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Serum retinol-binding protein 4 correlates with obesity, insulin resistance, and dyslipidemia in HIV-infected subjects receiving highly active antiretroviral therapy

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Abstract

Highly active antiretroviral therapy (HAART) contributes to the development of metabolic complications including dyslipidemia, insulin resistance (IR), and lipodystrophy (LD). Recent studies reported that retinol-binding protein 4 (RBP4) is associated with IR, dyslipidemia, and obesity in non–HIV-infected populations. The aim of this study was to evaluate the associations between RBP4 and LD or metabolic abnormalities in HIV-infected subjects receiving HAART. We performed a cross-sectional study with 113 HIV-infected subjects receiving HAART for more than 6 months. Body composition and abdominal fat were measured by bioelectrical impedance analysis and ultrasonography, and fasting serum RBP4 was measured by enzyme-linked immunosorbent assay. Retinol-binding protein 4 levels in subjects with LD were similar to those without LD (P = .839). Retinol-binding protein 4 had significantly positive correlations with waist circumference (r = 0.298, P = .002), waist-to-hip ratio (r = 0.336, P = .001), body mass index (r = 0.310, P = .002), total body fat mass (r = 0.323, P = .001), total cholesterol (r = 0.188, P = .048), log (triglyceride) (r = 0.269, P = .004), and log (homeostasis model assessment of IR) (r = 0.207, P = .036), and negative correlations with quantitative insulin sensitivity check index (r = −0.209, P = .034) after adjustment for age and sex. In stepwise multivariate linear regression analysis, waist-to-hip ratio was the most significant independent predictor of increased RBP4 (standardized β = .351, P = .001). These results suggest that serum RBP4 is associated with obesity, IR, and dyslipidemia in HIV-infected subjects receiving HAART.
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1. Introduction

Highly active antiretroviral therapy (HAART) has significantly improved the survival rate and quality of life for human immunodeficiency virus type 1 (HIV-1)–infected subjects [1], but it contributes to the development of metabolic complications including dyslipidemia, insulin resistance (IR), and lipodystrophy (LD) [2]. These metabolic and morphologic abnormalities in HIV-infected subjects can result in a higher prevalence of metabolic syndrome and cardiovascular disease (CVD) [3,4]. Therefore, early detection of metabolic abnormalities may be necessary to reduce the risks of CVD in subjects receiving HAART.

Adipose tissue is considered an active endocrine organ that secretes adipocytokines such as adiponectin and leptin, impacting IR, type 2 diabetes mellitus (T2DM), obesity, and CVD [5]. Several studies reported the association of low circulating adiponectin and leptin levels with metabolic abnormalities such as LD, IR, and alteration of lipid profiles in HIV-infected subjects receiving HAART [6-8].

Retinol-binding protein 4 (RBP4) is a recently discovered adipocytokine that is secreted by adipocytes and liver. It specifically binds to retinol (vitamin A) and transports retinol in circulation [9]. Newer studies have reported that RBP4 is associated with IR [10,11], lipid metabolism [12], obesity [13], ectopic fat accumulation [14], and the components of the metabolic syndrome [11,12,15] in various populations. Schindler et al [16] first investigated the effect of HAART on

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plasma RBP4 levels in treatment-naive HIV-infected subjects, but it has not been well established whether serum RBP4 levels are associated with LD or various metabolic abnormalities in HIV-infected subjects receiving HAART.

In the present study, we aimed to evaluate the associations between serum RBP4 levels and IR, lipid profiles, obesity, or LD in subjects receiving HAART.

2. Methods

2.1. Subjects and study design

A cross-sectional study was performed with 113 HIV-infected Koreans at Severance Hospital, a 2000-bed, university-affiliated, tertiary care hospital in Seoul, Republic of Korea. We prospectively enrolled HIV-1–infected subjects who had been receiving HAART for at least 6 months. Patients were eligible if they never received peroxisome proliferator-activated receptor–γ agonists, statins, fibrates, hypoglycemic agents, or antihypertensive drugs. We excluded subjects who received antiobesity medications or corticosteroids; had any opportunistic infection or malignancy while undergoing current treatment; or had underlying conditions of chronic liver or renal disease according to the *International Classification of Diseases, 10th Revision* [17], history of T2DM, hypertension, or CVD. This study was approved by the Institutional Review Board of the Clinical Research Institute of Severance Hospital. Written informed consent was obtained from all participants.

2.2. Laboratory measurements

We obtained blood samples after a 12-hour overnight fast. All parameters, except RBP4, were immediately measured after sampling. For RBP4 analysis, blood samples were immediately centrifuged after collection and stored at −70°C until tested.

Plasma glucose, total cholesterol (Total-C), and triglyceride (TG) were determined using an enzymatic colorimetric assay (Hitachi, Tokyo, Japan). High-density lipoprotein cholesterol (HDL-C) was measured using lipoprotein electrophoresis (Hitachi), and low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula [18], except in patients with TG higher than 400 mg/dL. Fasting insulin was measured by radioimmunoassay kit (DAINABOT, Tokyo, Japan). Homeostasis model assessment of insulin resistance (HOMA-IR) as a marker for IR was calculated according to the following formula: [fasting glucose (in millimoles per liter) × fasting insulin (in microunits per milliliter)/22.5] [19]. Quantitative insulin sensitivity check index (QUICKI) as a marker for insulin sensitivity was calculated using the following formula: 1/[log (fasting insulin) (in microunits per milliliter) + log (fasting glucose) (in milligrams per deciliter)] [20].

Serum RBP4 was measured using the commercially available human RBP4 sandwich enzyme-linked immunosorbent assay (ELISA) kit (Immundiagnostik, Bensheim, Germany), and optical densities were measured at 450 nm (Molecular Devices V-MAX 220 VAC ELISA reader; Molecular Devices, Palo Alto, CA). The assay was conducted according to the manufacturer's instructions. Inter- and intraassay variations were 9.8% and 5%, respectively.

Plasma HIV-RNA was determined by standard real-time polymerase chain reaction with a lower detection limit of 40 copies per milliliter (COBAS AMPLICOR HIV-1 MONITOR, version 2.42; Roche, Basel, Switzerland), and peripheral blood CD4+ T-lymphocyte count was measured by FACScan flow cytometry (Beckman Coulter, Fullerton, CA). If HIV-RNA was undetectable, we expressed the value as 39 copies per milliliter.

2.3. Anthropometric or ultrasonographic assessment of body composition or abdominal fat and carotid intima-media thickness measurement

Systolic or diastolic blood pressure (BP) and waist circumference (WCr) were measured by physical examination. Waist circumference was taken with a nonelastic measuring tape placed directly on the skin at the midpoint between the lowest tip of the rib and upper tip of the iliac crest in the upright position.

Body composition was measured using direct segmental multifrequency bioelectrical impedance instrument with 8-point tactile electrodes (Inbody 4.0; Biospace, Seoul, Korea) [21,22]. Waist-to-hip ratio (WHR), body mass index (BMI), and total body fat mass were obtained by bioelectrical impedance analysis (BIA) [22].

Lipodystrophy was diagnosed based on the clinical definition of the US Division of AIDS table for adverse events (2004 version) by a single clinician, and all cases with severity grade of 1 or more were included [23]. *Mixed LD* was defined as when both lipoatrophy and abnormal fat accumulation were simultaneously present in the same patient [3].

Abdominal ultrasonography (US) was performed by a single specialist to evaluate abdominal fat thickness. Vertical scanning was performed along the abdominal median from the xiphoid process to the umbilicus to measure the maximal thickness of the preperitoneal fat at the anterior surface of the liver and minimal thickness of the abdominal subcutaneous fat [24]. We also measured the *maximal thickness of the abdominal subcutaneous fat* defined as the distance from the echogenic line between the skin and subcutaneous tissue to the external face of the rectus abdominis muscle and *intraabdominal fat distance* (IAD) defined as the distance from the linea alba or internal face of the rectus abdominis muscle to the anterior wall of the abdominal aorta by the same vertical scanning [25,26]. The abdominal wall fat index (AFI) was calculated as the ratio of the maximal thickness of the preperitoneal fat to the minimal thickness of the abdominal subcutaneous fat [24]. The abdominal *visceral*

to subcutaneous fat area ratio (VSR) was defined as the ratio of IAD to the maximal thickness of the abdominal subcutaneous fat [25,26].

Carotid US was performed by a single specialist to evaluate intima-media thickness (IMT). Bilateral common carotid arteries (CCA) were scanned obliquely from the

Table 1
Comparison of clinical and metabolic characteristics according to RBP4 quartiles

Variables	Total (N = 113)	Q1 (n = 28) <26.25 μg/mL	Q2 (n = 29) 26.25-30.90 μg/mL	Q3 (n = 28) 30.91-36.59 μg/mL	Q4 (n = 28) >36.59 μg/mL	*P* value
Age (y)	39.9 ± 10.6	38.4 ± 8.3	38.3 ± 9.3	42.5 ± 13.0	40.5 ± 10.9	.388[a]
Sex, male	108 (95.6)	26 (92.9)	28 (96.6)	27 (96.4)	27 (96.4)	.546[b]
Body composition parameters[c]						
WCr (cm)	82.7 ± 7.4	79.4 ± 6.6	82.2 ± 6.7	83.3 ± 6.9	86.0 ± 8.1	.011[a]
WHR	0.85 ± 0.05	0.83 ± 0.04	0.84 ± 0.04	0.86 ± 0.03	0.87 ± 0.06	.004[a]
BMI (kg/m^2)	23.1 ± 3.0	21.9 ± 2.6	22.6 ± 2.8	23.4 ± 2.2	24.4 ± 3.6	.014[a]
Total body fat mass (kg)	13.5 ± 5.7	11.79 ± 4.50	12.11 ± 4.25	13.92 ± 3.98	16.07 ± 7.98	.024[a]
Systolic BP (mm Hg)	131.0 ± 16.6	128.0 ± 17.5	127.8 ± 12.4	130.8 ± 12.8	137.3 ± 21.0	.123[a]
Diastolic BP (mm Hg)	78.1 ± 12.0	76.4 ± 12.2	74.5 ± 10.8	82.4 ± 10.9	78.9 ± 13.0	.092[a]
Fasting glucose (mmol/L)	5.27 (4.94-5.91)	5.16 (4.94-5.92)	5.27 (4.80-5.74)	5.24 (4.81-5.97)	5.38 (5.01-6.31)	.529[d]
Total-C (mmol/L)	4.74 ± 1.10	4.61 ± 1.08	4.64 ± 1.21	4.62 ± 1.16	5.11 ± 0.91	.242[a]
LDL-C (mmol/L)	2.06 ± 0.99	2.23 ± 0.67	2.00 ± 1.00	2.10 ± 1.01	1.90 ± 1.21	.646[a]
HDL-C (mmol/L)	1.24 ± 0.31	1.30 ± 0.36	1.23 ± 0.28	1.20 ± 0.26	1.24 ± 0.33	.691[a]
TG (mmol/L)	2.45 (1.66-4.40)	1.94 (1.21-2.84)	2.27 (1.67-4.64)	2.63 (1.64-3.72)	3.52 (2.20-5.57)	.011[d]
HOMA-IR	3.47 (1.69-7.48)	2.02 (1.42-4.70)	3.54 (1.36-9.10)	3.56 (2.15-8.45)	3.95 (2.62-7.59)	.130[d]
QUICKI	0.32 ± 0.05	0.339 ± 0.045	0.325 ± 0.052	0.319 ± 0.045	0.313 ± 0.040	.216[a]
Carotid IMT (mm)	0.545 (0.500-0.605)	0.553 (0.528-0.580)	0.515 (0.486-0.613)	0.558 (0.513-0.638)	0.540 (0.488-0.653)	.547[d]
Abdominal fat parameters[e]						
IAD (mm)	38.28 (30.26-53.23)	32.71 (26.66-48.23)	38.28 (28.13-56.13)	38.28 (32.65-54.35)	45.30 (30.42-54.16)	.234[d]
Abdominal wall fat index	1.22 (0.78-1.83)	1.02 (0.72-1.61)	1.27 (0.81-2.44)	1.06 (0.81-1.79)	1.44 (0.76-2.10)	.699[d]
VSR	2.96 (2.06-3.92)	3.23 (1.92-3.90)	3.59 (2.08-4.34)	2.51 (2.06-4.88)	2.67 (1.98-3.11)	.314[d]
LD, yes	33 (29.2)	8 (28.6)	9 (31.0)	7 (25.0)	9 (32.1)	.905[b]
Known duration of HIV infection (mo)	27.0 (13.0-51.5)	30.0 (16.8-48.8)	37.0 (17.0-58.0)	26.5 (12.3-53.3)	26.0 (13.0-54.8)	.874[d]
CD4+ T-lymphocyte count (/μL)	431.9 ± 191.0	390.3 ± 178.9	450.4 ± 203.4	489.7 ± 164.2	396.4 ± 206.0	.162[a]
Plasma HIV-RNA (copies/mL)	39.0 (39.0-39.0)	39.0 (39.0-214.5)	39.0 (39.0-43.1)	39.0 (39.0-39.0)	39.0 (39.0-45.8)	.383[d]
HAART regimen on RBP4 analysis						
NNRTI-based	37 (32.7)	8 (28.6)	6 (20.7)	14 (50.0)	9 (32.1)	.310[b]
PI-based	76 (67.3)	20 (71.4)	23 (79.3)	14 (50.0)	19 (67.9)	
Boosted PI	60 (53.1)	14 (50.0)	18 (62.1)	12 (42.9)	16 (57.1)	.240[b]
Unboosted PI	16 (14.2)	6 (21.4)	5 (17.2)	2 (7.1)	3 (10.7)	
Use of PI for >6 mo[f], yes	67 (59.3)	18 (64.3)	18 (62.1)	14 (50.0)	17 (60.7)	.584[b]
Use of d4T or ddI for >6 mo[f], yes	43 (38.1)	15 (53.6)	10 (34.5)	5 (17.9)	13 (46.4)	.358[b]
Total duration of HAART (mo)	20.0 (10.0-36.0)	23.5 (15.0-28.8)	19.0 (9.0-43.0)	14.0 (9.0-27.5)	23.0 (12.0-45.8)	.473[d]
Total duration of NNRTI-based HAART (mo)	13.7 ± 12.3	16.7 ± 14.1	16.5 ± 13.9	12.3 ± 10.4	11.6 ± 12.8	.639[a]
Total duration of PI-based HAART (mo)	14.0 (0.0-26.0)	16.0 (0.0-25.0)	17.0 (2.0-37.0)	8.5 (0.0-18.8)	18.0 (1.3-30.8)	.261[d]

Results are expressed as mean ± SD, median (interquartile range), or number (percentage). d4T indicates stavudine; ddI, didanosine.

[a] One-way ANOVA.

[b] *P* values by linear-by-linear association of χ^2 test.

[c] Measured by BIA.

[d] Kruskal-Wallis test.

[e] Measured by US.

[f] These variables mean continuous exposure history of PI or d4T or ddI for more than 6 months before RBP4 measurement.

anterior and posterior directions, and IMT was measured on the far wall of the bilateral CCA about 10 mm proximal to the bifurcation of the carotid artery [27]. The average distance between the inner echogenic line representing the luminal-intimal interface and the outer echogenic line representing the media-adventitia interface was calculated by automatic IMT measurement software (Intimascope; Media Cross, Tokyo, Japan) [27,28]. The mean of bilateral average CCA IMT was used as the carotid IMT in our analysis. For carotid and abdominal US, a high-resolution real-time B-mode US with a 10-MHz linear probe (LOGIQ 7; GE Medical Systems, Milwaukee, WI) was used.

2.4. Statistical analysis

All statistical analyses were performed using SPSS 13.0 software (SPSS, Chicago, IL). We performed 1-sample Kolmogorov-Smirnov test for all continuous variables to verify the normal distribution. Normally distributed data were expressed as the mean ± SD, whereas variables with a skewed distribution were represented as the median (interquartile range). Categorical variables were reported by number (percentage). Student *t* test and 1-way analysis of variance were used to compare the mean value of continuous variables normally distributed between independent groups. For variables with skewed distributions, we used Kruskal-Wallis test. To analyze the differences in nominal variables between groups, we performed χ^2 test. Pearson zero-order or partial correlation coefficient was used to analyze the univariate correlation between RBP4 and metabolic parameters. Fasting glucose, TG, HOMA-IR, IMT, IAD, AFI, VSR, known duration of HIV infection, and total duration of HAART or protease inhibitor (PI)–based HAART were log-transformed because of their skewed distributions in correlation analysis. To identify metabolic parameters most strongly associated with RBP4, multivariate stepwise linear regression analysis was performed with age, sex, and variables with *P* value of less than .10 in age- and sex-adjusted partial correlation analysis. In this model, a probability of F value of 0.05 or 0.10 was used as the cutoff point for entry or removal of successively entered variables, respectively. All *P* values were 2-tailed, and *P* less than .05 was considered to be statistically significant.

Table 2
Difference in serum RBP4 levels according to LD and regimens of HAART

	RBP4 (μg/mL)	*P* values
LD		
No (n = 80)	32.49 ± 9.65	.839[a]
Yes (n = 33)	32.09 ± 8.61	
Lipoatrophy (n = 14)	30.93 ± 6.87	.810[b]
Abnormal fat accumulation (n = 8)	33.04 ± 10.69	
Mixed type (n = 11)	32.89 ± 9.62	
Current HAART regimens on RBP4 analysis		
NNRTI-based (n = 37)	33.34 ± 10.42	.442[a]
PI-based (n = 76)	31.90 ± 8.77	
Boosted PI (n = 60)	32.33 ± 8.94	.413[a]
Unboosted PI (n = 16)	30.30 ± 8.16	
History of continuous PI exposure for >6 mo before RBP4 analysis		
No (n = 46)	32.82 ± 9.95	.672[a]
Yes (n = 67)	32.06 ± 8.93	
History of continuous d4T or ddI exposure for >6 mo before RBP4 analysis		
No (n = 70)	32.27 ± 8.15	.891[a]
Yes (n = 43)	32.54 ± 11.06	

Results are expressed as mean ± SD.
[a] Student *t* test.
[b] One-way ANOVA.

Table 3
Correlation between serum RBP4 levels and clinical or metabolic variables

	RBP4 (μg/mL)			
	Not adjusted		Age- and sex-adjusted	
	r	*P*	*r*	*P*
Age (y)	0.127	.181	–	–
WCr (cm)	0.308	.001	0.298	.002
WHR	0.349	<.001	0.336	.001
BMI (kg/m^2)	0.300	.003	0.310	.002
Total body fat mass (kg)	0.274	.006	0.323	.001
Systolic BP (mm Hg)	0.152	.121	0.133	.179
Diastolic BP (mm Hg)	0.060	.542	0.041	.678
Log (fasting glucose [mmol/L])[a]	0.119	.208	0.109	.256
Total-C (mmol/L)	0.191	.043	0.188	.048
LDL-C (mmol/L)	−0.148	.120	−0.144	.133
HDL-C (mmol/L)	0.011	.911	0.056	.561
Log (TG [mmol/L])[a]	0.303	.001	0.269	.004
Log (HOMA-IR)[a]	0.197	.043	0.207	.036
QUICKI	−0.201	.040	−0.209	.034
Log (carotid IMT [mm])[a]	0.003	.975	−0.044	.675
Log (IAD[b] [mm])[a]	0.243	.016	0.250	.013
Log (abdominal wall fat index[b])	0.103	.338	0.087	.428
Log (VSR[b])	−0.052	.611	−0.085	.412
Log (total duration of HAART [mo])[a]	0.057	.548	0.062	.521
Total duration of NNRTI-based HAART (mo)	−0.108	.448	−0.052	.719
Log (total duration of PI-based HAART [mo])[a]	0.018	.875	0.033	.772
Log (known duration of HIV infection [mo])[a]	0.034	.718	0.031	.746

r means the correlation coefficient.
[a] Logarithmic transformation was performed because of skewed distribution.
[b] Measured by US.

3. Results

3.1. Analysis according to RBP4 quartiles

The clinical and metabolic characteristics of all participants are included in Table 1. Waist circumference (*P* = .011), WHR (*P* = .004), BMI (*P* = .014), and total body fat mass (*P* = .024) were significantly increased with increasing

RBP4 quartiles. Subjects included in the higher RBP4 quartile had significantly higher TG ($P = .011$). Homeostasis model assessment of insulin resistance had a tendency to increase with higher RBP4 quartiles, and QUICKI had a tendency to decrease with higher RBP4 quartiles. However, the difference of these variables between quartiles did not reach statistical significance ($P = .130$ and .216, respectively). There were no significant differences in age, sex, BP, glucose, CD4+ T-lymphocyte count, HIV-RNA, known duration of HIV infection, current HAART regimens on RBP4 analysis, total duration of HAART or nonnucleoside analogue reverse transcriptase inhibitor (NNRTI)– or PI-based HAART, presence of LD, carotid IMT, and abdominal fat parameters between quartiles (Table 1).

3.2. Serum RBP4 levels according to LD and HAART

Retinol-binding protein 4 in subjects with LD was similar to those without LD ($P = .839$). In an analysis of groups according to LD type, there was no significant difference in RBP4. In addition, RBP4 was not significantly different between groups according to current HAART regimens on RBP4 measurement and continuous exposure history of PI or stavudine or didanosine for more than 6 months before RBP4 measurement (Table 2). The similarities of RBP4 between groups for LD and HAART were sustained after adjustment for age and sex without significant changes in P value (data were not shown).

3.3. Correlation between RBP4 and clinical or metabolic parameters

Retinol-binding protein 4 had significantly positive correlations with WCr ($r = 0.308$, $P = .001$), WHR ($r = 0.349$, $P < .001$), BMI ($r = 0.300$, $P = .003$), total body fat mass ($r = 0.274$, $P = .006$), Total-C ($r = 0.191$, $P = .043$), log (TG) ($r = 0.303$, $P = .001$), log (HOMA-IR) ($r = 0.197$, $P = .043$), and log (IAD) ($r = 0.243$, $P = .016$) without adjustment. In addition, RBP4 was significantly negatively correlated with QUICKI ($r = -0.201$, $P = .040$) without adjustment. After adjustment for age and sex, the significant correlations with RBP4 were sustained in all of the above variables. Age, BP, glucose, LDL-C, HDL-C, carotid IMT, AFI, VSR, total duration of HAART or NNRTI- or PI-based HAART, and known duration of HIV infection had no significant correlation with RBP4 with or without adjustment (Table 3).

In stepwise multivariate linear regression analysis, we identified WHR as the most significant independent predictor of increased RBP4 levels (standardized $\beta = .351$, $P = .001$) (Table 4).

Table 4
Stepwise multivariate linear regression analysis to identify variables with strongest correlation for serum RBP4 levels

Independent variables	Standardized regression coefficients	t	P values
WHR	0.351	3.57	.001
Excluded variables in model			
Age (y)	−0.009	−0.09	.932
Sex	−0.144	−1.47	.145
WCr (cm)	0.145	1.00	.320
BMI (kg/m^2)	0.007	0.04	.967
Total body fat mass (kg)	−0.036	−0.20	.840
Total-C (mmol/L)	0.049	0.48	.633
Log (TG [mmol/L])	0.173	1.62	.109
Log (HOMA-IR)	0.115	1.13	.261
QUICKI	−0.123	−1.22	.227
Log (IAD [mm])	0.134	1.18	.239

Dependent variables are RBP4 levels. Independent variables are age, sex, and variables with P value of less than .10 in age- and sex-adjusted partial correlation analysis. In this model, a probability of F value of 0.05 or 0.10 was used as the cutoff point for entry or removal of successively entered variables, respectively.

4. Discussion

We found a positive correlation between RBP4 and TG or Total-C, like the studies performed in non–HIV-infected individuals [10,11,13,29]. Our RBP4 levels were associated with IR as indicated by the positive correlation with HOMA-IR and negative association with QUICKI, which is consistent with results from previous studies in non–HIV-infected populations [11-15]. In addition, RBP4 had significant positive correlations with parameters reflecting abdominal visceral adiposity or central obesity such as WCr, WHR, and IAD and those reflecting total body fat or obesity such as BMI and total body fat mass, the known risk factors for IR and CVD [30]. However, we did not find a significant correlation in this first study that evaluated the association between RBP4 and carotid IMT. In our results, RBP4 correlated with Total-C, HOMA-IR, QUICKI, or IAD only on univariate analysis, but not on quartile analysis. These discrepancies might be due to the lower statistical power caused by a relatively small sample size because Total-C, HOMA-IR, and IAD had a tendency to increase and QUICKI had a tendency to decrease with higher RBP quartiles in general despite statistical insignificance.

Because most of our subjects had relatively low WCr and BMI, this group consisted of subjects with overall lean body compositions, although we did not restrict the enrollment of patients with high WCr or BMI. These characteristics of study participants may result from racial differences because this was conducted in Korea. However, the relatively increased median HOMA-IR [10,12,13] and normal median fasting glucose levels suggest that the rate of subjects with IR despite normoglycemia and nonobesity was high.

There are only 2 studies evaluating RBP4 in HIV-infected subjects [16,31]. Schindler et al [16] reported that HAART induces the increase of RBP4 in drug-naive subjects, and Haider et al [31] reported that rosiglitazone decreases RBP4. The report of Schindler et al described that RBP4 had a positive correlation with TG and Total-C, but no correlation with any parameter of glucose metabolism and body

composition in a small number of subjects without the detailed data [16]. To our knowledge, this is the first detailed report evaluating the relationship between serum RBP4 and various metabolic profiles including carotid IMT in subjects receiving HAART. Unlike the report of Schindler et al, the total duration of HAART was not associated with RBP4 in our study. However, the study of Schindler et al might be unable to universalize this association because they investigated only 14 treatment-naive subjects and did not determine whether the linear association is sustained for more than 1 year after HAART [16]. In addition, the effects of various regimens of HAART on different metabolic abnormalities and RBP4 should not be overlooked.

Serum RBP4 was recently found to be associated with visceral fat [13,32] and ectopic fat accumulation in non–HIV-infected humans [14], suggesting that there might be a link between LD and RBP4. However, in the present study, there were no significant differences in RBP4 between groups with and without LD or between groups according to LD type. Further study is warranted to establish whether a clinical and pathogenic link exists between RBP4 and LD in a larger cohort.

Protease inhibitor exposure may theoretically have an association with increased RBP4 because PI blocks glucose transporter 4 in vitro and the blockade of glucose transporter 4 seems to be an inducer of RBP4 secretion by adipocytes [11,33]. However, in this study, the exposure to PIs as well as NNRTIs or thymidine analogues was not associated with RBP4. Each nucleoside analogue reverse transcriptase inhibitor, PI, or NNRTI drug has remarkably various effects on lipid profiles, glucose metabolism, mitochondrial toxicity, IR, and parameters that can directly affect the secretion of adipocytokines such as fat redistribution and adipocyte growth, apoptosis, or differentiation [34-37]. Furthermore, PIs may decrease plasma retinol levels and directly elevate RBP4 or retinoic acid levels by altering retinoid signaling and metabolism or through an effect on cytochrome P450 [38]. However, the effects of PIs on retinoic acid synthesis also differ depending on drug type [38]. Therefore, specific regimens of HAART that are composed of combinations of more than 3 antiretroviral drugs may result in the development of various metabolic abnormalities or different RBP4 levels, as our results from crude cross-sectional analyses for antiretroviral drugs did not reveal the precise correlation between HAART and RBP4.

There were some limitations to this study. We used BIA and US for evaluating body composition and abdominal adiposity. However, previous reports revealed that the various parameters of body composition measured by BIA or US strongly correlated with those measured by dual-energy x-ray absorptiometry or computed tomography scan [26,39,40]. We could not concisely evaluate whether subjects had impaired glucose tolerance or T2DM because we did not perform a 2-hour oral glucose tolerance test. The limitation of HOMA-IR/QUICKI as a surrogate marker of IR/insulin sensitivity instead of the more correct euglycemic-hyperinsulinemic clamp also could not be overlooked. In addition, ELISA was used for measurement of RBP4. However, a previous study had reported a strong correlation between ELISA and Western blot [12], and the important findings of this study do not depend on absolute RBP4 concentrations. We did not compare RBP4 between subjects receiving HAART and drug-naive HIV-infected or HIV-negative individuals. Furthermore, because this was not a longitudinal but a cross-sectional study, we did not assess the changes in RBP4 and metabolic parameters during defined intervals after HAART initiation in the same subjects. Therefore, our results could not truly determine causal relationships between RBP4 and various metabolic abnormalities induced by HAART. Long-term follow-up of a larger sample set is needed to evaluate exactly whether RBP4 is associated with HAART duration or specific antiretroviral drugs and useful as a diagnostic marker for LD or a predictive factor for the development of metabolic complications, and to analyze the causality between RBP4 and the development of various metabolic abnormalities by a specific regimen of HAART.

The correlation of RBP4 with metabolic parameters may differ according to basic characteristics of the study population, which include race, age, ratio of sexes, and baseline degrees of obesity, central adiposity, LD, etc. To prove the roles of RBP4 as the pathogenesis or surrogate marker of metabolic abnormalities in subjects receiving HAART, further study should be performed in various study populations because our subjects were relatively young, truly lean, and predominantly male.

In conclusion, our study reveals that fasting serum RBP4 levels are associated with obesity, IR, and dyslipidemia in HIV-infected subjects receiving HAART. The measurement of RBP4 could allow us to collect valuable information on metabolic abnormalities in patients receiving HAART.

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All authors declare that there are no conflicts of interest associated with this manuscript.

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