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Effects of pioglitazone on serum fetuin-A levels in patients with type 2 diabetes mellitus

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Abstract

Fetuin-A (α2-Heremans-Schmid glycoprotein), a circulating glycoprotein, can inhibit insulin signaling both in vivo and in vitro. Recently, we and another independent group have shown that fetuin-A is positively associated with insulin resistance in humans. Furthermore, it has been reported that higher fetuin-A levels are associated with metabolic syndrome and atherogenic lipid profiles. These data suggest that fetuin-A might be a regulator of insulin resistance and/or metabolic syndrome. However, it is not clear how fetuin-A levels are regulated. To address this, we investigated the effects of representative insulin-sensitizing therapies such as pioglitazone, metformin, and aerobic exercise on fetuin-A levels. Twenty-seven patients with type 2 diabetes mellitus were divided into pioglitazone-treated (Pio), metformin-treated (Met), and exercise-treated (Ex) groups. Ten patients in the Pio group and 9 patients in the Met group took 15 or 30 mg/d pioglitazone or 500 or 750 mg/d metformin, respectively, for 6 months. Eight patients in the Ex group underwent a 3-month aerobic exercise program. Serum fetuin-A levels were measured before and after each intervention. Intervention significantly decreased hemoglobin A_{1c} in all groups. After treatment, serum fetuin-A levels significantly decreased in the Pio group (291.2 ± 57.7 to 253.1 ± 43.9 μg/mL, $P = .006$), whereas there were no changes in serum fetuin-A after intervention in either the Met or the Ex groups. We hypothesize that pioglitazone could partially ameliorate insulin resistance via modulating fetuin-A levels.

1. Introduction

Insulin resistance plays an important role in the pathogenesis of type 2 diabetes mellitus and related atherosclerotic diseases. Although the precise mechanism of insulin resistance is still unknown, improving insulin sensitivity is a beneficial approach for both treating diabetes and preventing cardiovascular diseases. For this purpose, insulin-sensitizing agents such as pioglitazone (one of the thiazolidinedione derivatives) and metformin are widely used. Pioglitazone, a peroxisome proliferator–activated receptor γ agonist, has been proposed to act on adipose tissue and to improve dysregulated "adipokine" profiles in the insulin-resistant state. On the other hand, metformin has been reported to stimulate adenosine monophosphate–activated kinase, resulting in an enhancement of insulin sensitivity in skeletal muscle and a decrease in hepatic glucose output in liver. In addition to drugs, aerobic exercise is also an important strategy for improving insulin resistance in the treatment of diabetes [1].

Fetuin-A (α2-Heremans-Schmid glycoprotein) is a circulating glycoprotein mainly synthesized by the liver. Several reports have suggested that fetuin-A can inhibit insulin receptor autophosphorylation and subsequent downstream signaling in vitro [2-5]. It has also been reported that fetuin-A–deficient mice demonstrate enhanced insulin sensitivity [6,7]. In humans, we have recently shown that fetuin-A is positively associated with insulin resistance [8]. Likewise, another independent group has demonstrated that fetuin-A is correlated with insulin resistance and fat accumulation in the liver [9]. Furthermore, it has been reported that higher fetuin-A levels are associated with metabolic syndrome and atherogenic lipid profiles in patients with coronary artery disease [10]. These data suggest that fetuin-A might be a

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Table 1
Clinical characteristics at baseline and after treatment in the pioglitazone, metformin, and exercise group

	Pioglitazone		Metformin		Exercise	
	Baseline	After treatment	Baseline	After treatment	Baseline	After treatment
n (male/female)	10 (7/3)	–	9 (4/5)	–	8 (5/3)	–
Age (y)	63 ± 10	–	62 ± 6	–	62 ± 18	–
Duration (y)	8 ± 8	–	7 ± 8	–	5 ± 6	–
BMI (kg/m^2)	26.8 ± 3.5	28.0 ± 3.8 *	29.1 ± 4.7	28.8 ± 4.5	27.4 ± 4.2	26.3 ± 3.5
SBP (mm HG)	133 ± 19	128 ± 17	143 ± 22	143 ± 23	134 ± 15	132 ± 17
FPG (mmol/L)	8.8 ± 2.0	7.4 ± 1.1	12.0 ± 3.9	9.3 ± 2.8 *	7.7 ± 1.8 †	6.2 ± 0.9 *
HbA_{1c} (%)	8.0 ± 0.8	7.0 ± 0.5 *	9.5 ± 2.2	8.0 ± 1.9 *	7.8 ± 0.9	6.2 ± 0.7 *
HOMA-IR	3.5 ± 1.6	2.4 ± 1.2	6.3 ± 3.0	4.2 ± 2.8 *	5.1 ± 4.0	3.3 ± 3.1
TC (mmol/L)	5.0 ± 0.8	5.2 ± 1.2	5.6 ± 0.5	5.2 ± 0.4	5.0 ± 0.6	4.9 ± 0.9
TG (mmol/L)	2.4 ± 2.0	1.9 ± 1.4	2.3 ± 1.0	1.8 ± 1.0 *	1.7 ± 0.6	1.5 ± 0.6
HDL cholesterol (mmol/L)	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.5 ± 0.3 *	1.1 ± 0.4	1.2 ± 0.3 *

All values are mean ± SD. SBP indicates systolic blood pressure; TC, total cholesterol; TG, triglyceride.
* $P < .05$ vs baseline data in each group.
† $P < .05$ metformin group vs exercise group at baseline.

regulator of insulin resistance and/or metabolic syndrome. However, it is not clear how fetuin-A levels are regulated.

To address this, we investigated the effects of representative insulin-sensitizing treatments such as pioglitazone, metformin, and aerobic exercise on fetuin-A levels in patients with type 2 diabetes mellitus.

2. Research design and methods

Twenty-seven subjects with type 2 diabetes mellitus (16 men and 11 women) were selected for the present study from patients who attend our diabetes center at Osaka City University Hospital. The diagnosis of diabetes was based on a previous history of diabetes or on the American Diabetes Association criteria. Subjects with renal dysfunction (serum creatinine >110 μmol/L) were excluded. In this study, serum creatinine levels ranged from 31.8 to 91.1 μmol/L (mean, 58.2 ± 14.6 μmol/L). None of subjects showed overt proteinuria (23 subjects with normoalbuminuria and 4 subjects with microalbuminuria). We excluded subjects with inflammation such as acute infection, autoimmune diseases, and malignant tumors. No subjects received insulin therapy, and no medications were altered during this study. The subjects were consecutively divided into pioglitazone-treated (Pio), metformin- (Met), and exercise-treated (Ex) groups (not randomized). Ten diabetic subjects in the Pio group (7 men and 3 women) took 15 or 30 mg/d pioglitazone for 6 months. In the Met group (4 men and 5 women), the subjects were administered 500 or 750 mg/d metformin for 6 months. Eight subjects in the Ex group (5 men and 3 women) were hospitalized for 2 weeks and instructed to perform ergometer exercise for 40 min/d, 5 times a week. The exercise intensity was determined by the analysis of expired gas in each subject. Anaerobic threshold was determined according to the method of Wasserman et al [11]. Heart rate (HR) at anaerobic threshold point was recorded and used as a target HR. The mean target HR in the Ex group was 103 ± 16 beats per minute. After discharge from the hospital, they were instructed to visit a gymnasium and to perform the same exercise prescription 3 to 5 times a week for 3 months. All exercise sessions were supervised and recorded by an instructor. Informed consent was obtained from all participants, and the study was approved by the ethics committee of the Osaka City University Hospital. Plasma glucose was measured using the glucose oxidase method; and plasma insulin, by immunoradiometric assay (ARCHITECT Insulin; Abbott, Chicago, IL). Serum total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol were measured using enzymatic methods adapted for an autoanalyzer (Hitachi 7450; Hitachi, Tokyo, Japan). Serum fetuin-A was measured by a commercially available enzyme-linked immunosorbent assay kit (BioVender Laboratory Medicine, Modrice, Czech Republic) as previously reported [8,12]. The inter- and intraassay coefficient of variations were less than 10%, and the minimum detection limit for fetuin-A is 3.5 μg/mL. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting plasma glucose (FPG) and fasting insulin resistance index levels using the following formula: HOMA-IR = fasting insulin resistance index in microunits per milliliter × FPG in millimoles per liter/22.5. All values are the means ± SD. Statistical analysis was performed using the Stat View 5 system (SAS Institute, Cary, NC) for Windows (Microsoft, Redmond, WA). Paired and unpaired t tests, χ^2 tests, 1-way

Table 2
Simple linear regression analysis of the association between fetuin-A and various factors at baseline

	r	P
Age	−0.230	.249
BMI	−0.103	.608
FPG	0.049	.809
HbA_{1c}	0.038	.853
Log (HOMA)	0.192	.337
TC	−0.224	.262
TG	−0.314	.111
HDL cholesterol	−0.417	.031

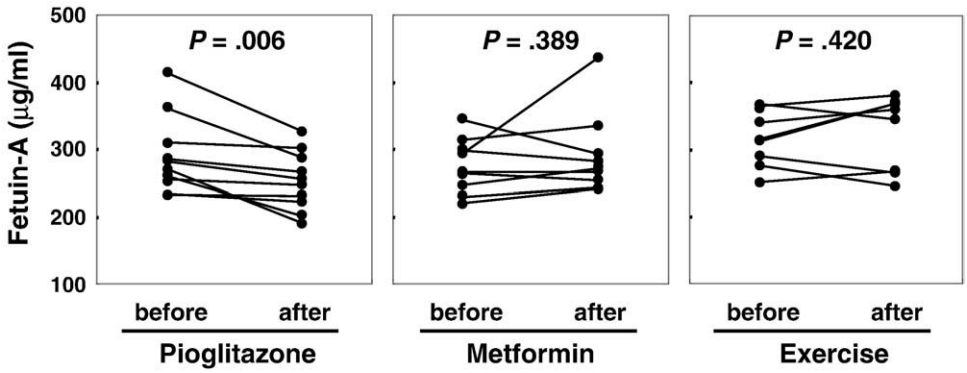

Fig. 1. Effects of pioglitazone, metformin, and exercise on serum fetuin-A levels in patients with type 2 diabetes mellitus. Treatment with pioglitazone for 6 months significantly reduced serum fetuin-A levels (291.2 ± 57.7 to 253.1 ± 43.9 μg/mL, $P = .006$). However, there were no changes in serum fetuin-A after intervention by either metformin for 6 months or aerobic exercise for 3 months (275.1 ± 40.4 to 291.6 ± 62.4 μg/mL, $P = .389$ and 314.7 ± 42.1 to 325.0 ± 55.5 μg/mL, $P = .420$, respectively).

analyses of variance (Scheffe type), and Kruskal-Wallis tests were used where appropriate. P values of less than .05 were considered to be statistically significant.

3. Results

Clinical characteristics of all patients are shown in Table 1. There were no differences in age, sex, duration of diabetes, body mass index (BMI), systolic blood pressure, hemoglobin A_{1c} (HbA_{1c}), HOMA-IR, total cholesterol, triglyceride, and HDL cholesterol among all groups at baseline. Fasting plasma glucose was significantly lower in the Ex group than in the Met group at baseline. We first examined the association between fetuin-A and various factors in all 27 subjects at baseline (Table 2). As previously reported [8], fetuin-A levels were not associated with log (HOMA) in subjects with type 2 diabetes mellitus. However, fetuin-A levels were inversely related to HDL levels. Intervention significantly decreased HbA_{1c} in all groups. The BMI significantly increased in the Pio group but did not change in the Met and the Ex groups. The HOMA-IR was improved in the Met group. In the Pio and the Ex groups, the HOMA-IR tended to decrease, although not to a significant extent. Systolic blood pressure and total cholesterol level were not changed by intervention in all groups. Before the intervention, there were no differences in fetuin-A levels among all groups. From before to after treatment, serum fetuin-A levels significantly decreased in the Pio group (291.2 ± 57.7 to 253.1 ± 43.9 μg/ml, $P = .006$) (Fig. 1), whereas there were no changes in serum fetuin-A after intervention in either the Met or the Ex group (275.1 ± 40.4 to 291.6 ± 62.4 μg/mL, $P = .389$ and 314.7 ± 42.1 to 325.0 ± 55.5 μg/mL, $P = .420$, respectively).

4. Discussion

This is the first study to demonstrate the effects of insulin-sensitizing therapies on serum fetuin-A, a regulator of insulin resistance and/or metabolic syndrome. We found that pioglitazone reduced serum fetuin-A levels in patients with type 2 diabetes mellitus, whereas the other insulin-sensitizing therapies, metformin and aerobic exercise, did not affect fetuin-A levels. How pioglitazone reduces fetuin-A levels is unclear. In a recent study focusing on the association between hepatic steatosis and fetuin-A secreted by the liver, Stefan et al [9] found that improved hepatic steatosis with amelioration of insulin resistance under a lifestyle intervention was accompanied by a decrease in fetuin-A levels. Because liver fat content is positively associated with insulin resistance, they suggest a potential role for fetuin-A as a link between fatty liver and insulin resistance. Interestingly, several reports have shown that thiazolidinediones including pioglitazone improve the hepatocellular lipid content, whereas metformin did not affect it [13,14]. Taken together, it is possible that pioglitazone could regulate fetuin-A levels through reducing hepatic lipid content, resulting in partial improvement of insulin resistance.

Another important point to consider is the possible involvement of adiponectin with reduction of fetuin-A levels after pioglitazone treatment. It is well known that thiazolidinediones including pioglitazone increase plasma adiponectin levels [15]. Interestingly, adiponectin acts directly on the liver through adiponectin receptors, mainly AdipoR2 [15]. Therefore, it is hypothesized that pioglitazone could decrease fetuin-A levels through an adiponectin-dependent mechanism. This hypothesis needs to be tested in larger future studies.

At baseline, there was no association between fetuin-A levels and the log (HOMA) insulin resistance index. This is compatible with our previous report [8]. So far, the positive relationships between fetuin-A levels and insulin resistance have been limited to nondiabetic subjects [8,9]. Therefore, the significance of fetuin-A in insulin resistance is unknown in overt diabetes. In this study, we demonstrated that medication could modulate fetuin-A levels. Patients with overt diabetes often have concomitant diseases such as hypertension, dyslipidemia, etc; and as such, they are

prescribed medications not only for their diabetes but for these other diseases as well. Thus, hyperglycemia in itself or some medications may be affecting fetuin-A levels and/or functions, resulting in no association between fetuin-A and insulin resistance in patients with overt diabetes. Taking into consideration the unknown impacts of fetuin-A in overt diabetes, it remains possible that pioglitazone improves insulin resistance independently of a reduction in fetuin-A levels. To clarify the role of fetuin-A in insulin resistance, subjects without overt diabetes should be used as a cohort.

There are several other limitations of this study. Importantly, the number of subjects was small. This makes it difficult to investigate underlying factors involved in the mechanisms in question. Furthermore, the subjects were not randomly assigned to each group in this study; and interventions were not performed under blinded conditions. Considering the small sample size and the lack of randomization, we cannot exclude the possibility that the reduction of fetuin-A levels in the Pio group may have resulted from other confounding factors. In addition, we cannot reach definite conclusions in consideration of the following situations. First, the duration of intervention in the Ex group was much shorter than that in the previous report [9]. Exercise intervention did not lead to significant body weight loss in our study. Second, the doses of metformin were relatively low because higher doses are not available in Japan. Thus, intervention under different conditions, for example, longer period of exercise or higher doses of metformin, may alter fetuin-A levels to a greater extent. A randomized blinded study with a larger number of subjects will be needed to confirm our preliminary findings and to address these limitations.

Pioglitazone is known to improve various cardiovascular risk markers independently of its effect on glycemic control [16]. A randomized, double-blinded, multicenter trial demonstrated that pioglitazone slowed progression of atherosclerosis compared with glimepiride in subjects with type 2 diabetes mellitus [17]. On the other hand, recent reports have suggested an involvement of fetuin-A with early-stage atherosclerosis evaluated by ultrasonography in subjects with normal renal function [12,18], although it seems to play a protective role for advanced atherosclerosis with calcification especially in patients with chronic kidney disease as a calcification inhibitor [19,20]. Fiore et al [18] have reported that fetuin-A positively correlated with intima-media thickness, a representation of morphologic changes of the arterial wall. Furthermore, we have recently shown the positive association of fetuin-A levels with arterial stiffness, a functional property of atherosclerosis [12]. Based on these observations, pioglitazone could play a protective role in atherosclerosis, beyond its effect on glycemic control, via lowering fetuin-A levels.

In conclusion, treatment with pioglitazone reduced serum fetuin-A levels in patients with type 2 diabetes mellitus. However, our observations require confirmation because of the small sample size. Larger randomized and blinded trials will be necessary for this purpose. Further work is also needed to investigate whether fetuin-A is involved in the pleiotropic effects of pioglitazone on insulin resistance and related atherosclerotic diseases.

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