RESEARCH ARTICLE

The Relationship of Fetuin-A, Adiponectin, Retinol Binding Protein-4 (RBP-4) and High Sensitivity C-Reactive Protein (hsCRP) with Insulin Resistance (HOMA-IR) in Obese Non Diabetic Men

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Abstract

BACKGROUND: Central obesity is the accumulation of visceral (intra-abdominal) fat and is strongly known to be associated with insulin resistance and type 2 diabetes mellitus (T2DM). Obesity can cause adipocyte hypertrophy that results in dysregulation of adipokine expression. The abnormal function of adipocytes may play an important role in the development of a chronic low-grade proinflammatory state associated with obesity. Adiponectin, retinol binding protein (RBP)-4 and fetuin-A play a role in the pathophysiology of insulin resistance. Expression of fetuin-A is increased due to fat accumulation in the liver. Elevated concentration of fetuin-A in the circulation can impair insulin signaling in muscle and liver as well as suppress adiponectin secretion, although its molecular mechanism is still unclear. The aim of this study was to identify the relationship of fetuin-A, adiponectin, RBP-4 and hsCRP with insulin resistance in obese non diabetic men.

METHODS: This was an observational study with a cross-sectional design. The study subjects were 64 men with non diabetic abdominal obesity, characterized by waist circumference of 98.47 ± 5.88 cm and fasting blood glucose of 85.75 ± 8.36 mg/dL.

RESULTS: This study showed that fetuin-A was positively correlated with HOMA-IR in obese non diabetic men with insulin resistance ($r = 0.128$; $p = 0.570$), although not significant. Fetuin-A was found to be correlated with adiponectin, RBP-4 and hsCRP ($r = 0.150$; $p = 0.233$; $r = 0.050$; $p = 0.711$; $r = -0.04$; $p = 0.445$), although not significant.

CONCLUSIONS: The concentration of fetuin-A showed a tendency to be positively correlated with HOMA-IR and with RBP-4 in obese non diabetic men, although statistically not significant. The concentration of fetuin-A showed a tendency to be negatively correlated with adiponectin and hsCRP although statistically not significant. There was no interrelationship between fetuin-A, adiponectin, RBP-4, hsCRP and HOMA-IR. Elevated concentrations of fetuin-A were noted in obese subjects, which in turn might impair insulin signaling. This finding might suggest that fetuin-A may represent a new target for the prevention of insulin resistance. Further studies might be needed on obese population with fatty liver.

KEYWORDS: Fetuin-A, Adiponectin, RBP-4, hsCRP, Insulin Resistance

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Introduction

Obesity can occur because there is imbalance between calorie intake and energy expenditure. This positive energy imbalance induces intracellular triglyceride stores resulting in hypertrophy and hyperplasia of adipocyte. Obesity also induce the dysregulation of adipokine secretion and expression from visceral adipose tissue, which can promote insulin resistance and low grade systemic inflammation (1)

Inflammatory markers such as C-reactive protein (CRP) and interleukin (IL)-6 are increased in obese individuals as compared with normal subjects. Adipose tissue with lipodistrophy affects the secretion of several adipokine including leptin, adiponectin, resistin, and tumor necrosis factor (TNF)-α (2).

Not all forms of obesity can cause metabolic syndrome. Several studies have shown that only visceral obesity or central obesity can increase the risk of metabolic syndrome (3). Visceral or central obesity is characterized by the accumulation of adipose tissue inside the abdominal cavity, associated with the incidence of insulin resistance that often leads to the development of T2DM (4).

Adiponectin is an essential adipokine that is produced abundantly in adipocytes. Adiponectin is also known to play an important role in insulin's mechanism of actio action where hypoadiponectinemia is associated with the risk of insulin resistance (5).

The other adipokine that also plays a role in causing insulin resistance is RBP-4. RBP-4 increases insulin resistance by inhibiting insulin signaling in muscle and by increasing hepatic glucose output (6). Central obesity can also cause increased fat accumulation in the liver. Expression of fetuin-A is increased when there is fat accumulation in the liver. Elevated circulating concentrations of fetuin-A cause impairment of insulin signaling in the muscle and liver, triggering inflammation in adipose tissue and suppressing adiponectin production; however, its molecular mechanism is unclear. Adiponectin and RBP-4 are interconnected in a state of obesity and insulin resistance, thus it is suggested that fetuin-A also influences RBP-4 (7).

The HOMA (Homeostasis Model Assessment) model is used to estimate insulin resistance from fasting plasma insulin and glucose concentrations. The equations widely used are: HOMA-IR = (FPI x FPG)/22.5 for insulin resistance (IR) when FPI is fasting plasma insulin concentration (mU/L) and FPG is fasting plasma glucose (mmol/L).

Methods

This was an observational study with a cross sectional design done on 64 obese non diabetic male volunteers. Data collection was carried out from January to March 2010. The study proposal was approved by the Health Research Ethics Committee of the Faculty of Medicine, University of Hasanuddin, Makassar, Indonesia with registry number 084/ H4.8.4.5.31/PP36-KOMETIK/2012.

SUBJECTS

The study subjects were recruited from clients coming to Prodia Clinical Laboratory, Malang, Indonesia, for general medical check-up as well as from employees and relatives of this Laboratory. Each subject was given explanation about the study and signed an informed consent, prior to the commencement of the study. Inclusion and exclusion criteria of the study subjects were as follows:

- **Inclusion criteria:** adult male subjects aged 30-60 years, equipped General Medical Check Up (GMC) panel test in the last 6 months, central obesity meeting the criteria of waist circumference ≥ 90 cm.

- **Exclusion criteria:** patients with diabetes (fasting blood sugar > 126 mg / dL), alcohol consumption during the past six months before the study, those diagnosed of having active hepatitis A, B, C , chronic liver disease, fatty liver, liver cirrhosis (based on the result of medical check up in the past six months before the study), patients with renal failure: GFR < 60 mL/ min, patients with fever and other acute inflammation (based on the result of hsCRP > 10 mg/L)

ASSAY OF BIOCHEMICAL MARKERS

Fetuin-A was measured by Enzyme Linked Immuno Assay (ELISA) kit from Bio Vendor Laboratory Medicine (RD 191037100). Adiponectin was measured by ELISA kit from Daichi Pure Chemical, and RBP-4 concentrations was measured by ELISA kit from Quantikine (DRB400). Fasting insulin and hsCRP were measured by Chemiluminescence Immuno Assay kit from DPC on the Immulite® (Dade-Behring, Los Angeles, CA, USA). All assays were performed according to the manufacturers' instructions at Prodia Clinical Laboratory.

DATA ANALYSIS

Statistical analyses were performed with the SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL, USA). Distributions of continuous variables were assessed for

normality using the Kolmogorov-Smirnov. Correlations between variables were analyzed using univariate and bivariate analysis. We also used regression analysis between variables. The results were narrated and explained by tables. For statistical tests, we used 5% significance.

Statistical analysis was done using SPSS software for Windows version 15.0 with significance level at $p < 0.05$. The bivariate correlation was assessed by Spearman's Rho test. Comparison of BMP-2 between groups were analyzed using Mann-Whitney U test. Non parametric partial correlation was tested using Spearman analysis.

Results

Initially 96 subjects were recruited in the study, eventually 64 subjects remained in the analysis and 32 subjects were excluded. Basic clinical and biochemical variables were shown in Table 1. Subjects characteristics were divided into two groups: normal subjects and subjects with insulin resistance based on HOMA-IR value cut off 2.0.

Results of the correlation analysis of waist circumference with HOMA-IR and fetuin-A, log adiponectin, RBP-4 and log hsCRP using Pearson correlation test are shown in Table 2. We found that waist circumference was positively correlated with RBP-4 concentration ($r = 0.340$ and $p = 0.007$), but not correlated with the concentrations of fetuin-A, log adiponectin and log hsCRP.

The correlations between HOMA-IR and the concentrations of fetuin-A, log adiponectin, RBP-4 and log hsCRP are shown in Table 3. Data on the variables for Adiponectin and hsCRP were not normally distributed, so we used the logarithmic data in the analysis.

Results of a further analysis on both groups with variable fetuin-A, adiponectin, RBP-4 and hsCRP using Spearman correlation test are shown in Table 4.

Table 1. Basic Clinical and Biochemical Variables

Variable	Normal (HOMA-IR* < 2.0)	Insulin Resistance (HOMA-IR* ≥ 2.0)	Total	p
N	42	22	64	
Age (year)	42.38 ± 7.39	48.86 ± 6.00	44.61 ± 7.56	0.001**
Height (cm)	168.60 ± 6.99	168.66 ± 6.50	168.53 ± 6.59	0.903
Weight (kg)	78.14 ± 8.61	84.05 ± 10.23	80.17 ± 9.54	0.018*
Waist circumference (cm)	96.90 ± 5.33	101.45 ± 5.83	98.47 ± 5.88	0.003**
Systolic blood pressure (mmHg)	118.50 ± 14.40	125.59 ± 20.79	120.94 ± 17.04	0.115
Diastolic blood pressure (mm Hg)	80.71 ± 9.41	82.86 ± 12.89	81.45 ± 10.68	0.449
Fasting glucose (mg/dL)	83.47 ± 7.51	90.09 ± 8.33	85.75 ± 8.36	0.002**
Fasting insulin (mU/L)	5.93 ± 1.97	13.90 ± 5.59	8,67 ± 5,24	0.000**
HOMA-IR	1.22 ± 0.41	3.09 ± 1.28	1.86 ± 1.21	-
Adiponectin (µg/mL)	2.89 ± 1.43	2.59 ± 1.21	2.79 ± 1.36	0.393
RBP-4 (µg/mL)	40.10 ± 11.68	38.34 ± 6.49	39.49 ± 10.18	0.513
Fetuin-A (µg/mL)	390.66 ± 110.12	484.91 ± 185.60	452.52 ± 168.81	0.033*
hsCRP (mg/L)	2.00 ± 2.05	2.08 ± 2.02	2.03 ± 2.03	0.883

* HOMA-IR = homeostasis model assessment insulin resistance; RBP-4 = retinol binding protein-4; hsCRP = high sensitive C-reactive protein.

** Significant at $p < 0.05$.

Table 2. Correlation of waist circumference with HOMA-IR and concentrations of fetuin-A, log adiponectin, RBP-4 and log hsCRP

Variable	Waist Circumference (cm)	
	r	p
HOMA-IR	0.240	0.052
Fetuin-A (µg/mL)	0.020	0.901
Log Adiponectin (µg/mL	-0.130	0.290
RBP-4 (µg/mL)	0.340	0.007**
Log hsCRP (mg/L)	0.200	0.111

** Significant at $p < 0.05$.

Table 3. Relationship of HOMA-IR with concentrations of fetuin-A, log Adiponectin, RBP-4 and log hsCRP

Variable	HOMA-IR		Fetuin-A		Log Adiponectin		RBP-4		Log hsCRP	
	r	p	r	p	r	p	r	p	r	p
HOMA-IR	1.000	-	-0.220	0.075	-0.090	0.466	-0.080	0.537	0.090	0.445
Fetuin-A	-0.220	0.075	1.000	-	0.150	0.233	0.050	0.711	-0.040	0.738
Log Adiponectin	-0.090	0.466	0.150	0.233	1.000	-	-0.030	0.846	-0.240	0.059
RBP-4	-0.080	0.537	0.050	0.711	-0.030	0.846	1.000	-	-0.130	0.315
Log hsCRP	0.090	0.445	-0.040	0.738	-0.240	0.059	-0.130	0.315	1.000	-

HOMA-IR = homeostasis model assessment of insulin resistance; RBP-4 = retinol binding protein-4; hsCRP = high sensitive C reactive protein.
Significant at $p < 0.05$.

Table 4. Correlation of HOMA-IR with fetuin-A, adiponectin, RBP-4 and hsCRP in both groups

Variable	HOMA-IR < 2		HOMA-IR ≥ 2	
	r	p	r	p
Fetuin-A	-0.019	0.453	0.128	0.570
Adiponectin	-0.167	0.290	0.107	0.636
RBP-4	0.328	0.034*	-0.202	0.368
hsCRP	-0.175	0.266	0.152	0.500

** Significant at $p < 0.05$.

For a further analysis we divided the subjects into groups of those with waist circumference ≤ 98 cm and those with waist circumference > 98 cm, and we used Spearman correlation test on other groups with other variables that we categorized based on median value of the variables fetuin-A (399.65 μg/mL), adiponectin (2.39 μg/mL), RBP-4 (37.89 μg/mL) and hsCRP (1.13 mg/L); the results are shown in Table 5.

For further observation, we divided the subjects into those with age ≤ 45 years and those with age > 45 years, and performed the correlation test between each group to the combined variables (low adiponectin ≤ 2.39 μg/mL, high RBP-4 >37.89 μg/mL and high fetuin A > 399.65 μg/mL). We found that in the non diabetic male subjects with age above 45 years and having low concentration adiponectin and high concentration RBP-4, the risk of insulin resistance increased to 7.84–13 times (Table 6).

Table 5. Correlation between waist circumference groups and variable groups

Variable groups	Waist Circumference groups ≤ 98 cm and > 98	
	r	p
HOMA-IR	0.310	0.013*
Fetuin-A	0.130	0.324
Adiponectin	-0.060	0.623
RBP-4	0.060	0.623
hsCRP	0.125	0.324

* Significant at p < 0.05.

Table 6. Estimates of risk insulin resistance among subjects by age groups and variables

Variable		p	r	OR	95%C.I	
					Lower	Upper
Low Adiponectin	Age ≤ 45 Age > 45	0.005*	0.456	7.840	1.650	37.400
High RBP-4	Age ≤ 45 Age > 45	0.008*	0.459	13.000	1.390	121.380
High fetuin-A	Age ≤ 45 Age > 45	0.109	0.289	4.200	0.690	25.260
High hsCRP	Age ≤ 45 Age > 45	0.171	0.248	3.110	0.620	15.710

* Significant at p < 0.05.

Discussion

This study was performed to determine the correlation of fetuin-A, adiponectin, RBP-4 and hsCRP with the incidence of insulin resistance due to central obesity in non-diabetic men. The subjects were limited to men to prevent the possibility of bias due to the influence of hormones (8).

The results of our study showed a weak correlation between waist circumference and the incidence of insulin resistance (HOMA-IR > 2.0) with r = 0.240 and p = 0.052. HOMA-IR tended to increase in the group of subjects with waist circumference > 98 cm (r = 0.310 and p = 0.013) compared with the group of subjects with waist circumference ≤ 98 cm.

A cross-sectional study performed by Wahrenberg on 2746 patients found that waist circumference correlated positively with HOMA-IR (9). In our study, a positive correlation between waist circumference and RBP-4 concentrations (r = 0.340 and p = 0.007) was found. This result was consistent with that of the study performed by Gavi, which indicated a correlation of RBP-4 with waist-to-hip ratio and central obesity (10).

In this study we also found that waist circumference was not correlated with adiponectin concentrations (r = -0.130 and p = 0.290), but the correlation was found in the subjects with waist circumference > 98 cm, although not significant. Similar results were found by Koska, where hypoadiponectinemia in patients with non-diabetes mellitus were associated with increased intra-hepatic fat and intra-myocellular, so it was not directly associated with waist circumference (11).

We didn't find a correlation between waist circumference and fetuin-A concentration (r = 0.020 and p = 0.901), but subjects with waist circumferences > 98 cm showed a tendency of increased fetuin A concentrations. Circulating fetuin-A correlated positively with liver fat in humans in cross sectional and longitudinal analysis and was not directly associated with waist circumference (12).

We didn't find a correlation between fetuin A and HOMA-IR both in normal and Insulin Resistance (IR) groups. But the trend showed a positive correlation between fetuin-A and HOMA-IR in the IR group. Concentrations of fetuin-A both in normal and IR groups were in the normal range for healthy adults (450–600 μg/mL) (13,14). We thought that Fetuin-A was associated with insulin resistance unaffected by status of glucose metabolism. Further studies are needed to explain more clearly the mechanisms between fetuin-A and insulin resistance.

By student T-test, adiponectin concentrations were found to be slightly decreased in subjects with IR (2.59 g/mL compared to 2.89 g/mL in normal group), but it did not correlate with HOMA-IR both in the normal group (r = -0.167 and p = 0.290) and IR group (r = 0.107 and p = 0.636). Adiponectin gene expression and circulating adiponectin concentrations are lower in patients with T2DM than in non diabetic individuals (15). In this study, our subjects was non diabetic men.

This study showed that fetuin-A did not correlate with adiponectin (r = 0.150 and p = 0.233) and RBP-4 (r = 0.050 and p = 0.711). This was not similar with the result of the study by Hennige, in which they found elevated circulating concentrations of fetuin-A triggering inflammation in adipose tissue and suppression of adiponectin production (7). The possible explanation of this finding is in this study healthy male subjects and patients with central obesity did not suffer from fatty liver disease and the concentrations of fetuin-A were still in the normal range.

RBP-4 concentrations in this study was in the range of 24.17 to 91.83 ug /mL and this did not correlate with HOMA-IR (r = -0.202 and p = 0.368). Shea's study did not find significant correlation between HOMA-IR categorical and concentrations of RBP-4 in the circulation (16). Pancreatic islet cells would make compensatory mechanism by enhancing insulin secretion in conditions of insulin resistance. Increased RBP-4 is estimated to occur in individuals who are not sensitive to the compensation mechanism. RBP-4 is also known to bind to transthyretin (Thyroxine transport protein/TTR). TTR was known to play a role in β-cell stimulation. Circulating RBP-4 was highly bound to TTR. RBP-4 may negatively affect ß-cell function directly or by preventing the binding of TTR to its receptor (17). In the study of Mills, RBP-4 was strongly associated with retinol and not associated with insulin resistance in fasted obese adults (18). Since RBP4 is the principal transport protein for retinol (vitamin A) in the circulation, it give possibility that alterations of retinol metabolism might influence RBP4 (19). At present, there are no compelling data to suggest that dietary vitamin A contributes to the elevation in serum RBP4 levels observed in group with HOMA-IR < 2.0

We found that fetuin-A were not significantly associated with hs-CRP in our study. Actually, the association of fetuin-A and hs-CRP was remained controversial. In human, high serum fetuin-A levels were found to be positively associated with metabolic syndrome and hsCRP. The other study indicated that endogenous fetuin-A can attenuate the inflammatory response (20). However, the association of fetuin-A and hs-CRP in the

pathogenesis of low-grade inflammation needs to be studied further.

In this study we also found that the group of obese non diabetic subjects with age > 45 years and low adiponectin and high RBP-4, showed increased relative risk to insulin resistance.

Conclusion

Fetuin-A concentrations showed a tendency to be correlated positively with HOMA-IR and with RBP-4 although statistically not significant. Fetuin-A concentrations showed a tendency to be correlated negatively with adiponectin and hsCRP, although statistically not significant. There was no interrelationship of fetuin-A, adiponectin, RBP-4 and hsCRP with HOMA-IR. We suggest that similar further studies be conducted on a greater number of the Indonesian population who are non diabetic but having severe obesity.

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