Original Article

Serum Fetuin-A is an Independent Marker of Insulin Resistance in Japanese Men

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***Aim*: Fetuin-A, also known as alpha2-Heremans Schmid glycoprotein, is an abundant plasma protein synthesized predominantly in the liver. Fetuin-A inhibits insulin receptor autophosphorylation, which is mediated by its intrinsic tyrosine kinase activity. In this study, we examined the association between the serum fetuin-A level and insulin resistance in Japanese men.**

***Methods*: We recruited 300 unrelated Japanese men without known chronic diseases, such as diabetes mellitus, or a history of regular drug use, and who underwent health examinations. From a 75-g oral glucose tolerance test, the study population included 194 individuals with normal glucose tolerance, 91 with impaired glucose tolerance and/or impaired fasting glucose, and 15 with diabetes mellitus. Serum fetuin-A concentrations were measured using an ELISA kit.**

***Results*: Serum fetuin-A concentrations were positively correlated with fasting insulin levels ($r=0.269$, $p<0.001$), HOMA-IR ($r=0.274$, $p<0.001$) and LDL-cholesterol ($r=0.172$, $p<0.01$), and negatively correlated with HDL-cholesterol concentrations ($r=-0.191$, $p<0.001$). Fetuin-A concentrations were also positively correlated with serum leptin ($r=0.150$, $p<0.01$) and negatively with adiponectin concentrations ($r=-0.208$, $p<0.001$). Stepwise regression analyses confirmed that the fetuin-A concentration was independently associated with the fasting insulin level and HOMA-IR, as were body mass index, triglyceride, LDL-cholesterol, leptin and adiponectin concentrations.**

***Conclusion*: Our data suggest that increased serum fetuin-A levels constitute an independent marker of insulin resistance and an atherogenic lipid profile in Japanese men.**

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Key words; Fetuin-A, α2-Heremans Schmid glycoprotein, AHSG, Insulin resistance

Introduction

Type 2 diabetes is characterized by inadequate insulin secretion and insulin resistance in the target tissues[1]. Genes and the environment both contribute to the development of disease[2]. Insulin mediates its actions through phosphorylation of the insulin receptor (IR), a transmembrane-spanning tyrosine kinase (TK) receptor. Binding of insulin to the IR activates its intrinsic TK activity and, subsequently, tyrosine phosphorylation of several substrates, such as insulin receptor substrate (IRS) and Shc, which mediate the metabolic and mitogenic effects of insulin[3]. Various factors, including fatty acids and cytokines, have been shown to influence the effect of insulin through insulin-signaling molecules or through other pathways that interfere with the insulin-signaling pathway[4].

Fetuin-A, also known as alpha2-Heremans Schmid glycoprotein (AHSG), is an abundant plasma protein synthesized predominantly in the liver[5]. Fetuin-A regulates calcium homeostasis[6-9] and inhibits IR autophosphorylation, which is mediated by its intrinsic TK activity[10-12]. Acute injection of human

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recombinant fetuin-A inhibits insulin-stimulated tyrosine phosphorylation of the IR β-subunit and IRS-1 in rat liver and skeletal muscle[12]. Fetuin-A-deficient mice exhibit significantly enhanced insulin sensitivity and are resistant to weight gain on a high fat diet[13]. Fetuin-A knockout mice were also resistant to the age-related decrease in insulin sensitivity[14]. The human AHSG gene is located at chromosome 3q27, which has been identified as a susceptibility locus for type 2 diabetes and metabolic syndrome[15-17]. It was recently shown that serum fetuin-A levels are significantly associated with insulin sensitivity in non-diabetic humans[18, 19]. These observations strongly support the hypothesis that fetuin-A plays a physiological role in the regulation of insulin signaling and energy homeostasis.

The aim of this study was to investigate the associations between serum fetuin-A levels and insulin resistance and factors related to insulin resistance, such as adiposity, lipid profiles and adipokines, in Japanese men.

Materials and Methods

Study Population

We recruited 300 unrelated Japanese men who underwent a health examination at Kochi Red Cross Hospital (mean age ± SD: 52.4 ± 10.9 years). Subjects with renal insufficiency (serum creatinine > 1.2 mg/dL), liver disorders, infectious disease, chronic inflammatory disease or a history of cardiovascular disease, were excluded from the study. Subjects with a history of diabetes mellitus or regular use of any kind of drugs were also excluded from the study. All subjects underwent a 75-g oral glucose tolerance test (75-g OGTT), and were classified as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT), impaired fasting glucose (IFG) or diabetes mellitus (DM), according to World Health Organization criteria[20]. Metabolic syndrome (MetS) was defined according to the Japanese criteria for men: waist circumference ≥ 85 cm and the presence of two or more of the following factors: dyslipidemia (fasting triglycerides level ≥ 150 mg/dL and/or high-density lipoprotein-cholesterol (HDL-C) level < 40 mg/dL), elevated blood pressure (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg), and fasting glucose level ≥ 110 mg/dL[21]. The study was approved by the Ethics Committee of Kochi Red Cross Hospital and written informed consent was obtained from all participants.

Fasting blood samples were obtained from all subjects and were separated and stored at −80℃ until use.

Measurement of Serum Fetuin-A Concentrations

Serum fetuin-A concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit (BioVender Laboratory Medicine, Brno, Czech Republic). Briefly, sera were diluted 10,000-fold and pipetted into wells in a microtiter plate, the surface of which was coated with polyclonal anti-human fetuin-A-specific antibodies. After incubation, horseradish peroxidase-conjugated polyclonal anti-human fetuin-A antibodies were added to the wells. After incubation, washing and color development steps, absorbance was measured using an automated plate reader at 450 nm. The concentrations of diluted sera were read from a standard curve for human fetuin-A, and the actual concentrations were calculated. All samples were measured in parallel and in duplicate. The intra-assay and inter-assay coefficients of variation were below 10%.

Serum Adiponectin, Leptin and Other Clinical Parameters

Serum adiponectin and leptin concentrations were measured using appropriate ELISA kits (R&D Systems, Minneapolis, MN, USA). Serum insulin and highly sensitive C-reactive protein (hsCRP) concentrations were measured by a paramagnetic particle chemiluminescence immunoassay (Fujirebio, Tokyo, Japan) and a highly sensitive nephelometric assay (Dade Behring, Liederbach, Germany), respectively. Serum lipid concentrations were measured enzymatically using an autoanalyzer. The low-density lipoprotein-cholesterol (LDL-C) concentration was estimated using Friedewald's equation; LDL-C = total cholesterol − HDL-C − triglycerides/5. The non-HDL-cholesterol (non-HDL-C) concentration was calculated (total cholesterol − HDL-C). The estimated glomerular filtration rate (eGFR) was calculated from serum creatinine (Cr) with an original equation devised for Japanese individuals[22]: $eGFR = 194 \times Cr^{-1.094} \times Age^{-0.287}$. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = fasting serum insulin (mU/L) × fasting plasma glucose (mmol/L)/22.5[23].

Statistical Analyses

All data are presented as the means ± SD. Groups were compared using one-way analysis of variance with Fisher's protected least significant difference test. Single linear univariate correlations were evaluated by Pearson's correlation coefficient. Multiple regression analyses with a fasting insulin level or HOMA-IR as dependent variables were conducted using a stepwise forward selection method. The *F*-value for the inclusion and exclusion of variables was set at 4.0. Statisti-

Table 1. Clinical characteristics of the study subjects

Variable	NGT	IGT	IFG	IGT plus IFG	DM
Number	194	49	20	22	15
Age (years)	51 ± 11	55 ± 10**	54 ± 8	57 ± 10*	57 ± 10*
Body mass index (kg/m^2)	23.3 ± 2.6	23.7 ± 3.2	25.2 ± 2.4**	24.4 ± 3.3	24.5 ± 2.6
Waist circumference (cm)	83 ± 7	85 ± 8	86 ± 6	86 ± 7	87 ± 6*
Systolic blood pressure (mmHg)	126 ± 11	129 ± 12	132 ± 9*	135 ± 13***	130 ± 12
Diastolic blood pressure (mmHg)	77 ± 6	79 ± 7	81 ± 4**	83 ± 7***	79 ± 7
Fasting plasma glucose (mg/dL)	97 ± 6	99 ± 7*	114 ± 5$^{*** \dagger\dagger}$	118 ± 7$^{*** \dagger\dagger}$	123 ± 21$^{*** \dagger\dagger \ddagger \S}$
2-h plasma glucose (mg/dL)	109 ± 17	157 ± 11$^{*** \ddagger}$	114 ± 17	165 ± 17$^{*** \ddagger}$	235 ± 32$^{*** \dagger\dagger \ddagger \P}$
Fasting serum insulin (μU/mL)	4.6 ± 2.3	4.8 ± 2.8	6.0 ± 3.5*	6.1 ± 4.0*	6.4 ± 3.1*†
HOMA-IR	1.1 ± 0.6	1.2 ± 0.7	1.7 ± 1.0***	1.8 ± 1.2$^{*** \dagger\dagger}$	1.9 ± 0.9$^{*** \dagger\dagger}$
Total cholesterol (mg/dL)	209 ± 32	208 ± 32	218 ± 38	219 ± 31	221 ± 30
Triglycerides (mg/dL)	137 ± 106	141 ± 91	153 ± 144	180 ± 139	150 ± 57
HDL-cholesterol (mg/dL)	56 ± 15	56 ± 13	54 ± 9	60 ± 14	56 ± 10
LDL-cholesterol (mg/dL)	125 ± 33	125 ± 32	134 ± 35	123 ± 37	135 ± 27
Non-HDL-cholesterol (mg/dL)	153 ± 34	153 ± 34	165 ± 39	159 ± 30	165 ± 33

Data are presented as the number or mean ± SD. NGT: normal glucose tolerance, IGT: impaired glucose tolerance, IFG: impaired fasting glucose, DM: diabetes mellitus. 2-h plasma glucose: 2-h plasma glucose during the OGTT. HOMA-IR: homeostasis model assessment of insulin resistance. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. NGT; $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.001$ vs. IGT; $^{\ddagger}p<0.001$ vs. IFG; $^{\S}p<0.05$, $^{\P}p<0.001$ vs. IGT plus IFG.

cal significance was defined as $p<0.05$.

Results

Clinical Characteristics of the Study Subjects

Based on the results of the 75-g OGTT, the 300 participants were classified as follows: 194 with NGT, 49 with IGT, 20 with IFG, 22 with IGT plus IFG, and 15 with DM. The clinical characteristics of the participants in this study are shown in **Table 1**. The mean age was higher in IGT, IGT plus IFG, DM subjects than in NGT subjects. Mean body mass index (BMI) was higher in IFG subjects, and waist circumference was higher in DM subjects than in NGT subjects. Systolic and diastolic blood pressures were higher in IFG and IGT plus IFG subjects than in NGT subjects. Fasting serum insulin and HOMA-IR were significantly higher in IFG, IGT plus IFG, and DM subjects than in NGT subjects.

Serum Fetuin-A, hsCRP, Adiponectin and Leptin Concentrations

The distribution of fetuin-A concentrations is shown in **Fig. 1**. The mean ± SD fetuin-A concentration was 27.1 ± 6.2 mg/dL. Serum fetuin-A concentrations in DM subjects ($n=15$) were significantly higher than in all non-DM subjects combined (NGT, IGT, IFG and IGT plus IFG; $n=285$) (30.4 ± 7.6 vs. 27.0 ± 6.1 mg/dL, respectively, $p<0.05$), although the number of subjects with DM was very small. In comparison to subjects with DM, fetuin-A concentrations tended to be lower in those with NGT and IFG, and were significantly lower in the IGT subgroups (**Table 2**). In contrast, significantly lower adiponectin concentrations were detected in subjects with DM than in those with NGT, IGT and IGT plus IFG (**Table 2**). On the other hand, there was no difference in hsCRP or leptin concentration between the five groups classified by the 75-g OGTT.

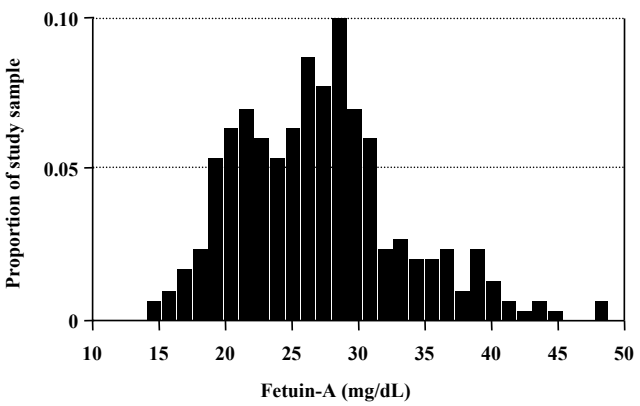

Fig. 1. Distribution of serum fetuin-A concentrations in Japanese men who underwent a 75-g oral glucose tolerance test during a health examination ($n=300$).

Associations Between Fetuin-A Quartiles and Clinical Parameters

The associations between fetuin-A quartiles and

Table 2. Serum fetuin-A, C-reactive protein, adiponectin and leptin concentrations

Variable	NGT	IGT	IFG	IGT plus IFG	DM
Number	194	49	20	22	15
Fetuin-A (mg/dL)	27.2 ± 6.1	25.8 ± 5.3	26.3 ± 7.5	28.3 ± 7.1	30.4 ± 7.6 $^{*\,\S}$
C-reactive protein (mg/L)	0.13 ± 0.28	0.13 ± 0.13	0.19 ± 0.40	0.23 ± 0.49	0.15 ± 0.19
Adiponectin (μg/mL)	6.4 ± 4.4	6.3 ± 4.7	5.6 ± 2.9	6.5 ± 4.0	3.6 ± 1.2 $^{\dagger\,\ddagger}$
Leptin (ng/mL)	5.0 ± 5.3	4.9 ± 5.9	6.1 ± 5.5	5.3 ± 4.9	5.7 ± 5.9

Data are presented as the number or mean ± SD. NGT: normal glucose tolerance, IGT: impaired glucose tolerance, IFG: impaired fasting glucose, DM: diabetes mellitus. $^{*}p = 0.06$ vs. NGT and IFG; $^{\S}p < 0.05$ vs. IGT; $^{\dagger}p < 0.05$ vs. IGT and IGT plus IFG; $^{\ddagger}p < 0.01$ vs. NGT.

Table 3. Characteristics of the study participants according to quartiles of serum fetuin-A level

Variable	Fetuin-A quartile				p [a]
	1 (≤22.4 mg/dL)	2 (22.5–26.6 mg/dL)	3 (26.7–30.2 mg/dL)	4 (≥30.3 mg/dL)	
Age (years)	52 ± 10	53 ± 14	53 ± 10	52 ± 9	NS
Body mass index (kg/m^2)	23.1 ± 2.9	24.0 ± 3.0	23.6 ± 2.3	23.9 ± 2.8	NS
Waist circumference (cm)	83 ± 7	85 ± 8	83 ± 7	85 ± 7	NS
Systolic blood pressure (mmHg)	128 ± 13	129 ± 12	126 ± 10	127 ± 11	NS
Diastolic blood pressure (mmHg)	79 ± 7	79 ± 7	77 ± 6	78 ± 7	NS
Fasting plasma glucose (mg/dL)	100 ± 11	102 ± 11	99 ± 8	103 ± 14	< 0.1
2h plasma glucose (mg/dL)	122 ± 33	130 ± 33	123 ± 29	135 ± 45	< 0.1
Fasting serum insulin (μU/mL)	4.0 ± 1.9	5.0 ± 3.0 *	4.7 ± 2.7	6.0 ± 2.8 $^{***\,\dagger}$	< 0.001
HOMA-IR	1.0 ± 0.5	1.3 ± 0.8 *	1.2 ± 0.7	1.5 ± 0.8 $^{***\,\dagger}$	< 0.001
Total cholesterol (mg/dL)	206 ± 34	209 ± 29	213 ± 31	215 ± 35	NS
Triglycerides (mg/dL)	134 ± 115	147 ± 125	141 ± 105	148 ± 83	NS
HDL-cholesterol (mg/dL)	60 ± 15	56 ± 16	56 ± 13	53 ± 12 **	< 0.05
LDL-cholesterol (mg/dL)	119 ± 36	123 ± 30	129 ± 31	133 ± 33	< 0.1
Non-HDL-cholesterol (mg/dL)	146 ± 36	152 ± 32	158 ± 32 *	162 ± 35 **	< 0.05
Albumin (g/dL)	4.5 ± 0.2	4.5 ± 0.2	4.5 ± 0.3	4.6 ± 0.2	NS
Estimated GFR (mL/min)	81 ± 13	82 ± 16	77 ± 13	80 ± 19	NS
C-reactive protein (mg/L)	0.16 ± 0.37	0.17 ± 0.32	0.15 ± 0.27	0.11 ± 0.12	NS
Adiponectin (μg/mL)	7.3 ± 3.8	6.4 ± 5.6	6.0 ± 3.4	5.1 ± 3.6 **	< 0.05
Leptin (ng/mL)	4.0 ± 3.9	5.7 ± 6.2	4.7 ± 5.2	6.1 ± 5.7	< 0.1
Metabolic syndrome (n, %)	11 (14.7)	16 (21.3)	6 (8.0)	15 (20.0)	NS
Diabetes mellitus (n, %)	3 (4.0)	2 (2.7)	1 (1.3)	9 (12.0) §	< 0.05

Data are presented as the number (%) or mean ± SD. [a] Frequencies of metabolic syndrome and diabetes mellitus were tested by χ^2-test and the others by analysis of variance. 2-h plasma glucose: 2-h plasma glucose during the OGTT, GFR: glomerular filtration rate. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. fetuin-A quartile 1; $^{\dagger}p < 0.05$ vs. fetuin-A quartile 2 and $p < 0.01$ vs. fetuin-A quartile 3; $^{\S}p < 0.05$ vs. fetuin-A quartile 2 and $p < 0.01$ vs. fetuin-A quartile 3.

clinical parameters were evaluated and are shown in **Table 3**. The mean ages were similar among the quartiles of fetuin-A. Compared with the lowest quartile group, the higher fetuin-A quartile groups had higher fasting insulin, HOMA-IR and non-HDL-C levels, and lower HDL-C and adiponectin levels. The prevalence of DM was higher in the highest quartile of fetuin-A.

Associations Between Serum Fetuin-A Concentrations and Clinical Parameters

The results of simple linear regression analyses between serum fetuin-A concentrations and clinical parameters are summarized in **Table 4**. Serum fetuin-A levels were positively correlated with fasting serum insulin (**Fig. 2**, **left**), HOMA-IR (**Fig. 2**, **right**), LDL-C (**Fig. 3**, **left**), non-HDL-C (**Fig. 3**, **middle**) and leptin concentrations (**Fig. 4**, **right**), and nega-

Table 4. Simple linear regression analysis of serum fetuin-A concentration in the total study population

Variable	r	p
Age	−0.027	NS
Body mass index	0.094	NS
Waist circumference	0.066	NS
Systolic blood pressure	−0.043	NS
Diastolic blood pressure	−0.036	NS
Fasting plasma glucose	0.079	NS
2h plasma glucose	0.070	NS
Fasting serum insulin	0.269	<0.001
HOMA-IR	0.274	<0.001
Total cholesterol	0.130	<0.05
Triglycerides	0.055	NS
HDL-cholesterol	−0.191	<0.001
LDL-cholesterol	0.172	<0.01
Non-HDL-cholesterol	0.201	<0.001
Albumin	0.088	NS
C-reactive protein	−0.062	NS
Adiponectin	−0.208	<0.001
Leptin	0.150	<0.01

Simple linear regression analysis was performed in all subjects ($n=300$). r: correlation coefficient.

tively with HDL-C (**Fig. 3**, **right**) and adiponectin concentrations (**Fig. 4**, **left**). Exclusion of the 15 subjects with DM from the study population did not affect these significant correlations between fetuin-A levels and fasting insulin, HOMA-IR, HDL-C, LDL-C, non-HDL-C, adiponectin and leptin concentrations (data not shown). Significant positive correlations between HOMA-IR and fetuin-A levels were preserved in subjects with NGT ($n=194$, $r=0.280$, $p<0.001$) and in subjects with impaired glucose regulation (IGT, IFG and IGT plus IFG; $n=91$, $r=0.255$, $p=0.01$). HOMA-IR was found to be independently associated with fetuin-A levels ($\beta=0.221$, $F=14.590$), as were LDL-C ($\beta=0.126$, $F=5.139$) and HDL-C concentrations ($\beta=-0.116$, $F=4.088$), in stepwise regression analysis of the fetuin-A level using the significant variables listed in **Table 4** as explanatory factors (total $R^2=0.104$, $p<0.001$). Similar results were obtained in a model that included the fasting insulin level instead of HOMA-IR as an explanatory variable (data not shown).

We also examined whether the serum fetuin-A levels were associated with MetS in this study population. In 147 of 300 subjects, the waist circumference was 85 cm or over (visceral obesity), and 48 of 147 obese subjects were considered to have MetS according to the Japanese criteria. The serum fetuin-A concentrations in non-obese ($n=153$), non-MetS obese ($n=99$) and MetS ($n=48$) subjects were 26.9 ± 6.6, 27.4 ± 5.5 and 27.4 ± 6.6 mg/dL, respectively, and there were no differences among groups. The results did not change when subjects with DM were excluded (data not shown).

Associations Between Insulin Resistance and Clinical Parameters

Stepwise multiple regression analyses for the fasting insulin level and HOMA-IR, both of which are markers for insulin resistance, were performed using many of the factors listed in **Table 4** as explanatory factors. As a result, the serum fetuin-A level was independently associated with the fasting insulin level and with HOMA-IR, as were BMI, leptin, adiponectin, triglycerides and LDL-C (**Table 5**). Diastolic blood pressure was only significantly associated with HOMA-IR. In models including waist circumference instead of BMI as an explanatory variable, fetuin-A was significantly associated with fasting insulin ($\beta=0.134$, $F=10.106$; total $R^2=0.517$, $p<0.001$) and HOMA-IR ($\beta=0.145$, $F=11.107$; total $R^2=0.492$, $p<0.001$). Furthermore, in models including non-HDL-C instead of LDL-C as an explanatory variable, the fetuin-A level showed similar associations with fasting insulin ($\beta=0.142$, $F=12.513$; total $R^2=0.564$, $p<0.001$) and HOMA-IR ($\beta=0.155$, $F=14.064$; total $R^2=0.543$, $p<0.001$). Exclusion of subjects with DM did not affect the significant associations between fetuin-A and fasting insulin ($\beta=0.124$, $F=9.106$; total $R^2=0.561$, $p<0.001$) or HOMA-IR ($\beta=0.143$, $F=11.193$; total $R^2=0.519$, $p<0.001$).

Discussion

We have demonstrated that elevated serum fetuin-A is significantly associated with insulin resistance, as assessed by the fasting insulin level and HOMA-IR in 300 Japanese men. Mori *et al.* showed that serum fetuin-A levels were significantly correlated with log-transformed HOMA-IR in 160 non-diabetic Japanese subjects[19]. In the present study, we showed for the first time that the association between serum fetuin-A and insulin resistance remained significant after adjustment for adipokine and hsCRP levels, in addition to other factors associated with insulin resistance. This confirms that the serum fetuin-A level is independently associated with insulin resistance in Japanese subjects.

In our study, the serum fetuin-A concentration was higher in subjects with newly diagnosed DM than in all subjects without DM, although the number of

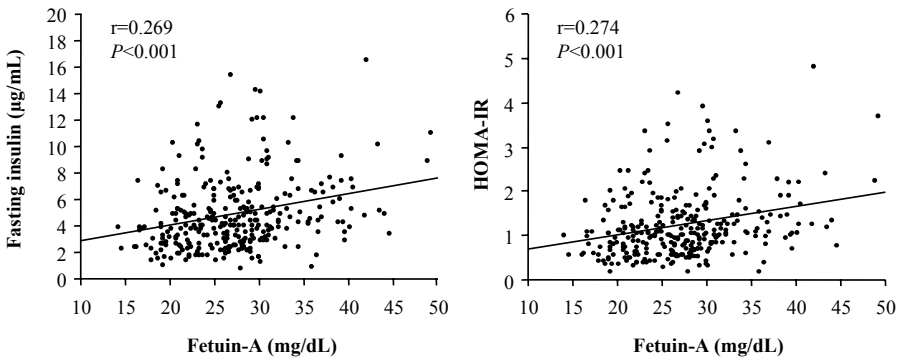

Fig. 2. Correlations between serum fetuin-A concentrations and fasting insulin or HOMA-IR in the complete study population (n=300). Single linear univariate correlations were evaluated by Pearson's correlation coefficient.

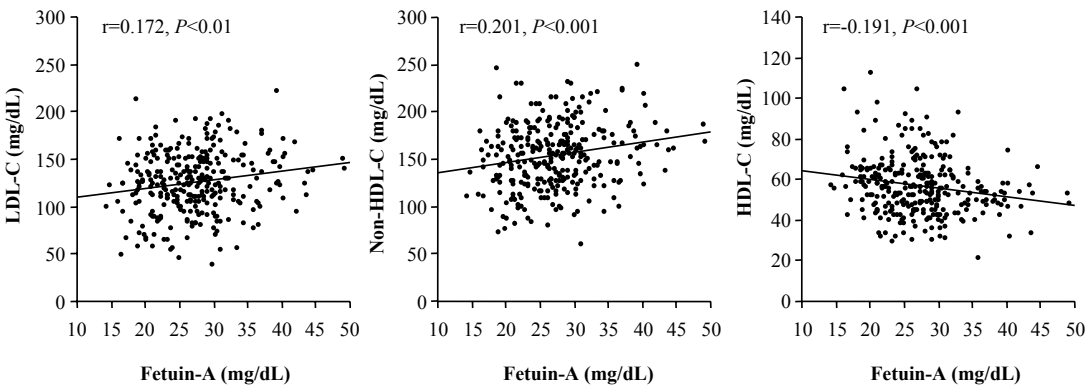

Fig. 3. Correlations between serum fetuin-A concentrations and LDL-cholesterol, non-HDL-cholesterol and HDL-cholesterol concentrations in the complete study population (n=300). Single linear univariate correlations were evaluated by Pearson's correlation coefficient.

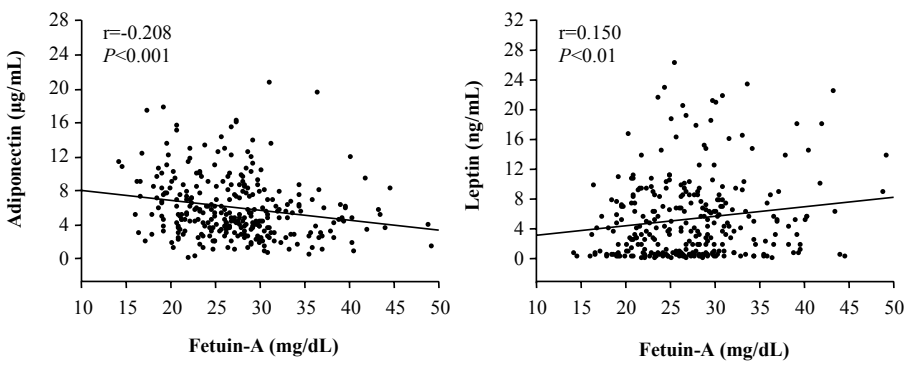

Fig. 4. Correlations between serum fetuin-A concentrations and adiponectin or leptin concentrations in the complete study population (n=300). Single linear univariate correlations were evaluated by Pearson's correlation coefficient.

subjects with DM was very small. Positive correlations between fetuin-A and fasting plasma glucose and HbA1C have been shown in subjects without DM[24]. On the other hand, there was no difference in the mean serum fetuin-A levels between subjects without versus with type 2 DM, despite the significant correlation between serum fetuin-A and insulin resistance in subjects without DM[19]. Thus, the cross-sectional

Table 5. Stepwise regression analyses for fasting insulin level and HOMA-IR in the total study population

Variables	Fasting insulin			HOMA-IR		
	β	F	p	β	F	p
Fetuin-A	0.142	12.511	<0.001	0.155	14.067	<0.001
Body mass index	0.314	38.676	<0.001	0.304	32.311	<0.001
Leptin	0.347	50.188	<0.001	0.309	37.815	<0.001
Triglycerides	0.174	15.514	<0.001	0.170	13.918	<0.001
LDL-cholesterol	0.098	5.387	<0.05	0.109	6.283	<0.05
Adiponectin	−0.094	5.083	<0.05	−0.096	5.005	<0.05
Diastolic blood pressure	0.072	1.539	NS	0.087	4.393	<0.05
Systolic blood pressure	0.061	1.079	NS	0.009	0.022	NS
HDL-cholesterol	−0.049	0.692	NS	−0.031	0.273	NS
Age	0.034	0.341	NS	0.023	0.159	NS
C-reactive protein	−0.003	0.003	NS	−0.011	0.035	NS
Total R^2	0.564	$p<0.001$		0.543	$p<0.001$	

Stepwise multiple regression analysis was performed in all subjects ($n=300$). F-values for the inclusion and exclusion of variables were set at 4.0 at each step. β: standardized partial regression coefficient. Significant variables ($p<0.05$) were included in the model for adjustment and calculation of total R^2.

association between the circulating fetuin-A level and DM is rather complex.

To date, two prospective studies have investigated the associations between fetuin-A and the risk for DM. It was shown that the fetuin-A level is associated with incidental DM in a longitudinal study with 6 years of follow-up of well-functioning older persons[25]. Another large prospective, population-based study with 7 years of follow-up also showed significant associations between fetuin-A and increased risk for DM, particularly in individuals with elevated plasma glucose levels within the non-diabetic range[24]. These earlier studies, together with our cross-sectional data showing an independent association between fetuin-A and insulin resistance, support the hypothesis that fetuin-A contributes to the future development of DM by inducing insulin resistance.

We have shown that high concentrations of glucose induce transactivation of the AHSG gene which encodes fetuin-A, and enhance fetuin-A protein expression in cultured human hepatoma cells[26]; however, factors other than glucose levels (e.g. insulin levels and inflammation) could also influence the synthesis or degradation of fetuin-A. In fact, interleukin (IL)-6, IL-1β and tumor necrosis factor-α (TNF-α) downregulate fetuin-A synthesis[27, 28]. Thus, serum fetuin-A levels in subjects with DM may vary among clinical settings. Furthermore, some genetic polymorphisms can influence circulating fetuin-A levels[29, 30]; therefore, these factors should be taken into consideration to confirm the relationship between fetuin-A and DM.

Fetuin-A-knockout mice exhibited not only enhanced insulin sensitivity but also resistance to the adipogenic effect of a high-fat diet[13], suggesting that fetuin-A might regulate adipogenesis. In accordance with this, higher fetuin-A levels were shown to be associated with increased visceral adiposity in well-functioning elderly persons[25] and with a higher prevalence of MetS[31]. However, in our present study, a significant association with MetS was not detected, and serum fetuin-A was not associated with adiposity per se, as assessed by BMI or waist circumference. The inclusion criteria for their study required a medical history of coronary artery disease (CAD)[31], which may explain the higher prevalence of MetS in their study sample. This is quite different from our study, in which subjects with chronic diseases, such as CAD, or those with regular use of any drugs, including statins and antihypertensive drugs, were excluded. Negative associations between fetuin-A and BMI and waist circumference were also shown in 2,164 subjects without DM[24]. This suggests that, at least in a general population, fetuin-A is not associated with adiposity per se, but directly induces insulin resistance in insulin-sensitive tissues.

We found that fetuin-A is negatively correlated with adiponectin levels and positively correlated with leptin levels; both of these adipokines have marked effects on glucose metabolism. An inverse correlation between fetuin-A and adiponectin has already been shown in a study of 49 healthy subjects[32]. Further-

more, in the same paper, it was shown that fetuin-A promotes cytokine expression in monocytes and adipocytes, and suppresses the production of adiponectin. In our multiple regression analysis, the association between fetuin-A and insulin resistance was not affected by adipokine levels; however, we cannot reject the possibility that fetuin-A may induce insulin resistance, partially by suppressing of adiponectin production.

A significant association between serum fetuin-A and atherogenic lipid profiles has been reported in subjects with CAD but not DM[31]. Our present study also showed that the level of fetuin-A is associated with the atherogenic lipid profile in men without any cardiovascular diseases. Fetuin-A, as a phosphorylation substrate, inhibits IR TK activity[10-12], which results in impaired insulin action and could induce dyslipidemia by increasing lipolysis from adipose tissue; however, we could not detect a direct correlation between fetuin-A levels and triglyceride concentrations. Therefore, it is possible that another factor may promote the elevation in fetuin-A and LDL-C levels; for example, transcriptional factors that regulate cholesterol homeostasis (e.g. sterol regulatory element binding protein[33]) could be involved in the regulation of hepatic synthesis of fetuin-A. Further studies are needed to elucidate the underlying mechanisms.

Significant positive correlations between fetuin-A and hsCRP have been shown in subjects without DM, with or without CAD[24, 31]; however, we could not detect a significant association between hsCRP and fetuin-A in apparently healthy Japanese men. Fetuin-A is known to induce proinflammatory cytokines, such as IL-6, IL-1β and TNF-α[32]. On the other hand, fetuin-A is a negative acute phase protein and its expression is suppressed by these cytokines[27, 28]. Thus, the cross-sectional correlation between fetuin-A and CRP is complex, and the association between fetuin-A and CRP may alter depending on the type, grade or phase of disease.

There are several limitations to our study. First, the sample size was relatively small; therefore, the number of subjects in each subgroup (particularly the IFG, IGT plus IFG and DM groups) was too small to reach definitive conclusions for the comparisons among subgroups. For example, there was no difference in the fetuin-A level between NGT and IFG, although HOMA-IR in IFG was significantly higher than in NGT. This may be due to the small number of IFG subjects because significant correlations between HOMA-IR and fetuin-A were preserved both in subjects with NGT and in subjects with impaired glucose regulation. Second, the cross-sectional design does not allow for causal inference or evaluation of the direction of an association. Third, our study participants were only men; therefore, our results may not be generalized to women. Finally, we cannot rule out the possibility that other unmeasured factors, which may affect fetuin-A and insulin resistance, could explain their correlation.

In summary, higher fetuin-A concentrations showed a strong and independent association with insulin resistance and atherogenic lipid profiles in Japanese men. These findings, in conjunction with previous studies in animals and humans, support the hypothesis that fetuin-A directly inhibits insulin signaling. However, our association study is underpowered to reach definitive conclusions because of the small number of study subjects. Longitudinal studies based on a larger population are needed to confirm these findings.

Conclusion

We demonstrated that elevated levels of serum fetuin-A are significantly associated with insulin resistance, as assessed by the fasting insulin level and HOMA-IR in Japanese men. The association between serum fetuin-A and insulin resistance remained significant after adjustment for adipokine concentrations and other factors known to be associated with insulin resistance. These data suggest that the serum fetuin-A level is an independent marker of insulin resistance in Japanese subjects.

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