Original Article

Usefulness of C-Reactive Protein to High-Molecular-Weight Adiponectin Ratio to Predict Insulin Resistance and Metabolic Syndrome in Japanese Men

Yoshifumi Saisho[1], Hiroshi Hirose[1, 2], Yoshie Seino[1], Ikuo Saito[2], and Hiroshi Itoh[1]

[1]Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan
[2]Health Center, Keio University School of Medicine, Tokyo, Japan

***Aim*: We questioned whether the ratio of C-reactive protein to high-molecular-weight adiponectin (C/A ratio), compared to each value alone, is more useful to predict insulin resistance and/or metabolic syndrome.**
***Methods*: We measured serum CRP and HMW adiponectin levels in 841 Japanese men who had participated in an annual health checkup. Correlations of the C/A ratio with metabolic parameters were assessed, and its predictive values for insulin resistance and MetS were compared with CRP or HMW adiponectin alone.**
***Results*: The C/A ratio was higher in subjects with MetS ($n=114$) than in those without MetS (0.46 ± 0.67 vs. 0.23 ± 0.39, $p<0.0001$). The C/A ratio was correlated with a larger number of metabolic parameters than CRP, but the correlation was comparable to HMW adiponectin. Likewise, the area under the curve of the C/A ratio in receiver operator characteristic analysis for MetS was greater than that of CRP, but comparable to that of HMW adiponectin. However, the AUC of the C/A ratio in ROC analysis for insulin resistance (HOMA-IR >2.5) was greater than that of CRP or HMW adiponectin alone.**
***Conclusion*: While the C/A ratio provided little advantage to predict MetS, it might be more useful to predict insulin resistance than CRP or HMW adiponectin alone.**

J Atheroscler Thromb, 2010; 17:944-952.

Key words; C-reactive protein, HMW adiponectin, Metabolic syndrome, Insulin resistance

Introduction

Cardiovascular disease (CVD) and cancer are leading causes of death in developed countries. Since over 300,000 people die from CVD in Japan every year, it is important to detect individuals at a high risk for CVD to encourage prevention. While obesity, hypertension, hyperglycemia and dyslipidemia are known risk factors of CVD, studies have revealed that a cluster of these metabolic abnormalities is itself an important risk factor of CVD, which has now been recognized as metabolic syndrome (MetS)[1].

Insulin resistance, visceral obesity and chronic inflammation have been proposed to be the central pathogenesis of MetS[1]; therefore, various inflammatory markers have been reported to be indicators of MetS and CVD[2-4]. Among them, C-reactive protein (CRP) is one of the most widely used markers[2-4]. Increased CRP has been shown to be associated with obesity[5] and MetS[6]. Higher CRP levels have been shown to predict CVD[7-10].

Since visceral obesity is also a hallmark of MetS, adiponectin, an adipokine secreted from adipose tissue, has also been reported to be a marker of MetS[11]. Lower adiponectin levels have been shown to be associated with visceral obesity[12], insulin resistance[13] and type 2 diabetes[12, 14, 15]. Lower adiponectin levels also predict CVD[16]. Animal studies have shown that

Address for correspondence: Yoshifumi Saisho, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjukuku, Tokyo 160-8582, Japan
E-mail: saish@sc.itc.keio.ac.jp
Received: November 4, 2009
Accepted for publication: February 24, 2010

increased adiponectin prevents the development of atherosclerosis[17]. In particular, the high-molecular-weight (HMW) form of adiponectin has been proposed to be an active form[18-20]. We and other groups have shown that HMW adiponectin levels are more strongly correlated with MetS and CVD than total adiponectin levels[13, 21, 22]. Basu *et al*. have also shown that HMW, but not low-molecular-weight, adiponectin is selectively decreased in individuals with type 2 diabetes, suggesting that HMW adiponectin plays an important role in the development of CVD[23].

Although CRP and HMW adiponectin are thought to be independent risk factors of CVD, an additive effect of both markers to evaluate MetS remains to be established. Establishing a robust biomarker that can predict MetS instead of examining individual features will be important to screen populations of individuals, monitor the natural history of the disease, and measure the response to therapeutic interventions. We and others have shown a synergistic effect of CRP and HMW adiponectin to evaluate MetS in the general population[24] and type 2 diabetic subjects[25], suggesting a superior effect when both markers are combined. In contrast, Devaraj *et al*. have reported that CRP, HMW adiponectin and its ratio were comparable to predict MetS[26]. Therefore, in our large cohort of Japanese men, we examined whether the ratio of CRP to HMW adiponectin (C/A ratio) has any advantage to predict insulin resistance and/or MetS compared to each factor alone.

Methods

Subjects

This study included 841 Japanese male teachers and workers at Keio University aged 25 to 68 years (mean 48 ± 10 years, **Table 1**) who underwent an annual health checkup in 2007. Information regarding smoking, alcohol intake, physical activity and medication was obtained from questionnaires. Subjects with endocrine disease, such as Cushing syndrome, significant renal or hepatic disease, or inflammatory disease (or CRP > 10 mg/L) were excluded. Subjects receiving medication for dyslipidemia were also excluded. The present study was conducted according to the principles expressed in the Declaration of Helsinki. Informed consent was obtained from each subject after a full explanation of the purpose, nature and risk of all procedures used. The protocol was approved by the ethics review committees of the Health Center and the Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan.

Measurement

Systolic and diastolic blood pressures were measured in the sitting position after resting for at least 3 min. Blood samples were collected the morning after an overnight fast.

Plasma glucose and serum lipids were assayed by routine automated laboratory methods as described previously[13, 25, 27, 28]. Serum insulin concentration was measured by an enzyme immunoassay, using a commercially available kit (Tosoh, Tokyo, Japan), with intra- and interassay coefficients of 2.9 to 4.6% and 4.5 to 7.0%, respectively. The insulin resistance index was assessed by a homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated as fasting serum insulin (mU/L) × fasting plasma glucose (mmol/L)/22.5.

HMW adiponectin was measured using a commercially available kit (HMW adiponectin ELISA Kit; Fujirebio, Tokyo, Japan) as previously reported[13, 25, 27, 28]. This ELISA system does not need a denaturing step, and the monoclonal antibody (IH7) is reported to react specifically with the HMW form of adiponectin[29]. The dilution curve was parallel to the standard curve. Intra- and interassay coefficients were 2.4 to 3.0% and 4.2 to 5.1%, respectively. Serum CRP levels were measured by nephelometry, a latex particle-enhanced immunoassay (N Latex CRP Ⅱ; Dade Behring, Tokyo, Japan) with both intra- and interassay coefficients of < 5.0%.

Definition of Metabolic Syndrome (MetS)

MetS was defined according to the revised NCEP criteria[30] as 3 or more of 5 components in which the cut-off point of the waist circumference was modified for Japanese as ≥ 90 cm in men according to the recommendation by the International Diabetes Federation (IDF)[31]; the cut-off points of the other components were systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg for blood pressure, ≥ 150 mg/dL for triglycerides, < 40 mg/dL for HDL-cholesterol, and ≥ 100 mg/dL for fasting plasma glucose. Subjects receiving antihypertensive or hypoglycemic medication were considered to have the respective component. Japanese metabolic syndrome (JMetS) defined by the Examination Committee for Criteria of Metabolic Syndrome[32] was also examined. The criteria of JMetS is waist circumference ≥ 85 cm (for men) plus 2 or more of the following 3 components; systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg, triglycerides ≥ 150 mg/dL and/or HDL cholesterol < 40 mg/dL, and fasting plasma glucose ≥ 110 mg/dL.

Table 1. Characteristics of subjects

	Total	MetS		JMetS	
		(−)	(+)	(−)	(+)
N	841	727	114	746	95
Age (years)	48 ± 10	48 ± 10	51 ± 9***	48 ± 10	51 ± 9*
Height (m)	1.71 ± 0.06	1.70 ± 0.06	1.72 ± 0.06*	1.71 ± 0.06	1.72 ± 0.06
Weight (kg)	68.4 ± 9.9	66.8 ± 8.8	79.0 ± 10.5†	67.0 ± 8.9	79.6 ± 10.7†
BMI (kg/m^2)	23.5 ± 3.0	23.0 ± 2.7	26.8 ± 3.2†	23.0 ± 2.7	27.0 ± 3.3†
Waist circumference (cm)	83.3 ± 8.1	81.8 ± 7.2	92.6 ± 7.7†	82.0 ± 7.3	93.3 ± 7.4†
Systolic blood pressure (mmHg)	127 ± 18	125 ± 17	143 ± 17†	125 ± 17	145 ± 16†
Diastolic blood pressure (mmHg)	80 ± 12	78 ± 11	90 ± 11†	78 ± 12	91 ± 10†
Current smoking (%)	12.1	11.8	15.0	11.6	16.8
Alcohol intake (≥20g/day) (%)	25.3	23.9	34.2*	23.5	38.9**
No exercise (<150 min/week) (%)	59.8	59.5	61.6	59.2	64.2
Antihypertensives (%)	11.9	8.9	30.7†	9.5	30.5†
Oral hypoglycemic agents (%)	1.9	1.2	6.1†	1.3	6.3***
AST (IU/L)	24 ± 11	23 ± 7	32 ± 22†	23 ± 8	33 ± 23†
ALT (IU/L)	28 ± 21	25 ± 15	47 ± 39†	26 ± 16	47 ± 40†
γ-GTP (IU/L)	48 ± 49	42 ± 43	83 ± 67†	43 ± 43	85 ± 73†
ALP (IU/L)	208 ± 59	206 ± 59	222 ± 57**	206 ± 59	225 ± 60**
Glucose (mg/dL)	95 ± 17	92 ± 14	109 ± 28†	93 ± 13	111 ± 33†
Total cholesterol (mg/dL)	208 ± 31	206 ± 30	221 ± 30†	206 ± 31	220 ± 27†
LDL-cholesterol (mg/dL)	124 ± 29	123 ± 28	130 ± 31**	123 ± 28	128 ± 30
HDL-cholesterol (mg/dL)	59 ± 15	60 ± 14	48 ± 11†	60 ± 15	48 ± 11†
Triglyceride (mg/dL)	120 ± 85	104 ± 67	222 ± 112†	105 ± 67	236 ± 119†
Uric acid (mg/dL)	6.1 ± 1.2	6.1 ± 1.2	6.6 ± 1.3†	6.1 ± 1.2	6.6 ± 1.2†
Creatinine (mg/dL)	0.84 ± 0.12	0.84 ± 0.12	0.84 ± 0.13	0.84 ± 0.12	0.82 ± 0.13
Insulin (mU/L)	5.5 ± 4.9	5.1 ± 4.1	8.1 ± 8.0†	5.2 ± 4.1	8.1 ± 8.6†
HOMA-IR	1.31 ± 1.24	1.17 ± 1.02	2.16 ± 1.97†	1.20 ± 1.02	2.19 ± 2.13†
CRP (mg/L)	0.61 ± 0.79	0.58 ± 0.75	0.87 ± 0.98***	0.58 ± 0.75	0.93 ± 1.00†
HMW adiponectin (μg/mL)	3.84 ± 2.54	4.01 ± 2.59	2.69 ± 1.80†	4.00 ± 2.59	2.51 ± 1.59†
C/A ratio	0.26 ± 0.45	0.23 ± 0.39	0.46 ± 0.67†	0.23 ± 0.39	0.51 ± 0.72†

Values are the mean ± SD. MetS; metabolic syndrome by modified NCEP criteria[30, 31]. JMetS; metabolic syndrome by Japanese criteria[32].
*$p<0.05$, **$p<0.01$, ***$p<0.001$, †$p<0.0001$ vs. MetS(−) or JMetS(−).

Statistical Analysis

Comparisons between two groups were performed with Student *t*-tests. Relationships between two parameters were determined by simple regression analysis. Stepwise regression analysis was performed in a forward direction with *F* for the entry set to 4. Odds ratios were determined between first and third tertiles by logistic regression analysis. These statistical analyses were performed using the StatView program for Windows (version 5.0; SAS Institute Inc., Cary, NC, USA). Receiver operator characteristic (ROC) curves for insulin resistance and MetS were plotted and the area under the curve (AUC) of ROC curves was analyzed using the Statistical Package for the Social Sciences (version 17.0; SPSS, Chicago, IL, USA). Because AST, ALT, γ-GTP, HDL-cholesterol, triglyceride, insulin, HOMA-IR, CRP, HMW adiponectin and C/A ratio were normally distributed after logarithmic transformation, the logarithms of these parameters were used for analyses. All data are expressed as the mean ± S.D., and values of $p<0.05$ were considered significant.

Results

CRP, HMW Adiponectin and its Ratio in Subjects with or without MetS

Characteristics of the subjects are shown in **Table 1**. In all subjects, mean CRP and HMW adiponectin levels were 0.61 ± 0.79 mg/L and 3.84 ± 2.54 μg/mL,

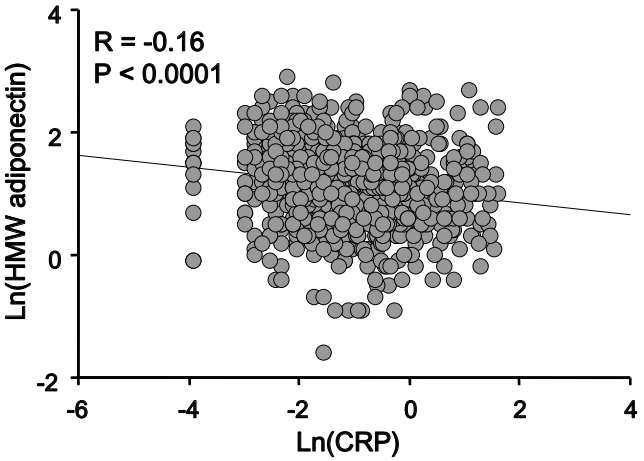

Fig. 1. Correlation between CRP and HMW adiponectin. There was a significant, but weak, correlation between CRP and HMW adiponectin.

respectively. A weak but significant negative correlation existed between CRP and HMW adiponectin ($r=-0.16$, $p<0.0001$, **Fig. 1**). Among them, 114 (13.6%) and 95 (11.3%) subjects were diagnosed with MetS and JMetS, respectively (**Table 1**). Subjects with MetS were more insulin resistant than those without MetS (HOMA-IR: 2.16±1.97 vs. 1.17±1.02, $p<0.0001$). CRP levels were significantly higher and HMW adiponectin levels were significantly lower in subjects with MetS than in those without MetS (CRP: 0.87±0.98 vs. 0.58±0.75 mg/L, $p<0.001$ and HMW adiponectin: 2.69±1.80 vs. 4.01±2.59 μg/mL, $p<0.0001$, respectively). As a result, the CRP to HMW adiponectin ratio (C/A ratio) was significantly higher in subjects with MetS than in those without MetS (0.46±0.67 vs. 0.23±0.39, $p<0.0001$). Similarly, the C/A ratio was also significantly higher in subjects with JMetS than in those without JMetS (0.51±0.72 vs. 0.23±0.39, $p<0.0001$, **Table 1**).

Correlations of CRP, HMW Adiponectin and its Ratio with Metabolic Parameters

As expected, both CRP and HMW adiponectin were significantly associated with various metabolic parameters, while HMW adiponectin appeared to correlate with a larger number of metabolic parameters, including blood pressure, compared to CRP (**Table 2**). The C/A ratio was also significantly correlated with various metabolic parameters and was comparable to HMW adiponectin: i.e., the C/A ratio was significantly positively correlated with age ($r=0.12$), BMI ($r=0.36$), waist circumference ($r=0.39$), systolic ($r=0.11$) and diastolic blood pressure ($r=0.15$), AST ($r=0.21$), ALT ($r=0.29$), γ-GTP ($r=0.31$), glucose ($r=0.19$), LDL-cholesterol ($r=0.14$), triglyceride ($r=0.32$), uric acid ($r=0.21$), insulin ($r=0.29$) and HOMA-IR ($r=0.31$), and was significantly negatively correlated with HDL-cholesterol ($r=-0.32$, **Table 2**).

ROC Analysis for Insulin Resistance and MetS

Stepwise regression analysis revealed that CRP and HMW adiponectin were independently associated with HOMA-IR after adjustment for age ($\beta=-0.23$, $F=46.8$ in HMW adiponectin, $\beta=0.19$, $F=33.5$ in CRP, $R^2=0.10$). CRP and HMW adiponectin were also independently associated with the number of MetS components after adjustment for age ($\beta=-0.28$, $F=77.2$ in HMW adiponectin, $\beta=0.13$, $F=15.6$ in CRP, $\beta=0.19$, $F=34.2$ in age, $R^2=0.16$).

To confirm the synergistic effects of HMW adiponectin on CRP, the subjects were then divided into tertiles based on CRP and HMW adiponectin levels, respectively. CRP levels of each tertile were as follows: lowest tertile: 0.21 mg/L or less, median tertile: 0.22 to 0.54 mg/L, highest tertile: 0.55 mg/L or more. HMW adiponectin levels of each tertile were as follows: lowest tertile: 2.4 μg/mL or less, median tertile: 2.5 to 4.2 μg/mL, highest tertile: 4.3 μg/mL or more. As a result, components of MetS and HOMA-IR were both highest in the highest CRP-lowest HMW adiponectin tertile group and lowest in the lowest CRP-highest HMW adiponectin tertile group (**Fig. 2**), while HMW adiponectin appeared to be a more dominant determinant for components of MetS than CRP (**Fig. 2A**). CRP and HMW adiponectin were independently associated with the both numbers of MetS components and HOMA-IR ($p<0.05$ by two-way ANOVA). The odds ratio (first tertile vs. third tertile) for insulin resistance (HOMA-IR >2.5) was higher for the C/A ratio (8.05, 95% CI; 3.11–20.85) than CRP (4.43, 95% CI; 2.09–9.38) or HMW adiponectin (3.34, 95% CI; 1.66–6.73) (**Table 3**); however, the odds ratio for MetS or JMetS in the C/A ratio (4.02 and 6.20, 95% CI; 2.31–7.01 and 3.17–12.11, respectively) was rather comparable to that in HMW adiponectin (3.70 and 5.39, 95% CI; 2.14–6.38 and 2.82–10.31, respectively) or CRP (2.18 and 2.36, 95% CI; 1.33–3.58 and 1.38–4.03, respectively) (**Table 3**).

Thus, to examine the superiority of the C/A ratio to CRP and HMW adiponectin alone, we performed a ROC analysis of CRP, HMW adiponectin and the C/A ratio to evaluate insulin resistance and MetS. The AUC of the C/A ratio for MetS (0.67, 95% CI; 0.62–0.72) was greater than that of CRP (0.61, 95% CI; 0.55–0.67), but comparable to that of HMW adiponectin (0.67, 95% CI; 0.62–0.72) (**Fig. 3A**). Similar

Table 2. Correlations with metabolic parameters

	CRP		HMW adiponectin		C/A ratio	
	r	*p*	*r*	*p*	*r*	*p*
Age	0.08	0.02	−0.13	0.0003	0.12	0.0003
BMI	0.22	<0.0001	−0.37	<0.0001	0.36	<0.0001
Waist circumference	0.27	<0.0001	−0.36	<0.0001	0.39	<0.0001
Systolic blood pressure	0.04	0.28	−0.17	<0.0001	0.11	0.001
Diastolic blood pressure	0.07	0.047	−0.19	<0.0001	0.15	<0.0001
AST	0.13	0.0003	−0.22	<0.0001	0.21	<0.0001
ALT	0.16	<0.0001	−0.33	<0.0001	0.29	<0.0001
γ-GTP	0.19	<0.0001	−0.32	<0.0001	0.31	<0.0001
ALP	0.11	0.001	−0.03	0.38	0.10	0.003
Glucose	0.12	0.0006	−0.19	<0.0001	0.19	<0.0001
Total cholesterol	0.02	0.63	−0.16	<0.0001	0.09	0.009
LDL-cholesterol	0.05	0.18	−0.22	<0.0001	0.14	<0.0001
HDL-cholesterol	−0.18	<0.0001	0.36	<0.0001	−0.32	<0.0001
Triglyceride	0.18	<0.0001	−0.37	<0.0001	0.32	<0.0001
Uric acid	0.14	<0.0001	−0.20	<0.0001	0.21	<0.0001
Creatinine	0.06	0.07	−0.07	0.04	0.09	0.01
Insulin	0.22	<0.0001	−0.23	<0.0001	0.29	<0.0001
HOMA-IR	0.23	<0.0001	−0.26	<0.0001	0.31	<0.0001

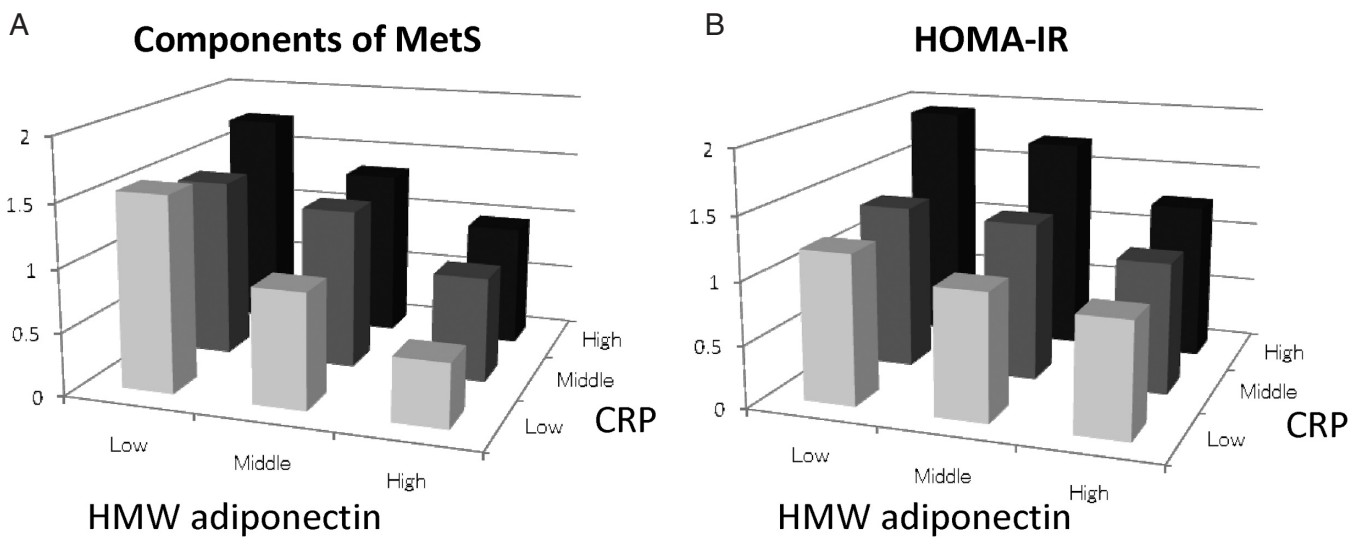

Fig. 2. Additive effects of HMW adiponectin on CRP. A) Number of MetS components and B) HOMA-IR. Components of MetS and HOMA-IR were both highest in the highest CRP-lowest HMW adiponectin tertile group and lowest in the lowest CRP-highest HMW adiponectin tertile group (A and B), while HMW adiponectin appears to be a more dominant determinant for components of MetS than CRP (A). CRP and HMW adiponectin were independently associated with both the number of MetS components and HOMA-IR ($p<0.05$ by two-way ANOVA).

results were obtained for JMetS (AUC of CRP: 0.63 (95% CI; 0.57–0.69), HMW adiponectin: 0.69 (95% CI; 0.64–0.75), C/A ratio: 0.70 (95% CI; 0.65–0.75)) (**Fig. 3B**). In contrast, although the 95% CI overlapped, the AUC of the C/A ratio for insulin resistance (0.71, 95% CI; 0.65–0.78) was greater than that of CRP (0.67, 95% CI; 0.60–0.74) or HMW adiponectin (0.67, 95% CI; 0.59–0.74) (**Fig. 3C**).

Table 3. Odds ratios for insulin resistance and MetS

	MetS	*p*	JMetS	*p*	Insulin resistance	*p*
CRP	2.18 (1.33–3.58)	0.002	2.36 (1.38–4.03)	0.002	4.43 (2.09–9.38)	0.0001
HMW adiponectin	3.70 (2.14–6.38)	<0.0001	5.39 (2.82–10.31)	<0.0001	3.34 (1.66–6.73)	0.0008
C/A ratio	4.02 (2.31–7.01)	<0.0001	6.20 (3.17–12.11)	<0.0001	8.05 (3.11–20.85)	<0.0001

Odds ratios were determined between first and third tertiles. ( ): 95% CI.
Insulin resistance was defined as HOMA-IR >2.5.

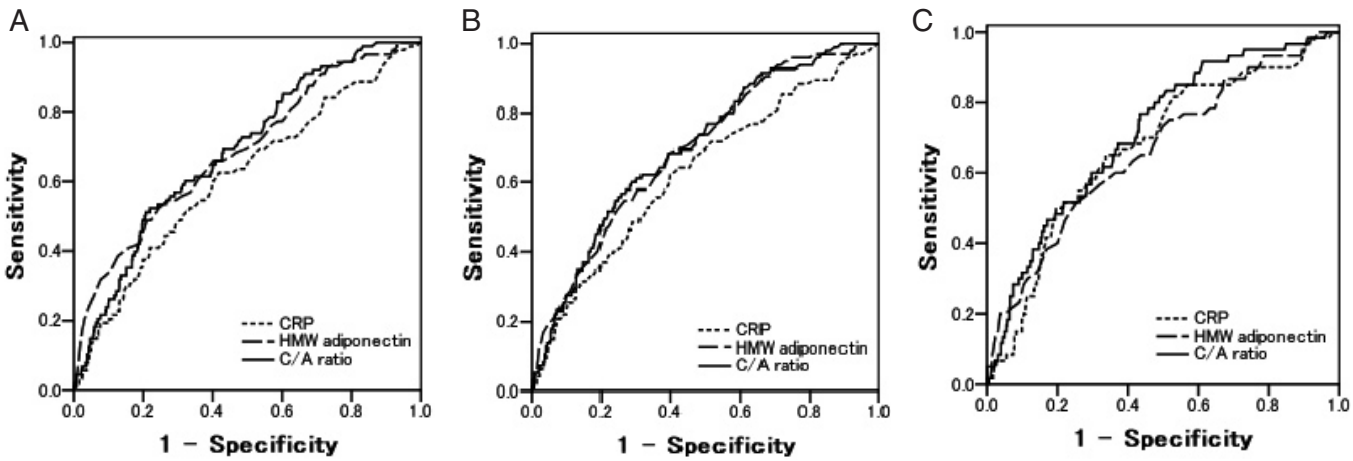

Fig. 3. The ROC curves of serum levels of CRP, HMW adiponectin and CRP to HMW adiponectin ratio (C/A ratio) for evaluation of MetS (A), JMetS (B) and insulin resistance (C). The AUC of the C/A ratio for evaluation of MetS (0.67, 95% CI; 0.62–0.72) was comparable to that of HMW adiponectin (0.67, 95% CI; 0.62–0.72), but greater than that of CRP (0.61, 95% CI; 0.55–0.67). Similarly, the AUC of the C/A ratio for evaluation of JMetS (0.70, 95% CI; 0.65–0.75) was comparable to that of HMW adiponectin (0.69, 95% CI; 0.64–0.75), but greater than that of CRP (0.63, 95% CI; 0.57–0.69). In contrast, the AUC of the C/A ratio for evaluation of insulin resistance (0.71, 95% CI; 0.65–0.78) was greater than that of CRP (0.67, 95% CI; 0.60–0.74) or HMW adiponectin (0.67, 95% CI; 0.59–0.74). The presence of insulin resistance was defined as HOMA-IR >2.5. Cut-off value, sensitivity and specificity were as follows: A) CRP: 0.42 mg/L, 0.59 and 0.60, HMW adiponectin: 2.85 μg/mL, 0.64 and 0.40, C/A ratio: 0.12, 0.68 and 0.58, B) CRP: 0.41 mg/L, 0.62 and 0.60, HMW adiponectin: 2.85 μg/mL, 0.68 and 0.60, C/A ratio: 0.14, 0.68 and 0.61, C) CRP: 0.50 mg/L, 0.65 and 0.66, HMW adiponectin: 2.45 μg/mL, 0.57 and 0.68, C/A ratio: 0.11, 0.82 and 0.51.

Discussion

In this study we reported that CRP and HMW adiponectin, an active form of adiponectin, synergistically reflected metabolic abnormalities. The C/A ratio showed little advantage to predict MetS, while it better predicted insulin resistance than CRP or HMW adiponectin alone.

Both CRP[6-10] and adiponectin[11, 13, 16, 21, 22] have been shown to be predictors of MetS and CVD. A decrease in CRP levels has been shown to correlate with the prevention of CVD[7-10]. Decreased adiponectin has also been reported to associate with the onset of CVD[16]. Moreover, an inverse correlation between CRP and adiponectin has been shown in blood and adipose tissue[33], and direct suppression of CRP by adiponectin has been reported[34], which suggests a direct correlation between CRP and adiponectin. Recently, Tabara *et al*. have shown synergistic effects of CRP and HMW adiponectin on metabolic abnormalities in older Japanese subjects[24], which indicate the superiority of a combination of both markers. We have also reported the synergistic effects of both markers on metabolic abnormalities in Japanese type 2 diabetic subjects[25]; however, these studies did not measure waist circumference to evaluate MetS. In contrast, Devaraj *et al*. have reported a comparison among CRP, HMW adiponectin and its ratio in the evaluation of MetS in 123 subjects with MetS and 91 subjects without MetS[26]. They have reported that the measurement of CRP is equivalent to the measurement of HMW adiponectin and its ratio to CRP in predicting MetS[26]. The present study utilized a larger sample size coupled with the measurement of waist circumference

to confirm that the C/A ratio is comparable to HMW adiponectin as a predictor of MetS. While both CRP and HMW adiponectin predict MetS, these results indicate that the additive effect of both markers for the prediction of MetS is minimal.

Nonetheless, our study showed that the C/A ratio reflects more insulin-resistant subjects than CRP or HMW adiponectin alone. In fact, recent studies have indicated different characteristics between CRP and MetS[35-37]. It has been reported that CRP predicted the development of CVD independently of MetS[38]; therefore, the addition of CRP to the criteria of MetS has been proposed[39]. In the present study, stepwise regression analysis also revealed that CRP and HMW adiponectin are independently associated with insulin resistance and the number of MetS components. It has been reported that CRP is more closely correlated with obesity itself (i.e., total fat) rather than visceral fat[35, 37], while HMW adiponectin is closely correlated with visceral fat[12]. Consistent with this, in the present study, the correlation between CRP and HMW adiponectin was significant, but weak. Moreover HMW adiponectin is more closely correlated with the number of MetS components or MetS itself than CRP, in line with the previous study[11]. Whereas insulin resistance, visceral obesity and chronic inflammation are all hallmarks of MetS, the precise mechanism of MetS remains to be elucidated[1]. It is possible that the combination of CRP and HMW adiponectin is more closely correlated with insulin resistance rather than MetS, as defined by the sum of each metabolic abnormality[30]. Indeed Meigs *et al*. have reported that metabolic syndrome without insulin resistance did not increase the risk for CVD[40]. Since insulin resistance itself is a strong predictor of the onset of CVD[41], the C/A ratio may have an advantage to detect subjects at risk for CVD compared to CRP or HMW adiponectin alone, possibly even better than MetS.

There are several limitations in this study. First, the cross-sectional study design; while no advantage was found for the C/A ratio to predict MetS, the possibility cannot be excluded. Likewise, while there was some advantage of the C/A ratio to predict insulin resistance, it remains unknown whether the C/A ratio has any advantage to predict the future development of CVD. Second, we recruited only Japanese male participants. Serum CRP or HMW adiponectin levels as well as adiposity differ between Asians and Caucasians and between genders; our results therefore may not be reflected in women or in other ethnicities. Also, since we collected participants from the general population, the sample size of MetS was relatively small. Moreover, the frequency of JMetS in our cohort was lower than that in the National Health and Nutrition Survey of Ministry of Health, Labour and Welfare, Japan in 2007 reporting 22.5% among adult men. This may be because our cohort consisted of men working at academic institutions, which might influence the ROC analysis for MetS.

In conclusion, while the C/A ratio provided little advantage to predict MetS, it might be more useful to predict the presence of insulin resistance than CRP or HMW adiponectin alone. It will be of interest to test whether the C/A ratio better predicts the development of CVD than CRP or HMW adiponectin alone. Further prospective studies will be warranted to clarify this question.

Acknowledgements

The authors thank Fujirebio Co. Inc. (Tokyo, Japan) for their technical assistance with the ELISA system for HMW-adiponectin. This study was supported in part by a Grant-in Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan (to H.H.), and by research grants from Keio University, Tokyo (to H.H.).

References

1) Kahn R, Buse J, Ferrannini E, Stern M: The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care, 2005; 28: 2289-2304
2) Athyros VG, Kakafika AI, Karagiannis A, Mikhailidis, DP: Do we need to consider inflammatory markers when we treat atherosclerotic disease? Atherosclerosis, 2008; 200: 1-12
3) Haffner SM: Abdominal adiposity and cardiometabolic risk: do we have all the answers? Am J Med, 2007; 120: S10-16; discussion S16-17
4) Corrado E, Rizzo M, Coppola G, Fattouch K, Novo G, Marturana I, Ferrara F, Novo S: An update on the role of markers of inflammation in atherosclerosis. J Atheroscler Thromb, 2010; 17: 1-11
5) Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB: Elevated C-reactive protein levels in overweight and obese adults. JAMA, 1999; 282: 2131-2135
6) Ford ES: The metabolic syndrome and C-reactive protein, fibrinogen, and leukocyte count: findings from the Third National Health and Nutrition Examination Survey. Atherosclerosis, 2003; 168: 351-358
7) Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA, Braunwald E: C-reactive protein levels and outcomes after statin therapy. N Engl J Med, 2005; 352: 20-28
8) Ridker PM, Hennekens CH, Buring JE, Rifai N: C-reactive protein and other markers of inflammation in the

prediction of cardiovascular disease in women. N Engl J Med, 2000; 342: 836-843
9) Ridker PM, Rifai N, Rose L, Buring JE, Cook NR: Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med, 2002; 347: 1557-1565
10) Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, Gallimore JR, Pepys MB: Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ, 2000; 321: 199-204
11) Matsushita K, Yatsuya H, Tamakoshi K, Wada K, Otsuka R, Takefuji S, Sugiura K, Kondo T, Murohara T, Toyoshima H: Comparison of circulating adiponectin and proinflammatory markers regarding their association with metabolic syndrome in Japanese men. Arterioscler Thromb Vasc Biol, 2006; 26: 871-876
12) Yatagai T, Nagasaka S, Taniguchi A, Fukushima M, Nakamura T, Kuroe A, Nakai Y, Ishibashi S: Hypoadiponectinemia is associated with visceral fat accumulation and insulin resistance in Japanese men with type 2 diabetes mellitus. Metabolism, 2003; 52: 1274-1278
13) Seino Y, Hirose H, Saito I, Itoh H: High molecular weight multimer form of adiponectin as a useful marker to evaluate insulin resistance and metabolic syndrome in Japanese men. Metabolism, 2007; 56: 1493-1499
14) Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J: Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet, 2002; 360: 57-58
15) Daimon M, Oizumi T, Saitoh T, Kameda W, Hirata A, Yamaguchi H, Ohnuma H, Igarashi M, Tominaga M, Kato T: Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese Population: the Funagata study. Diabetes Care, 2003; 26: 2015-2020
16) Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB: Plasma adiponectin levels and risk of myocardial infarction in men. JAMA, 2004; 291: 1730-1737
17) Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, Uchida S, Ito Y, Takakuwa K, Matsui J, Takata M, Eto K, Terauchi Y, Komeda K, Tsunoda M, Murakami K, Ohnishi Y, Naitoh T, Yamamura K, Ueyama Y, Froguel P, Kimura S, Nagai R, Kadowaki T: Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. J Biol Chem, 2003; 278: 2461-2468
18) Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T: Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem, 2003; 278: 40352-40363
19) Kobayashi H, Ouchi N, Kihara S, Walsh K, Kumada M, Abe Y, Funahashi T, Matsuzawa Y: Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. Circ Res, 2004; 94: e27-31
20) Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, Funahashi T, Walsh K: Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. J Biol Chem, 2004; 279: 1304-1309
21) Hara K, Horikoshi M, Yamauchi T, Yago H, Miyazaki O, Ebinuma H, Imai Y, Nagai R, Kadowaki T: Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care, 2006; 29: 1357-1362
22) Aso Y, Yamamoto R, Wakabayashi S, Uchida T, Takayanagi K, Takebayashi K, Okuno T, Inoue T, Node K, Tobe T, Inukai T, Nakano Y: Comparison of serum high-molecular weight (HMW) adiponectin with total adiponectin concentrations in type 2 diabetic patients with coronary artery disease using a novel enzyme-linked immunosorbent assay to detect HMW adiponectin. Diabetes, 2006; 55: 1954-1960
23) Basu R, Pajvani UB, Rizza RA, Scherer PE: Selective downregulation of the high molecular weight form of adiponectin in hyperinsulinemia and in type 2 diabetes: differential regulation from nondiabetic subjects. Diabetes, 2007; 56: 2174-2177
24) Tabara Y, Osawa H, Kawamoto R, Tachibana-Iimori R, Yamamoto M, Nakura J, Miki T, Makino H, Kohara K: Reduced high-molecular-weight adiponectin and elevated high-sensitivity C-reactive protein are synergistic risk factors for metabolic syndrome in a large-scale middle-aged to elderly population: the Shimanami Health Promoting Program Study. J Clin Endocrinol Metab, 2008; 93: 715-722
25) Saisho Y, Hirose H, Yamamoto Y, Nakatani H, Itoh H: Combination of C-reactive protein and high molecular weight (HMW)-adiponectin reflects further metabolic abnormalities compared with each of them alone in Japanese type 2 diabetic subjects. Endocr J, 2008; 55: 331-338
26) Devaraj S, Swarbrick MM, Singh U, Adams-Huet B, Havel PJ, Jialal I: CRP and adiponectin and its oligomers in the metabolic syndrome: evaluation of new laboratory-based biomarkers. Am J Clin Pathol, 2008; 129: 815-822
27) Seino Y, Hirose H, Saito I, Itoh H: High-molecular-weight adiponectin is a predictor of progression to metabolic syndrome: a population-based 6-year follow-up study in Japanese men. Metabolism, 2009; 58: 355-360
28) Komiya N, Hirose H, Kawabe H, Itoh H, Saito I: Effects of telmisartan therapy on metabolic profiles and serum high molecular weight (HMW)-adiponectin level in Japanese male hypertensive subjects with abdominal obesity. J Atheroscler Thromb, 2009; 16: 137-142
29) Nakano Y, Tajima S, Yoshimi A, Akiyama H, Tsushima M, Tanioka T, Negoro T, Tomita M, Tobe T: A novel enzyme-linked immunosorbent assay specific for high-molecular-weight adiponectin. J Lipid Res, 2006; 47: 1572-1582
30) Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F: Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation, 2005; 112: 2735-2752
31) Alberti KG, Zimmet P, Shaw J: Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med, 2006;

23: 469-480
32) The Examination Committee for Criteria of Metabolic Syndrome, Definition and criteria of metabolic syndrome. J Jpn Soc Intern Med, 2005; 94: 794-809 (in Japanese)
33) Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, Okamoto Y, Ohashi K, Nagaretani H, Kishida K, Nishizawa H, Maeda N, Kobayashi H, Hiraoka H, Matsuzawa Y: Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation, 2003; 107: 671-674
34) Devaraj S, Torok N, Dasu MR, Samols D, Jialal I: Adiponectin decreases C-reactive protein synthesis and secretion from endothelial cells: evidence for an adipose tissue-vascular loop. Arterioscler Thromb Vasc Biol, 2008; 28: 1368-1374
35) Kahn SE, Zinman B, Haffner SM, O'Neill MC, Kravitz BG, Yu D, Freed MI, Herman WH, Holman RR, Jones NP, Lachin JM, Viberti GC: Obesity is a major determinant of the association of C-reactive protein levels and the metabolic syndrome in type 2 diabetes. Diabetes, 2006; 55: 2357-2364
36) Timpson NJ, Lawlor DA, Harbord RM, Gaunt TR, Day IN, Palmer LJ, Hattersley AT, Ebrahim S, Lowe GD, Rumley A, Davey Smith G: C-reactive protein and its role in metabolic syndrome: mendelian randomisation study. Lancet, 2005; 366: 1954-1959
37) Aronson D, Bartha P, Zinder O, Kerner A, Markiewicz W, Avizohar O, Brook GJ, Levy Y: Obesity is the major determinant of elevated C-reactive protein in subjects with the metabolic syndrome. Int J Obes Relat Metab Disord, 2004; 28: 674-679
38) Ridker PM, Buring JE, Cook NR, Rifai N: C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation, 2003; 107: 391-397
39) Ridker PM, Wilson PW, Grundy SM: Should C-reactive protein be added to metabolic syndrome and to assessment of global cardiovascular risk? Circulation, 2004; 109: 2818-2825
40) Meigs JB, Rutter MK, Sullivan LM, Fox CS, D'Agostino RB Sr, Wilson PW: Impact of insulin resistance on risk of type 2 diabetes and cardiovascular disease in people with metabolic syndrome. Diabetes Care, 2007; 30: 1219-1225
41) Abbasi F, Brown BW Jr, Lamendola C, McLaughlin T, Reaven GM: Relationship between obesity, insulin resistance, and coronary heart disease risk. J Am Coll Cardiol, 2002; 40: 937-943