Apo-B100, body fat percentage and waist circumference in patients with T2DM.

## Methods

The present study was conducted in Tehran, Iran between October 2012 to June 2013. A total of 103 patients, with type 2 diabetes (FBS $\geq$ 126mg/dl, 2hpp $\geq$ 200mg/dl and HbA1c :6-8%) aged 35 to 60 years old, who were referred to the Endocrinology & Metabolism Research Institute, Tehran University of Medical Sciences. Ethical approval was provided by the committee of ethics in Tehran University of Medical science. Necessary information was obtained from all participates. All individuals were informed regarding the tests and their clinical significances before the study and written consent obtained from all study participants.

Exclusion criteria; included ,insulin and alcohol consumption, pregnancy, lactation, smoking, history of myocardial infarction , stroke, kidney disease, liver disease, cancer, thyroid disease, anemia, dialysis, consumption of warfarin and other anticoagulant drugs, estrogen, progesterone, NSAIDs and multi vitamin mineral during the study.

The participants completed a questionnaire about subjective information (weight, Height, BMI, duration of having diabetes mellitus, familial history of diabetes, type of drug consumed for diabetes) pertinent medical history, and socio-economic and demographic variables. Nutrient intakes were calculated from food frequency questionnaire and analyzed using the Nutritionist IV software (First Databank, San Bruno, Calif., USA) modified for Iranian foods. Body fat index was measured by Bioelectrical impedance analysis (BIA) method, using a Quad scan 4000 (Body Stat. UK). Waist circumference was measured in the midway of the superior iliac crest and the costal margin. Physical activity was assessed by the validated IPAQ questionnaire (21).

Blood samples were taken after overnight fasting and the serum separated and stored at  $.80^{\circ}\text{C}$  until analyzed. Serum fetuin-A levels was measured using a commercially available enzyme-linked immunosorbent assay (ELISA; Biovendor, Czech). Serum glucose was measured with a Cobas MIRA analyzer (Roche Diagnostic, Basel, Switzerland) by enzymatic method (Pars Azmon Co., Tehran, Iran). Apo-B100 and apo-A1 were measured by immunoturbidimetry (Pars Azmon Co., Tehran, Iran) with a Cobas MIRA analyzer with calibration traceable to the International Federation of Clinical Chemistry Primary Standards (22).

The IR status was evaluated by the homeostasis model assessment–insulin resistance (HOMA-IR) index. The HOMA-IR was calculated by the following formula: [fasting insulin (uIU/mL)  $\times$  fasting blood glucose (mmol/L)]/22.5 (23).

### Statistical methods

Correlation between plasma fetuin-A and other clinical parameters was analysed by Spearman's correlation analysis. Patients were divided into three groups by the tertiles of fetuin-A levels. One-way analysis of variance (ANOVA) was used to evaluate differences of means among tertiles of fetuin-A groups based on sex. The Statistical Package for Social Sciences (version 18.0; SPSS Inc., Chicago, IL, USA) was used for all analyses.

### Results

Mean  $\pm$  SD of variables such as age, BMI and weight were  $51.08 \pm 5.94$  years,  $28.19 \pm 4.58 \text{ Kg/m}^2$  and  $77.35 \pm 13.1$  Kg respectively. Based on IPAQ questionnaire, 49% of participants had mild activity and 47.1% and 3.8% intermediate and severe activity level respectively. The results of FFQ are shown in Table 1.

Table 2 shows the means of some measured variables in participants. Table 3 indicates the correlation between the parameters in our participants. The results showed that waist circumference had a significant positive correlation with body fat percent-

Table 1. Energy and nutrients intake of patients based on FFQ

Nutrient and energy	Mean $\pm$ SD
Energy (Kcal)	2411.36 $\pm$ 1203.89
Carbohydrate (gr)	389.08 $\pm$ 209.51
Protein (gr)	77.69 $\pm$ 48.05
Total Fat (gr)	70.98 $\pm$ 34.76
SFA (gr)	16.01 $\pm$ 7.64
MUFA (gr)	22.18 $\pm$ 10.87
PUFA (gr)	18.1 $\pm$ 9.64
Fiber (gr)	65.78 $\pm$ 40.65
Vitamin C (mg)	300.62 $\pm$ 254.53
Vitamin E (mg)	16.3 $\pm$ 6.29
Ca (mg)	1191.84 $\pm$ 710.07
P (mg)	1405.24 $\pm$ 963.96
Zn (mg)	12.11 $\pm$ 9.74
Fe (mg)	34.39 $\pm$ 22.21
Na (mg)	3946.1 $\pm$ 1790
K (mg)	4889.48 $\pm$ 3111.68

Table 2. Means of some measured variables in participates

	sex	
	Men (n= 56) mean± SD	Women (n=47) mean± SD
Waist Circumference (cm)	98±10.6	103.85±12.7
Fat percent (%)	24.7±3.7	41±5.91
Apo A-1(mg/dl)	144.05±32.91	151.87±37.97
ApoB100(mg/dl)	74.14±24.23	75.87±30.73
Fetuin A(ng/ml)	23.21±11.3	23.82±10
HOMA-IR	2.89±1.7	4±3.36

Table 3. Correlation between the parameters in diabetic patients

	waist Circumference (cm)		Fat percent (%)		Apo A-1 (mg/dl)		ApoB100 (mg/dl)		Fetuin A (ng/ml)		HOMA-IR	
	r	p*	r	p*	r	p*	r	p*	r	p*	r	p*
Waist Circumference (cm)	----	----	0.55	<0.001	-0.05	0.56	0.04	0.64	0.03	0.76	0.48	<0.001
Fat percent (%)	0.55	<0.001	----	----	0.2	0.04	0.26	<0.001	0.14	0.14	0.41	<0.001
Apo A-1(mg/dl)	-0.05	0.56	0.2	0.04	----	----	-0.63	<0.001	0.22	0.02	0.04	0.68
ApoB100(mg/dl)	0.04	0.64	0.26	<0.001	-0.63	<0.001	----	----	0.13	0.19	0.13	0.16
Fetuin A(ng/ml)	0.03	0.76	0.14	0.14	0.22	0.02	0.13	0.19	----	----	0.12	0.2
HOMA-IR	0.48	<0.001	0.41	<0.001	0.04	0.68	0.13	0.16	0.12	0.2	----	----

age ( $r=0.55$ ,  $p<0.001$ ) and HOMA-IR ( $r=0.48$ ,  $p<0.001$ ). The same trend was also apparent for body fat percentage with Apo A-1 ( $r= 0.2$ ,  $p= 0.04$ ), Apo B100 ( $r= 0.2$ ,  $p<0.001$ ) and HOMA-IR ( $r= 0.4$ ,  $p< 0.001$ ) as well. By contrast, Apo B-100 had a significant negative relationship with Apo A1 ( $r= - 0.63$ ,  $p< 0.001$ ) but significant positive correlation with body fat percentage ( $r= 0.26$ ,  $p< 0.001$ ). Fetuin A had just a significant positive correlation with Apo A-1 ( $r= 0.22$ ,  $p= 0.02$ ).

#### *HOMA-IR levels and other clinical parameters based on the tertiles of serum fetuin-A levels on sex*

The participants were divided into three groups according to the levels of serum fetuin-A. The characteristics of clinical parameters according to the fetuin-A tertile are shown in Table 4 for men and table 5 for women. Based on data in table 4, no significant differences were seen between the various tertiles of fetuin A with some measured parameters men groups. The mean levels of HOMA-IR were significantly increased progressively across fetuin-A tertiles ( $p$  for trend = 0.04) in women but not men.

#### **Discussion**

Fetuin-A was revealed to act as a natural inhibitor of the insulin receptor tyrosine kinase in liver and skeletal muscle, so fetuin knock-out mice exhibited improved insulin sensitivity (24). Different observational studies have suggested that high serum fetuin-A levels are linked with the presence or expansion of the metabolic syndrome suggesting fetuin-A as a risk factor for this situation (17-18). Furthermore, fetuin-A complexes with calcium and phosphorus in the circulation and prevents the precipitation of these minerals in serum (25-26). Fetuin-A is considered as marker for vascular inflammation and as one of the most powerful negative regulators of vascular calcification (27-28). In animals lacking the fetuin-A gene, the aorta was found to be released of calcification and fibrosis, while peripheral vessels in the skin and kidney presented evidence of widespread calcification, and the small artery involvement preceded the impairment of renal function (29).

In a large study in humans, high serum fetuin-A levels were establish to be positively allied with the metabolic syndrome (MS) and subclinical inflammation, proposing that fetuin-A may be causally involved in the pathophysiology of these disorders (15).

Table 4. Correlation of fetuin-A tertiles with some parameters in diabetic men

	Tertiles of Fetuin A*	Mean±SD	95% CI	p value#
waist Circumference (cm)	1	96.35±10.95	90.02-102.68	0.8
	2	98.65±10.49	94.11-103.19	
	3	98.4±10.91	93.29-103.51	
Fat percent (%)	1	23.65±3.11	21.85-25.44	0.35
	2	24.83±4.14	23.03-26.62	
	3	25.52±3.52	23.86-27.17	
Apo A-1 (mg/dl)	1	133.5±44.13	108.01-158.98	0.36
	2	149.3±27.48	137.41-161.19	
	3	145.4±29.44	131.61-159.18	
ApoB100 (mg/dl)	1	70.07±29.68	52.93-87.21	0.41
	2	79.39±23.32	69.3-89.47	
	3	70.95±21.02	61.11-80.78	
HOMA-IR	1	2.97±1.88	1.88-4.05	0.8
	2	2.72±1.41	2.11-3.33	
	3	3.05±1.93	2.14-3.95	

\*range of fetuin-A (ng/ml): tertile 1: 0-18.9, tertile 2: 19-24, tertile 3:>24, #ANOVA

Table 5. Correlation of fetuin- A tertiles with some parameters in diabetic women

	Tertiles of Fetuin A*	Mean±SD	95% CI	p value#
waist Circumference (cm)	1	102.8±12.89	96.76 -108.83	0/66
	2	102.5±12.1	94.80-110.19	
	3	106.33±13.4	98.91-113.75	
Fat percent (%)	1	40.02±5.3	37.54-42.5	0/12
	2	39.48±5.5	35.96-43	
	3	43.56±6.49	39.97-47.16	
Apo A-1 (mg/dl)	1	146.3±30.06	132.23-160.36	0/34
	2	146.25±18.62	134.41-158.08	
	3	163.8±54.91	133.38-194.21	
ApoB100 (mg/dl)	1	71.95±24.76	60.35-83.54	0/6
	2	74.16±13.9	65.33-83	
	3	82.46±45.25	57.40-107.52	
HOMA-IR	1	2.69±1.56	1.96-3.42	0/04
	2	4.77±2.85	2.96-6.58	
	3	5.4±4.73	2.77-8.02	

\*range of fetuin-A (ng/ml): tertile 1: 0-18.9, tertile 2: 19-24, tertile 3:>24, #ANOVA

The circulatory hepatic glycoprotein fetuin-A has a vital role in atherosclerosis accompanying with patients with diabetes. Defects due to diabetes, such as impairment of endothelium and platelet function, donate to the cellular actions that lead to atherosclerosis. Certain physiological or pathophysiological conditions such as malnutrition and inflammation can change the blood serum level of fetuin-A. A higher level of fetuin-A in the blood than normal may lead to insulin resistance since it inhibits the tyrosine phosphorylation of the insulin receptors. Hyperglycemia and insulin resistance damages the normal function of endothelium and platelets, which lead to inflammation, vasoconstriction and thrombosis. All these conditions enhance the risk of atherosclerosis. In other word, a low level of fetuin-A in the blood may lead to vascular cal-

cification and worsens the atherogenic condition (27).

Furthermore, our results indicated that increase in body fat percent and waist circumference led to enhancement of HOMA-IR in type 2 diabetes mellitus (T2DM). We did not found any significant correlation between fetuin-A and this parameter. In other hand, in the present study, the elevated serum fetuin-A levels seem not to be associated with IR, as assessed by the HOMA-IR in patients with T2DM.

Our findings clearly demonstrated that a significant positive correlation between fetuin-A tertile and HOMA-IR in women but not in men.

However, in our present study, a significant association with MS indicators such as increase in ApoB-100 and decrease in Apo A-1 were not detected, and serum fetuin-A

was not associated with adiposity per se, as assessed by BIA. Consistent with our study, Ishibashi et al (28) reported that a significant association fetuin-A with MS was not detected in Japanese men. Also, Roos et al (29) could not demonstrate an association of fetuin-A with MS in patients with manifesting coronary heart disease (CHD) in a 6-year follow up study. It is not clear why conflicting results have been described regarding the association of serum fetuin-A levels with the presence of MS. One possible explanation is that the patients with T2DM may already control the components of MS via lifestyle modification, anti-diabetic, anti-hypertensive or anti-lipidaemic medications such as statin medication more firmly than general population.

This study had some limitations. In multivariable regression analysis, not all possible confounding factors of fetuin-A could be designated for adjustment. Second, the cross-sectional design cannot resolve the problem of causal relationship.

### Conclusion

In conclusion it is important that diabetic patients modify their life style change for modification to improve metabolic syndrome coeries such as lipid profile and waist circumference. Because alteration of life style in these population can affect their treatment and insulin resistance.

### Conflict of interest

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

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