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High plasma fetuin-A levels are associated with metabolic syndrome among males but not females in a Japanese general population

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ABSTRACT

Aims: Fetuin-A, a protein exclusively secreted from the liver, is associated with insulin resistance and/or metabolic syndrome (MetS). However, few studies have examined this association in Japan. We investigated this issue in a Japanese general population.

Methods: We performed an epidemiological survey in a small community in Japan. The participants consisted of 659 subjects (253 males and 406 females). Fetuin-A levels were measured by a sandwich ELISA method and the modified NCEP-ATP III criteria were adopted to diagnose MetS. The homeostasis model assessment index (HOMA-IR) was calculated as a marker of insulin resistance.

Results: Statistically significant characteristics of the 659 subjects stratified by fetuin-A quartiles were male gender (inversely), age (inversely), insulin, HOMA-IR, uric acid (inversely), alcohol intake (inversely) and the prevalence of MetS. Mean fetuin-A levels were 249.7 ± 45.1 μg/ml in males and 262.7 ± 55.8 μg/ml in females. In males, the prevalence of MetS was 43.1%, and their mean HOMA-IR level was 1.1. In females, the prevalence of MetS was 17.7%, and their mean HOMA-IR level was 0.9. Multiple stepwise regression analyses showed that fetuin-A levels in males but not females were independently associated with MetS and LDL-c. Multiple logistic regression analysis of fetuin-A (quartile 1 vs. quartile 4) in males showed significant odds ratios of 1.009 (95% C.I.: 1.003–1.015) for MetS and 1.376 (95% C.I.: 1.027–1.844) for 1-SD increment increase in LDL-c.

Conclusions: High plasma fetuin-A levels were associated with MetS in community-dwelling Japanese males but not females.

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1. Introduction

Fetuin-A (alpha-2-Heremans-Schmid glycoprotein: AHSG) is a liver-synthesized protein that is secreted into serum and is a potent circulating inhibitor of the precipitation of calcium and phosphorus [1–3]. In animal studies, fetuin-A is a multifunctional molecule and acts as an antagonist of transforming growth factor β and cytokine-dependent osteogenesis [4,5]. Moreover, fetuin-A binds directly the extracellular domain of the insulin receptor and inhibits insulin receptor tyrosine kinase activity [5]. In animals, fetuin-A knockout mice are insulin-sensitive and resistant to weight gain [3,5]. Conversely, injection of fetuin-A into mice induces insulin-resistance [6].

In humans, high circulating fetuin-A levels have been linked to the metabolic syndrome (MetS) including obesity, fatty liver disease, atherogenic dyslipidemia and insulin resistance as hallmarks of MetS [7,8]. Reports from different populations (Caucasians vs. Asians [7–14], males vs. females [11,15], obese vs. non-obese subjects [13,14,16,17], non-diabetics vs. diabetics [18–20], young vs. old persons [18,20–23], and patients with cardiovascular diseases vs. general populations [24–27]) indicated that plasma fetuin-A is associated with insulin resistance or MetS and is an independent risk factor of type 2 diabetes [13,15,17–22]. However, there is little information available for this association in Japan. We investigated this issue in a Japanese general population.

2. Materials and methods

2.1. Study population

A total of 673 subjects (260 males and 413 females) aged over 40 years received a population-based health examination in a fishing community in southwestern Japan, Uku town, from 2009 to 2012. This town is an isolated island in Sasebo city, located in Nagasaki prefecture, and the total population is about 3700. Of these, we excluded 14 subjects whose fetuin-A data were missing or who rejected the blood tests. Consequently, 659 subjects (253 males and 406 females) were enrolled in this study.

2.2. Data collection

Height and weight were measured, and body mass index (BMI) was calculated as weight (kilograms) divided by the square of height (square meters) as an index of the presence or absence of obesity. Waist circumference was measured at the level of the umbilicus in a standing position. Blood pressure (BP) was measured twice with the subjects in the sitting (first) and supine (second) position. Vigorous physical activity and smoking were avoided for at least 30 min before BP measurements. The second BP with the fifth phase diastolic pressure was used for analysis.

Hypertensives were defined as those with BP ≥ 140/90 mmHg and/or those receiving antihypertensive medication.

Blood was drawn from the antecubital vein for determinations of lipids profiles (total cholesterol, high-density lipoprotein cholesterol [HDL-c], low-density lipoprotein cholesterol [LDL-c] and triglycerides), creatinine, uric acid (UA), fasting plasma glucose (FPG), insulin, glycated hemoglobin A_{1c} [HbA_{1c}(NGSP)/mmol/mol (IFCC)], liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (γ-GTP)], gender hormones (estradiol, testosterone and progesterone) and plasma fetuin-A levels in the morning after a 12-h fast. Fasting blood samples were centrifuged within 1 h after the collection. Estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease (MDRD) study equation modified with a Japanese coefficient [28]. Fetuin-A levels were measured by a sandwich ELISA method (BioVendor Laboratory Medicine, Brno, Czech Republic) [7]. Intra- and inter-assay coefficients of variation of fetuin-A at a commercially available laboratory (SRL Inc. Fukuoka, Japan) were 5.2% and 3.8%, respectively.

The homeostasis model assessment index (HOMA-IR) was calculated from FPG and insulin levels [FPG (mg/dl) × insulin (μU/ml)/405] as a marker of insulin resistance [29]. Subjects with type 2 diabetes mellitus were defined as those with FPG ≥ 126 mg/dl or HbA_{1c} ≥ 6.5%, or those taking oral hypoglycemic agents and/or receiving insulin injection. Subjects with dyslipidemia were defined as those with LDL-c ≥ 140 mg/dl and/or triglycerides ≥150 mg/dl and/or HDL-c < 40 mg/dl and/or those taking lipid-lowering drugs.

We defined the metabolic syndrome according to ATP III (Adult Treatment Panel III) [30]. ATP III identified 5 components of the metabolic syndrome [abdominal obesity; given as waist circumference (>101.6 cm for males, >88.9 cm for females)], triglycerides (≥150 mg/dl), HDL-c (<40 mg/dl for males, <50 mg/dl for females), blood pressure (≥130 and/or ≥85 mmHg) and FPG (≥110 mg/dl). However, Japanese are much smaller than people of western countries, therefore, it is not appropriate to use the criteria of abdominal obesity of ATP III. Accordingly, we adopted ≥85 cm for males and ≥90 cm for females of waist circumference, proposed by the Japanese Society for Obesity [31]. When three of the five listed criteria were met, a subject was diagnosed as having MetS.

The average consumption of alcohol was categorized into four groups [non (0 g), light (<23 g), moderate (23–45 g) and heavy (≥46 g)] [32].

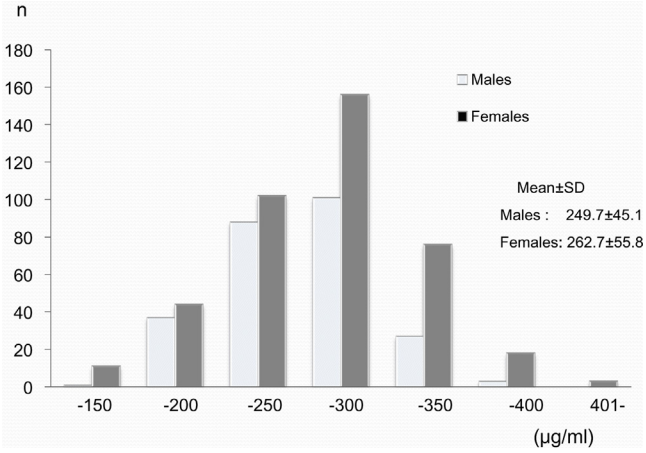

Fig. 1 – Plasma fetuin-A levels showing normal distributions in both genders.

This study was approved by the mayor and the welfare department of Uku town, as well as by the ethics committee of Kurume University. All participants gave informed consent.

2.3. Statistical analysis

Because of skewed distributions, natural logarithmic (ln) transformations were performed for triglycerides, insulin, HOMA-IR, estradiol, testosterone, and progesterone. Log-transformed values were reconverted to antilogarithm forms in the tables. The medications for hypertension, dyslipidemia and diabetes mellitus were coded as dummy variables. Gender, smoking habits and MetS were also coded as dummy variables. First, mean fetuin-A level was classified into quartiles. Second, we performed univariate analysis with fetuin-A as a dependent variable stratified by gender, and then we performed stepwise regression analysis in males using significant factors shown in univariate analysis. Finally, multiple logistic regression analysis of fetuin-A (quartile 1 vs. quartile 4) in males was performed. We estimated odds ratios and their 95% confidence intervals (C.I.) per 1-unit (approximately 1 SD) increase in the variable. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed using the SAS system (Release 9.3, SAS Institute, Cary, NC).

3. Results

Plasma fetuin-A levels had a normal distribution in both males and females (Fig. 1). Statistically significant characteristics of

Table 1 – Characteristics of study subjects in fetuin-A quartiles.

Characteristics	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*p*-Value
Number	163	163	167	166	
Male % (*n*:yes)	44.2 (72)	43.6 (71)	40.1 (67)	25.9 (43)	**0.002**
Fetuin-A (μg/ml)	190.7 ± 25.4	242.6 ± 9.9	273.4 ± 9.8	322.6 ± 30.6	**<0.001**
Age (years)	66.7 ± 9.2	67.2 ± 9.5	65.9 ± 10.1	63.8 ± 9.7	**0.010**
BMI (kg/m^2)	23.4 ± 3.1	23.7 ± 3.5	23.5 ± 3.4	24.0 ± 3.1	0.397
Waist (cm)	82.4 ± 8.9	83.2 ± 10.2	83.3 ± 10.7	83.3 ± 9.1	0.803
Systolic BP (mmHg)	135.6 ± 17.5	135.7 ± 19.1	137.4 ± 19.2	138.2 ± 20.5	0.526
Diastolic BP (mmHg)	81.1 ± 9.5	81.6 ± 10.0	81.8 ± 10.6	83.0 ± 11.6	0.386
FPG (mg/dl)	96.8 ± 16.9	96.2 ± 18.2	96.6 ± 15.8	96.5 ± 17.6	0.993
HbA_{1c} (NGSP) (%)	5.6 ± 0.5	5.6 ± 0.6	5.6 ± 0.6	5.6 ± 0.6	0.715
Insulin (μU/ml)[a]	3.6	4.0	3.9	4.5	**0.006**
Range	(0.5–15.4)	(0.5–69.2)	(0.8–14.8)	(1.0–24.6)	
HOMA-IR[a]	0.84	0.94	0.93	1.81	**0.019**
Range	(0.1–5.1)	(0.1–15.2)	(0.2–4.5)	(0.2–6.9)	
eGFR (ml/min/1.73 m^2)	74.4 ± 16.7	73.1 ± 14.9	71.2 ± 15.5	73.2 ± 16.1	0.323
Uric acid (mg/dl)	5.1 ± 1.4	4.8 ± 1.9	4.5 ± 2.0	4.2 ± 2.0	**<0.001**
γ-GTP (IU/l)	37.1 ± 38.7	35.6 ± 34.9	31.9 ± 35.7	34.4 ± 32.6	0.605
ALT (IU/l)	25.1 ± 12.7	23.9 ± 7.4	23.3 ± 6.7	26.9 ± 7.2	0.136
AST (IU/l)	20.9 ± 13.2	20.6 ± 10.2	20.1 ± 11.0	24.7 ± 18.7	0.067
Total-c (mg/dl)	199.5 ± 35.1	202.7 ± 32.5	205.5 ± 34.2	208.2 ± 38.6	0.138
HDL-c (mg/dl)	60.1 ± 15.8	60.3 ± 14.1	59.6 ± 16.6	61.7 ± 16.2	0.642
LDL-c (mg/dl)	118.6 ± 33.5	120.9 ± 28.6	124.8 ± 30.9	123.4 ± 35.1	0.312
Triglycerides (mg/dl)[a]	84.0	83.7	85.5	88.5	0.679
Range	(35–843)	(25–291)	(33–399)	(36–323)	
Estradiol (pg/ml)[a]	16.3	14.0	15.9	15.2	0.803
Range	(10–455)	(10–143)	(10–422)	(10–273)	
Testosterone (ng/ml)[a]	0.52	0.47	0.44	0.42	0.264
Range	(0.1–13.6)	(0.1–14.3)	(0.1–10.1)	(0.1–11.1)	
Progesterone (ng/ml)[a]	0.18	0.18	0.17	0.19	0.876
Range	(0.03–14.9)	(0.03–5.8)	(0.03–10.0)	(0.03–26.6)	
Alcohol intake % (*n*:yes)	49.7 (81)	42.3 (69)	38.9 (65)	33.1 (55)	**0.010**
None	47.8 (78)	55.8 (91)	60.4 (101)	65.7 (109)	**0.021**
Light (<23 g)	22.7 (37)	15.3 (25)	16.8 (28)	19.3 (32)	
Moderate (23–45 g)	15.9 (26)	12.9 (21)	13.8 (23)	9.6 (16)	
Heavy (≥46 g)	11.0 (18)	14.1 (23)	8.4 (14)	4.2 (7)	
Smoking % (*n*:yes)	31.9 (52)	29.4 (48)	28.1 (47)	20.5 (34)	0.123
DM % (*n*:yes)	6.7 (11)	8.0 (13)	10.8 (18)	8.4 (14)	0.612
Hypertension % (*n*:yes)	51.5 (84)	50.3 (82)	47.3 (79)	53.6 (89)	0.708
Dyslipidemia % (*n*:yes)	33.7 (55)	35.6 (58)	44.9 (75)	48.8 (81)	0.103
MetS % (*n*:yes)	23.3 (38)	29.4 (48)	29.3 (49)	33.7 (56)	**0.013**

Data are means (SD), geometric mean, range, or percent.

Abbreviations: BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistance; eGFR, estimated glomerular filtration rate; γ-GTP, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDL, low density lipoprotein; HDL, high density lipoprotein; DM, diabetes mellitus; MetS, metabolic syndrome.

The bold of *p* values was defined as $p < 0.05$.

[a] These variables are shown in the original scale after analysis using log (natural)-transformed values.

the 659 subjects stratified by fetuin-A quartiles were male gender (inversely), age (inversely), insulin, HOMA-IR, uric acid (inversely), alcohol intake (inversely) and the prevalence of MetS (Table 1). Because gender was a significant factor for fetuin-A levels, we analyzed the data stratified by gender. Demographics of 659 subjects stratified by gender were presented in Table 2. There was no significant difference of fetuin-A levels between males and females. Mean ± standard deviations in fetuin-A levels were 249.7 ± 45.1 μg/ml in males and 262.7 ± 55.8 μg/ml in females, respectively. In males, the prevalence of MetS was 43.1%, and their mean HOMA-IR level was 1.1. In females, the prevalence of MetS was 17.7%, and their mean HOMA-IR level was 0.9. The prevalence of alcohol intake, smoking habits and MetS in males was much higher than in females. Tables 3a and 3b showed univariate analyses with fetuin-A as a dependent variable stratified by gender. Plasma fetuin-A in males (Table 3a) was associated with BMI, waist circumference, systolic and diastolic BPs, insulin, HOMA-IR, total cholesterol, LDL-c, triglycerides and MetS. Fetuin-A levels in females (Table 3b) were associated only with uric acid ($p < 0.01$; inversely). Then, we performed multiple stepwise regression analyses to determine independent factors associated with fetuin-A levels in males (Table 4a). Fetuin-A levels in males were independently associated with MetS and LDL-c. We further investigated the associations using multiple logistic regression analysis of fetuin-A (quartile 1 vs. quartile 4) in males. Significant odds ratios were found in Table 4b [(odds ratio: 1.009; 95% C.I.: 1.003–1.015) in MetS and [(odds ratio: 1.376; 95% C.I.: 1.027–1.844) in 1-SD increment increase in LDL-c.]. The difference in fetuin-A levels was 18.2 μg/ml between the males with (260.1 μg/ml) and without (241.9 μg/ml) MetS. We also quantified the 13.9 μg/ml increase in fetuin-A corresponding to each 32.5 mg/dl increase in LDL-c.

Table 2 – Characteristics of study subjects.

Characteristics	Males (n = 253)	Females (n = 406)
Fetuin-A (μg/ml)	249.7 ± 45.1	262.7 ± 55.8
Age (years)	66.5 ± 9.2	65.6 ± 10.1
Body mass index (kg/m^2)	24.0 ± 3.0	23.4 ± 3.5
Waist (cm)	84.9 ± 8.9	81.9 ± 10.1
Systolic blood pressure (mmHg)	136.8 ± 18.3	136.7 ± 19.6
Diastolic blood pressure (mmHg)	83.4 ± 11.0	80.9 ± 10.1
Fasting plasma glucose (mg/dl)	103.7 ± 21.8	92.1 ± 11.2
HbA_{1c} (NGSP) (%)	5.7 ± 0.7	5.6 ± 0.5
Insulin (μU/ml)[a]	4.3	3.8
Range	(0.5–69.2)	(0.8–24.6)
HOMA-IR[a]	1.1	0.9
Range	(0.1–15.2)	(0.2–6.9)
eGFR (ml/min./1.73 m^2)	72.9 ± 16.1	73.0 ± 15.6
Uric acid (mg/dl)	5.3 ± 2.0	4.3 ± 1.7
γ-Glutamyl transpeptidase (IU/l)	50.3 ± 47.5	25.0 ± 20.0
Alanine aminotransferase (IU/l)	26.2 ± 11.4	23.9 ± 17.4
Aspartate aminotransferase (IU/l)	25.0 ± 15.5	19.4 ± 18.4
Total cholesterol (mg/dl)	190.1 ± 36.8	208.9 ± 33.2
HDL-cholesterol (mg/dl)	54.9 ± 15.8	62.7 ± 14.2
LDL-cholesterol (mg/dl)	116.8 ± 32.5	126.4 ± 32.2
Triglycerides (mg/dl)	113.6 ± 82.9	86.0 ± 35.1
Estradiol (pg/ml)[a]	23.4	20.8
	(10.0–64.0)	(10.0–455.0)
Testosterone (ng/ml)[a]	5.1	0.1
	(0.03–13.6)	(0.03–0.8)
Progesterone (ng/ml)[a]	0.3	0.4
	(0.03–0.8)	(0.03–26.6)
Alcohol intake % (n:yes)	74.0 (185)	21.3 (86)
None	25.7 (64)	78.5 (314)
Light (<23 g)	26.9 (67)	13.7 (55)
Moderate (23–45 g)	27.7 (69)	4.3 (17)
Heavy (≥46 g)	18.5 (46)	4.0 (16)
Current smoking % (n:yes)	64.7 (163)	4.4 (18)
Diabetes mellitus % (n:yes)	10.3 (26)	4.9 (20)
Hypertension % (n:yes)	45.8 (115)	37.7 (153)
Dyslipidemia % (n:yes)	18.3 (46)	24.3 (97)
MetS % (n:yes)	43.1 (109)	17.7 (72)

Data are means (SD), geometric mean, range, or percent.
Abbreviations: HOMA-IR, homeostasis model assessment-insulin resistance; eGFR, estimated glomerular filtration rate; MetS, metabolic syndrome.
[a] These variables are shown in the original scale after analysis using log (natural)-transformed values.

Table 3a – Univariate analyses with fetuin-A in males.

Characteristics	β	p
Age (years)	−0.29	0.34
Body mass index (kg/m^2)	2.62	**0.005**
Waist (cm)	0.81	**0.01**
Systolic blood pressure (mmHg)	0.32	**0.04**
Diastolic blood pressure (mmHg)	0.71	**0.006**
Fasting plasma glucose (mg/dl)	0.16	0.23
HbA_{1c} (NGSP) (%)	3.07	0.47
Insulin (μU/ml)[a]	13.53	**0.002**
HOMA-IR[a]	12.05	**0.002**
eGFR (ml/min/1.73 m^2)	−0.18	0.31
Uric acid (mg/dl)	−2.64	0.06
γ-Glutamyl transpeptidase (IU/l)	−0.03	0.64
Alanine aminotransferase (IU/l)	−0.07	0.79
Aspartate aminotransferase (IU/l)	0.36	0.06
Total cholesterol (mg/dl)	0.15	**0.049**
HDL-cholesterol (mg/dl)	−0.24	0.22
LDL-cholesterol (mg/dl)	0.24	**0.01**
Triglycerides (mg/dl)[a]	10.56	**0.049**
Estradiol (pg/ml)[a]	−0.02	0.95
Testosterone (ng/ml)[a]	−0.18	0.90
Progesterone (ng/ml)[a]	9.57	0.63
Alcohol intake (yes)	−10.51	0.10
Current smoking (yes)	−0.04	0.99
Diabetes mellitus (yes)	9.65	0.31
Hypertension (yes)	0.54	0.92
Dyslipidemia (yes)	5.18	0.48
MetS (yes)	17.68	**0.002**

Abbreviations: HOMA-IR, homeostasis model assessment-insulin resistance; eGFR, estimated glomerular filtration rate; MetS, metabolic syndrome.
The bold of p values was defined as $p < 0.05$.
[a] These variables are shown in the original scale after analysis using log (natural)-transformed values.

Table 3b – Univariate analyses with fetuin-A in females.

Characteristics	β	p
Age (years)	−0.44	0.11
Body mass index (kg/m^2)	−0.10	0.90
Waist (cm)	0.008	0.97
Systolic blood pressure (mmHg)	0.10	0.46
Diastolic blood pressure (mmHg)	0.16	0.55
Fasting plasma glucose (mg/dl)	0.20	0.57
HbA_{1c} (NGSP) (%)	2.29	0.77
Insulin (μU/ml)[a]	9.08	0.07
HOMA-IR[a]	8.00	0.08
eGFR (ml/min/1.73 m^2)	−0.15	0.42
Uric acid (mg/dl)	−4.68	**0.005**
γ-Glutamyl transpeptidase (IU/l)	0.27	0.06
Alanine aminotransferase (IU/l)	0.19	0.24
Aspartate aminotransferase (IU/l)	0.23	0.12
Total cholesterol (mg/dl)	0.07	0.42
HDL-cholesterol (mg/dl)	0.07	0.75
LDL-cholesterol (mg/dl)	0.03	0.71
Triglycerides (mg/dl)[a]	10.63	0.12
Estradiol (pg/ml)[a]	−0.004	0.95
Testosterone (ng/ml)[a]	−5.67	0.83
Progesterone (ng/ml)[a]	2.25	0.13
Alcohol intake (yes)	−10.73	0.12
Current smoking (yes)	3.99	0.77
Diabetes mellitus (yes)	2.97	0.81
Hypertension (yes)	4.22	0.47
Dyslipidemia (yes)	1.57	0.81
MetS (yes)	7.72	0.29

Abbreviations: HOMA-IR, homeostasis model assessment-insulin resistance; eGFR, estimated glomerular filtration rate; MetS, metabolic syndrome.
The bold of p value was defined as $p < 0.05$.
[a] These variables are shown in the original scale after analysis using log (natural)-transformed values.

4. Discussion

In this cross-sectional cohort study in a Japanese general population, multiple stepwise regression analysis revealed that plasma fetuin-A level was significantly and independently associated with MetS and LDL-c in males. The difference in fetuin-A levels was 18.2 μg/ml between the males with (260.1 μg/ml) and without (241.9 μg/ml) MetS. The 13.9 μg/ml increase in fetuin-A corresponded to each 32.5 mg/dl increase in LDL-c. Our data not only indicate statistical significance but also may suggest clinical implication of elevated fetuin-A in atherogenesis.

4.1. *Fetuin-A and MetS*

Our results are consistent with previous reports from other countries demonstrating a close association of plasma fetuin-A level with MetS [8,12,16,25]. From Japan, only two stdies have been reported [33,34]. In 2006, Mori et al. [33] reported for the first time the association of fetuin-A with insulin resistance (HOMA-IR) as well as BMI and triglycerides in a small number of non-diabetic subjects but not in diabetic subjects, and Ishibashi A, et al. [34], demonstrated that fetuin-A was associated with insulin resistance in Japanese men without apparent cardiovascular diseases. The latter did not examine female subjects. Here, we report the association of fetuin-A with not only insulin resistance but also MetS in a male but not female general population.

There may be a sex-specific association of fetuin-A with MetS [15]. Ishibashi et al. [34] reported the association of fetuin-A with insulin resistance in men and Laughlin et al. [27] reported the association of fetuin-A with type 2 diabetes only in females. As apparent from Table 1, there was a significant inverse relationship between fetuin-A levels and the prevalence of men. Accordingly, we performed analysis stratified by gender. A positive association of fetuin-A with MetS was found in males, but not in females. It is considered that men without MetS may have lower ($p < 0.001$) fetuin-A levels compared to women without MetS, due to the strong impact of BMI in all men but not in women. Thus, we reclassified the subjects into men with and without MetS and into women with and without MetS. Consequently, the

Table 4a – Multiple stepwise regression analysis for the correlates of fetuin-A in males.

Characteristics	β	p
MetS (yes)	17.68	0.002
LDL-cholesterol	0.20	0.02

Abbreviation: MetS, metabolic syndrome $R^2 = 0.176$.

Table 4b – Multiple logistic regression analysis of fetuin-A (quartile 1 vs. quartile 4) in males.

Dependent variable (increment)	Odds ratio	95% C.I.	*p*
MetS (yes)	1.009	1.003–1.015	0.002
LDL-cholesterol (32.5 mg/dl)[a]	1.376	1.027–1.844	0.032

Abbreviation: C.I., confidence interval.
[a] Odds ratio per 1-increment increase in variable.

fetuin-A levels in men without MetS (241.9 ± 41.4 μg/mL) were significantly lower ($p < 0.001$) than those in women without MetS (263.9 ± 57.3 μg/mL).

In order to investigate the underlying mechanisms for the sex difference, we measured several sex hormones. As shown in tables, sex hormones did not explain the sex difference. As fetuin-A knockout mice exhibit not only insulin resistance but also resistance to weight gain on a high fat diet, it is suggested fetuin-A may regulate adipogenesis [5]. In fact, there have been many reports indicating the association of fetuin-A with BMI in humans [8,12,16,18,20,23,26,27]. However, in our study, fetuin-A was not associated with BMI in females, probably due to the small number of obese females ($n = 113$). Thus, the negative association of fetuin-A with MetS in females may be ascribed to the absence of obesity, i.e., MetS prevalence (43.1% in males vs. 17.7% in females).

It may be interesting to refer reports from Asia other than Japan where the incidence of coronary artery disease is less than in Western countries [35]. Two reports from China [12,13] and one report from Korea [14] indicated the association of fetuin-A with insulin resistance and/or MetS. Thus, the association of fetuin-A with insulin resistance and/or MetS is rather universal and may not be influenced by genetics or life style. From this point of view, fetuin-A may be an interesting molecule. Previously, it was demonstrated that fetuin-A levels were associated with not only insulin resistance but also diabetes mellitus [12–14,17,18,21,22,36]. Although the number of diabetic subjects was small, we examined whether there was a difference of fetuin-A levels between non-diabetics and diabetics. The fetuin-A levels were 257.2 ± 52.2 μg/ml ($n = 603$), and 262.1 ± 53.4 μg/ml ($n = 56$) in non-diabetics and diabetics, respectively. Thus, fetuin-A levels were not elevated in diabetics. Our findings are consistent with some studies [10,14,20,25,33] but not with others [12,13,15,17–19,21–23,26,27,34]. Although we do not know why reported values of fetuin-A in diabetics were various, diabetes is a complicated condition in which many inflammatory and angiogenic factors are activated [17,19,37–40] that may influence fetuin-A levels.

Two reports [41,42] have shown that moderate alcohol consumption decrease plasma fetuin-A. Thus, alcohol intake is one of an important factor for fetuin-A levels. As apparent from Table 1, our data indicated that higher alcohol consumption was associated with lower plasma fetuin-A. It is unknown why alcohol consumption is associated with lower fetuin-A levels.

4.2. *Fetuin-A and LDL-c*

In this study, we found in males a positive association of fetuin-A with atherogenic profile (cholesterol and triglycerides). Because fetuin-A inhibits insulin actions, which lead to lipolysis and efflux of free fatty acids from adipose tissue, the association of fetuin-A with triglycerides may be understandable. Not only multiple stepwise regression analysis revealed a significant association of fetuin-A with LDL-c but also multiple logistic regression analysis revealed the 13.9 μg/ml increase in fetuin-A per each 32.5 mg/dl increase in LDL-c. The association of fetuin-A with cholesterol was not expected, however, a careful literature search revealed the positive association of fetuin-A with LDL-c in several reports [12–15,34]. Although mechanisms for the positive association of fetuin-A with LDL-c have not been clearly elucidated, some transcriptional factors that regulate cholesterol homeostasis such as sterol regulatory element binding protein may be involved in the regulation of hepatic synthesis of fetuin-A [43]. Whatever the mechanism are, elevations of fetuin-A were associated with MetS and hypercholesterolemia, which are strong risk factors for cerebro-cardiovascular diseases. In fact, many clinical studies reported a high incidence of cerebro-cardiovascular diseases in subjects with high fetuin-A levels [23–27].

4.3. *Limitations*

The present study has several limitations. First, this was a cross-sectional study and examined comparatively a small number of subjects. Thus, nothing conclusive for the association of fetuin-A with MetS is stated. Prospective studies with a large number of subjects are needed to investigate the role of fetuin-A in the development of MetS. Second, our population was relatively healthy, and most of them had BMI and HOMA-IR within normal limits. Moreover, the HOMA-IR is a weak estimate of insulin resistance. Third, since we did not have data for the prevalence of menopause and for sex hormone-binding globulin, we were not sure whether the sex difference for the association of fetuin-A levels with MetS was ascribed to sex hormones. Thus, it is necessary to investigate plasma fetuin-A levels in heterogeneous populations.

4.4. *Conclusions*

In conclusion, our data indicated that human circulating fetuin-A was positively and independently associated with MetS and LDL-c in males in a general population in Japan. Plasma fetuin-A may play a role in atherosclerotic diseases.

Conflicts of interest

None declared.

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