

Abdominal Visceral Fat Reduction Is Associated with Favorable Changes of Serum Retinol Binding Protein-4 in Nondiabetic Subjects

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Abstract. The adipocytokine retinol binding protein-4 (RBP4) has recently been shown to link obesity and insulin resistance, although their relationship remains controversial in human studies. The influence of weight reduction with changes of fat distribution on serum RBP4 concentration in nondiabetics is also unknown. We assessed the effect of weight reduction (especially abdominal visceral fat loss) on serum RBP4 levels after a structured weight-reduction program. We conducted a prospective intervention study consisting of a 16-week weight reduction program, including lifestyle modification and adjuvant appetite suppressants. A total of 52 nondiabetic subjects aged 37.4 ± 11 years with a body mass index of 27.4 ± 4 kg/m² were included. Serum RBP4 concentrations with other metabolic parameters and abdominal adipose tissue areas as determined by computed tomography scan were measured both before and 16 weeks after the weight reduction program. Subjects had a 10.9% loss of body weight accompanied by a 25.5% decrease in serum RBP4 levels, with improved insulin sensitivity after the program. The changes in RBP4 levels were significantly correlated with the amounts of abdominal visceral fat loss ($r = 0.38$, $p < 0.01$) but were not associated with the amount of total body fat loss or abdominal subcutaneous fat loss. Weight reduction, especially the loss of abdominal visceral fat, lowers serum RBP4 concentrations in nondiabetic subjects. The relationship between individual changes in RBP4 and abdominal visceral fat indicated that RBP4 may be involved in the beneficial effect of visceral fat reduction on the improvement of insulin resistance and metabolic syndrome.

Key words: Retinol binding protein-4, Visceral fat, Fat distribution, Weight reduction, Nondiabetic

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RECENT studies have revealed that adipocytes secrete a number of bioactive adipocytokines known to exert a wide variety of effects on glucose and lipid metabolism, energy homeostasis and cardiovascular function [1]. Obesity, especially visceral fat accumulation, alters adipocytokine secretion profiles, and obesity-related disorders are now recognized as a state of adipose tissue dysfunction [2]. Alteration in the secretion profile of adipocytokines plays a key role in the

pathogenesis of metabolic syndrome and its accompanying cardiovascular complications [1].

Retinol binding protein-4 (RBP4) is considered to be the primary carrier of retinol to tissues [3], and the urinary excretion of RBP4 is recommended as a useful marker for the detection of minor changes in renal dysfunction [4]. Recently RBP4 has been reported to be secreted from adipocytes independently from hepatic synthesis, and it provides a possible link between the expression of adipose-glucose transporter 4 (GLUT4) in adipocytes and insulin resistance [5]. RBP4 expression is increased in the adipose tissue of GLUT4 knockout mice, and the serum levels of RBP4 are elevated in insulin-resistant mice and in obese and type-2 diabetic subjects [6]. RBP4 concentrations were also correlated with lower insulin sensitivity, body mass in-

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dex (BMI), waist circumference, lipid profiles, and other components of metabolic syndrome [7]. However, despite the impressive results obtained in animal studies, there is considerable debate concerning the applicability in human studies. Furthermore, a lack of association between high RBP4 levels and obesity and insulin resistance has also been reported [8–11]. In a recent study, circulating RBP4 concentrations were similar in both lean and obese women [11], and neither abdominal subcutaneous RBP4 mRNA expression nor circulating RBP4 levels showed any correlation with the BMI [12]. This observation suggests a specific association between serum RBP4 levels and fat distribution rather than with a person's simple weight. In addition, weight changes exert major influences on circulating adipocytokine concentrations [13].

However, it is currently unclear whether RBP4 may exert a regulatory role of obesity related insulin resistance in weight reduction. One study reported that a five-percent weight loss improved the homeostasis model assessment (HOMA) index, even though there were no significant changes in RBP4 serum levels [11]. In contrast, severe calorie restriction reduced the levels of RBP4 in another study, but no relationship was observed between RBP4 and an improvement of insulin sensitivity [8].

Most of the previous studies did not use abdominal computed tomography (CT) or magnetic resonance imaging (MRI) to assess the patients' exact fat distribution, and very few studies have examined the effect of modest weight loss on serum RBP4 levels.

Therefore, the aim of this study was to assess the effect of modest weight reduction on serum RBP4 changes and their association with visceral adiposity, insulin resistance and metabolic parameters, using the accurate measurements of fat distribution and a structuralized weight reduction program.

Materials and methods

Subjects

The study was approved by the Yongdong-Severance Hospital and Ethics Committee, and written informed consent was obtained from all patients. Fifty-two subjects (40 females and 12 males) aged 37.4 ± 11.6 years with an average BMI of 23 kg/m^2 or greater participated in this study. All subjects were apparently healthy,

nonsmokers and low alcohol consumers (less than two drinks per week). None of the subjects had a past history of cardiovascular disease, diabetes, moderate to severe hypertension (resting blood pressure $>170/100 \text{ mmHg}$), renal impairment (serum creatinine $>120 \text{ }\mu\text{mol/L}$) or overt proteinuria by dipstick examination, obesity caused by an endocrine disorder, psychiatric disorders, a body weight fluctuation of more than 5 kg in the previous six months, or were taking any kind of medication.

Weight reduction program

The weight reduction program consisted of lifestyle modification and adjuvant pharmacotherapy using an appetite suppressant (10–15 mg of sibutramine). The subjects visited an obesity clinic twice per month and restricted their caloric intake to less than their usual intake by 600 kcal/day. Subjects were free to select the foods and beverages consumed, and food diaries were recorded at least five times per week during the 16-week study period. The physician and dietitians interviewed the subjects and re-evaluated their total energy and nutrient intake using the food records and also recommended proper nutrition. All patients were encouraged to achieve the goal of five hours of aerobic exercise (such as brisk walking, light jogging or stationary ergometer usage) per week. They were also administered 10–15 mg of sibutramine for a 16-week-period. The initial dose of 10 mg sibutramine was increased to 15 mg by the treating physician in patients experiencing an inadequate response (defined as $<2 \text{ kg}$ weight loss after four weeks of treatment).

Anthropometric measurements and computed tomography (CT)

Anthropometric measurements were taken with subjects in lightweight clothing and without shoes. Both their height to the nearest 0.1 cm and their weight to the nearest 0.1 kg were measured by an automatic height-weight scale. Percent body fat was measured by a total body DXA scan (Hologic QDR 1500; Delphi, Boston, MA, USA). Waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest. The abdominal adipose tissue area was quantified by CT (Tomoscan 350; Philips, Mahway, NJ, USA). With the subject in a supine position, a 10-mm CT slice scan was acquired

at the L4–L5 level to measure the total abdominal and visceral fat area. The visceral fat area was quantified by delineating the intra-abdominal cavity at the internal aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body. The subcutaneous fat area was calculated by subtracting the visceral fat area from the total abdominal fat area. Skeletal muscle attenuation was determined by measuring the mean value of all pixels within the range of 0 to 100 Hounsfield units (HU); fat areas fell in the range of –150 to –50 HU. To reduce variations in measurements, the same person measured both the anthropometric parameters and the CT scans throughout the study.

Metabolic parameters

At the beginning and at the end (16 weeks) of the study, each subject had serum samples taken after an overnight fast. Fasting serum glucose, total cholesterol, triglyceride and HDL-cholesterol levels were measured by enzymatic procedures using an autoanalyzer (Bayer, Terrytown, NY, USA). The LDL-cholesterol was calculated from the Friedewald equation if the serum triglyceride level was below 400 mg/dl [14]. Fasting insulin was measured by chemiluminescence immunoassay (Roche, Indianapolis, IN, USA). Insulin resistance was estimated by the homeostasis model assessment of the insulin resistance (HOMA-IR) index (fasting insulin [U/mL] $\times$ fasting glucose [mmol/L])/22.5). Serum RBP4 levels were measured using an enzyme-linked immunosorbent assay (Imunodiagnostic AG, Bensheim Germany), and the inter-assay and intra-assay variations were <6% and <10%, respectively.

Statistical analysis

All data were analyzed using the statistical program SAS 9.1 (SAS Institute, Cary, NC, USA). Data are described as the mean $\pm$ SD in the case of normal distribution and as median and interquartile range in the case of non-normal distribution. Paired t-tests (if normally distributed) and Wilcoxon rank-sum tests (in the case of non-normal distribution data) were used to assess the change of variables. Spearman correlation coefficients were calculated to evaluate the relationship between serum RBP4 levels and adiposity indices and metabolic variables in the before and the after weight reduction periods, respectively. Additionally, a

correlation analysis was done between changes (Δ) of adiposity, metabolic variables and changes in RBP4 levels (Δ RBP4). The calculation used for Δ values is as follows: (after weight reduction level–before weight reduction level). Statistical significance was defined at the 0.05 level of confidence.

Results

Effect of modest weight reduction on adiposity, metabolic parameters and serum RBP4

Subjects had a 10.9% weight reduction and significantly reduced abdominal visceral fat areas (VFAT) and subcutaneous fat areas (SFAT) after 16 weeks of weight reduction (Table 1). Analysis revealed that the modest change of weight significantly improved blood pressure and HOMA-IR and decreased the total cholesterol, LDL-cholesterol and triglyceride levels.

We also observed statistically significant changes with a 25.5% decrease in serum RBP4 levels (baseline: 20.0 ± 4.0 μ g/mL vs. after weight reduction: 14.9 ± 4.1 μ g/mL) (Fig. 1A). In particular, a lack of RBP4 reduction in this study was noted in only five subjects. To investigate whether Δ RBP4 can be explained by differences in the change of body fat mass or fat distribution, we performed additional analyses. If the subjects were stratified into two groups according to the magnitude of the changes (Δ) in BMI, total abdominal fat area (TFAT), VFAT, and SFAT during the entire program, there was no difference in the weight reduction-induced variation of RBP4 (Δ RBP4) between the groups (data not shown) except for the change in VFAT (Δ VFAT). (Fig. 1B).

Correlation of adiposity indices, metabolic parameters and serum RBP4 levels before and after weight reduction, and the correlation of changes in the adiposity index and Δ RBP4.

Before weight reduction, the serum RBP4 level was positively correlated with abdominal visceral fat area measured by CT and blood pressure, fasting insulin, HOMA-IR and triglycerides after adjustment for age and gender (Table 2). Similarly, after weight reduction the serum RBP4 level was positively correlated with the abdominal visceral fat area measured by CT and the fasting glucose, HOMA-IR and triglycerides

Table 1. Baseline and following weight reduction (WR) data and mean changes (%) in adiposity indices and metabolic parameters

Characteristics (N = 52)	Baseline	After WR	Change (%)	<i>p</i>
Age (years)	37.4 ± 11.6			
men/women (% of women)	(12/40) 76.9%			
Adiposity index				
Weight (kg)	73.6 ± 15.3	65.6 ± 14.4	−10.9	<0.01
BMI* (kg/m ²)	27.4 ± 4.0	24.4 ± 3.7	−10.9	<0.01
Waist (cm)	91.9 ± 9.5	83.1 ± 9.0	−9.6	<0.01
Total body fat %	33.8 ± 5.1	29.4 ± 5.5	−15.8	<0.01
Computed tomography				
Total fat area (cm ²)	342.3 ± 106.7	251.0 ± 92.5	−26.7	<0.01
Visceral fat area (cm ²)	97.7 ± 44.1	69.4 ± 37.8	−29.0	<0.01
Subcutaneous fat area (cm ²)	237.0 ± 76.6	182.4 ± 68.2	−23.0	<0.01
Metabolic variables				
Systolic BP† (mmHg)	123.4 ± 15.5	116.3 ± 13.8	−5.8	<0.01
Diastolic BP (mmHg)	74.0 ± 10.7	70.5 ± 9.0	−4.7	0.02
Fasting glucose (mmol/L)	4.97 ± 0.65	4.86 ± 0.43	−2.2	0.20
Fasting insulin (pmol/L)	56.9 ± 34.7	38.9 ± 21.5	−31.7	<0.01
HOMA-IR‡	1.83 ± 1.24	1.23 ± 0.70	−32.8	<0.01
Total cholesterol (mmol/L)	4.93 ± 0.84	4.47 ± 0.89	−9.3	<0.01
Triglyceride (mmol/L)	1.08 (0.71–1.72)	0.94 (0.70–1.23)	−13.1	<0.01
HDL-cholesterol§ (mmol/L)	1.38 ± 0.29	1.35 ± 0.27	−2.1	0.76
LDL-cholesterol# (mmol/L)	2.92 ± 0.80	2.65 ± 0.87	−9.2	<0.01

Data shown as mean ± S.D. or median with IQR (25th–75th percentile). *P*-values are calculated by t-test or Wilcoxon rank-sum test.

* Body mass index. † Blood pressure. ‡ Homeostasis Model Assessment Insulin Resistance. § High-density lipoprotein cholesterol. # Low-density lipoprotein cholesterol.

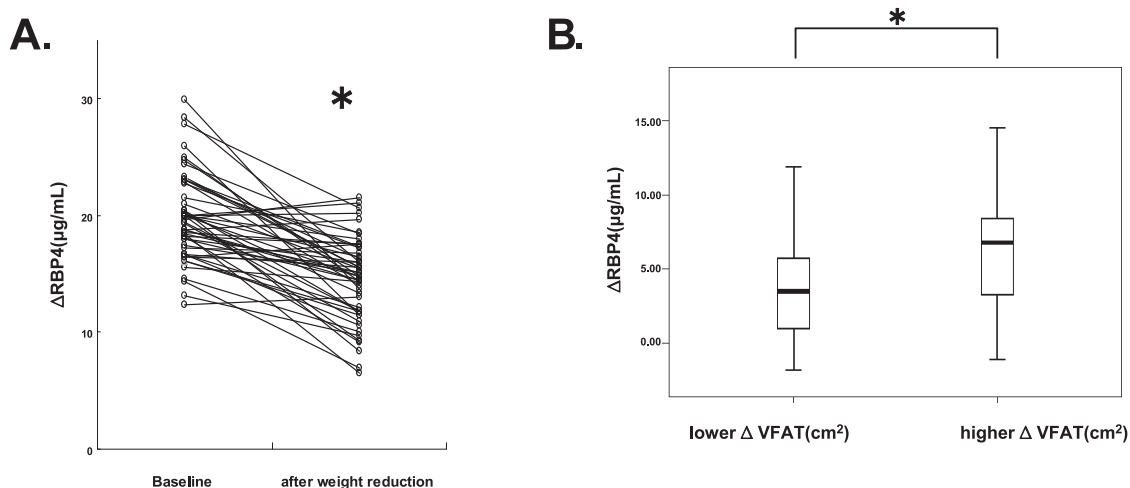


Fig. 1. Serum retinol binding protein-4 (RBP4) concentrations of each subjects at baseline and 16 weeks after weight reduction program (A), the Δ RBP4 of two groups, stratified according to the median value of Δ abdominal visceral fat area (VFAT) (B). A: Subjects showed significant decrease in mean serum RBP4 level after weight reduction (25.5%) (baseline: 20.0 ± 4.0 μ g/mL vs. after weight reduction: 14.9 ± 4.1 μ g/mL). * $p < 0.05$ vs. baseline. (paired t-test) B: Subjects were divided into two groups according to the median Δ VFAT value, lower Δ VFAT and higher Δ VFAT. *P* value was derived from Student's t-test. (* $p < 0.05$).

Table 2. Correlation of various adiposity indices, metabolic parameters and serum retinol binding protein-4 (RBP4) at baseline and at 16 weeks after beginning the weight reduction (WR) program. (age- and gender-adjusted)

	Baseline		After WR	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Adiposity				
Body mass index (kg/m ²)	0.29	0.07	0.27	0.16
Waist circumference (cm)	0.29	0.08	0.34	0.07
Total body fat %	0.05	0.79	0.09	0.66
Total fat area (cm ²)	0.27	0.10	0.30	0.12
Visceral fat area (cm ²)	0.43	<0.01	0.48	<0.01
Subcutaneous fat area (cm ²)	0.11	0.49	0.13	0.51
Metabolic variables				
Systolic BP* (mmHg)	0.38	0.02	0.03	0.88
Diastolic BP (mmHg)	0.53	<0.01	0.06	0.74
Fasting glucose (mmol/L)	0.26	0.11	0.47	<0.01
Fasting insulin (pmol/L)	0.34	0.03	0.37	0.05
HOMA-IR [†]	0.39	0.01	0.41	0.03
Total cholesterol (mmol/L)	0.11	0.50	0.09	0.64
Triglyceride (mmol/L)	0.36	0.02	0.51	<0.01
HDL cholesterol [§] (mmol/L)	-0.03	0.85	-0.21	0.27
LDL-cholesterol [#] (mmol/L)	-0.03	0.88	0.02	0.92

Coefficients (*r*) and *p*-values are calculated by the Spearman correlation model.

* Blood pressure. [†] Homeostasis Model Assessment Insulin Resistance. [§] High-density lipoprotein cholesterol. [#] Low-density lipoprotein cholesterol.

after adjustment for age and gender.

Further, when the changes were calculated as Δ values (the post-weight-reduction level minus the baseline level), Δ RBP4 levels were significantly correlated with Δ VFAT only among the adiposity indices. This result was unchanged after adjustments for age, gender and baseline values. There was no other significant correlation with the change of anthropometric parameters and other abdominal fat areas and Δ RBP4 (Fig. 2).

A weak relationship was found between Δ RBP4 and Δ insulin ($r = 0.25$, $p = 0.09$), Δ total-cholesterol ($r = 0.22$, $p = 0.16$) and Δ triglycerides ($r = 0.33$, $p = 0.06$). However, these weak relationships even vanished after an adjustment for Δ VFAT, indicating that the relationship is highly dependent on visceral abdominal fat accumulation (data not shown).

Discussion

The RBP4 has been shown to link obesity and insulin resistance in rodents [6] and recent report suggests that RBP4 plays an important role in insulin resistance and vascular complications not only in nondiabetic patients but also in type 2 diabetic patients [15].

In humans, however, the role of RBP4 is still controversial [7–11]. These incongruent results might suggest the complex roles of RBP4 in the pathophysiology of obesity and insulin resistance.

A number of clinical studies have demonstrated that visceral fat was more closely associated with the risk of insulin resistance, hypertension and hyperlipidemia than total body fat or subcutaneous fat [16], and also that visceral fat accumulation causes the dysregulation of adipocyte function [17]. In addition, the production and secretion of adipocytokines is considered to be dynamically regulated mainly by the patient's nutritional condition and lifestyle factors, such as physical activity [18].

Detailed knowledge regarding the association of obesity-related insulin resistance with RBP4 levels may need to be considered along with visceral adiposity and its effects on RBP4 levels during weight reduction programs.

The present study has demonstrated that moderate weight reduction significantly decreases serum RBP4 levels and has beneficial effects on insulin resistance and the lipid profile in nondiabetic subjects. Additionally, the changes in RBP4 were significantly correlated with abdominal visceral fat reduction.

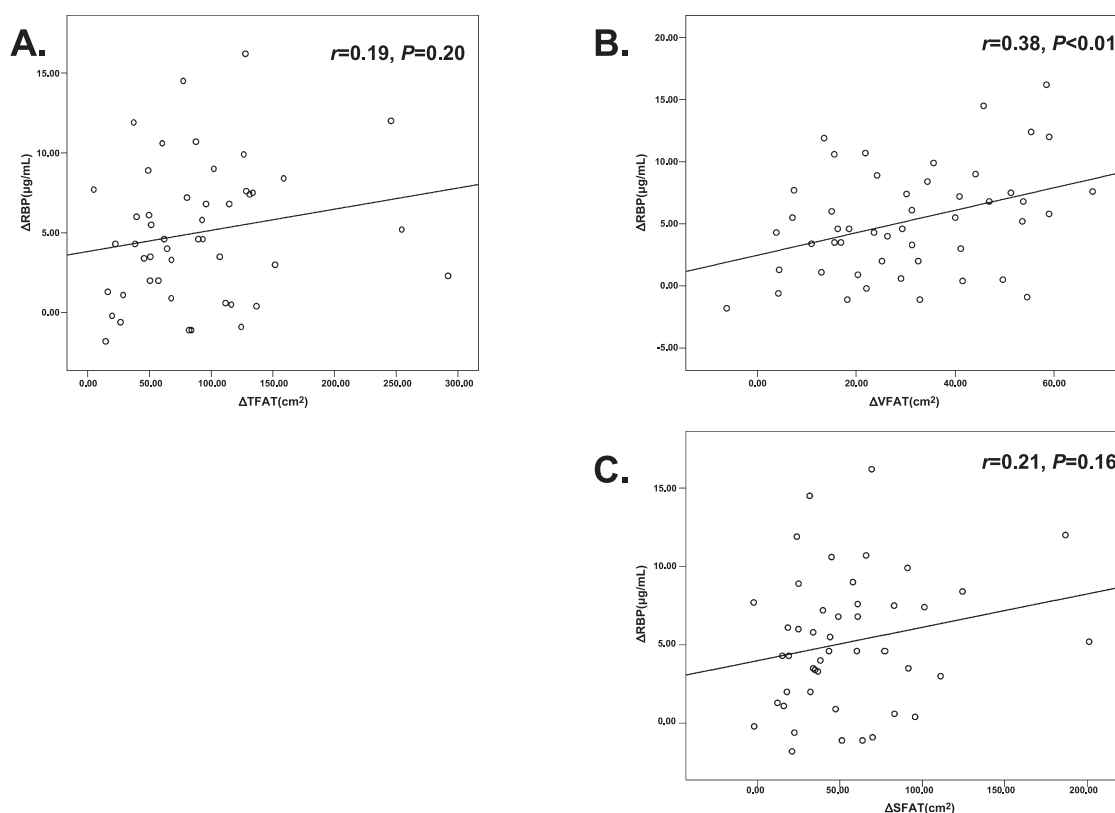


Fig. 2. The correlation between the change (Δ) of retinol binding protein-4 (RBP4) and total abdominal fat area (TFAT) (A), abdominal visceral fat area (VFAT) (B) and subcutaneous fat area (SFAT) (C) determined by computed tomography imaging. The calculation used for Δ values is as follows: (after weight reduction level-before weight reduction level). Coefficients (r) and p-values are calculated by the Spearman correlation model.

Previous studies are consistent with our study results that weight loss after calorie restriction or gastric banding surgery decreased RBP4 concentrations [8, 19]. In contrast, Janke *et al.* did not detect a difference in serum RBP4 concentrations according to the patient's obesity status, furthermore, mild weight loss was not associated with a significant decrease in RBP4 serum levels [11].

Disagreements with our findings may reflect differences in the magnitude of weight loss. Moreover, the previous study performed body composition measurements using a less-sensitive method and not with the more accurate CT, as we did in our study.

When our study subjects were stratified into two groups according to the magnitude of the changes in various adiposity indices, significant differences in the Δ RBP4 were observed only between the two groups stratified by Δ VFAT. We also found that the Δ VFAT was significantly correlated with the Δ RBP4. These results supported the previous findings of several

cross-sectional studies [20, 21] and suggested that the variability of the RBP4 response in the weight reduction programs might be caused by the variability of VFAT loss in the study subjects. Furthermore, the weak relation in the changes of RBP4 and insulin, as well as in lipid parameters even disappeared after an adjustment for reduction of visceral fat. These results suggested that there may be a mechanical link between reduced RBP4, visceral fat and metabolic risks.

The current study does not identify a precise mechanism for the changes of RBP4 in visceral fat reduction. One possible mechanism could argue for RBP4 production in visceral adipose tissue. In a very recent study, RBP4 was found to be more preferentially expressed in visceral than in subcutaneous adipose tissue, and RBP4 mRNA was negatively correlated with GLUT4 mRNA in visceral fat but not in subcutaneous fat [22]. Another potential link between RBP4 and visceral fat was suggested by the coordination of other adipocytokines expressed in the visceral fat. The

changes in RBP4 and visfatin were correlated in HIV-positive subjects receiving rosiglitazone [23], and the RBP4 reduction in obese children by lifestyle intervention was closely associated with the magnitude of the decrease in IL-6 [24]. The crosstalk and its mechanism between these adipocytokines need to be elucidated in further studies.

Our study is limited by the relatively small sample size and the fact we did not measure intrahepatic fat. Because the liver is the major source of RBP4 synthesis [25] and is also related to insulin resistance [26], it is possible that intrahepatic fat might contribute to serum RBP4 levels in human subjects. Further studies are therefore required to determine the difference behind the relationship of hepatic and visceral abdominal fat and RBP4. Since our study is not a placebo-controlled study, possible direct effect of sibutramine

on serum RBP4 cannot be excluded at present. The direct effect of sibutramine on lowering serum RBP4 levels needs further investigations.

In summary, moderate weight reduction lowers serum RBP4 concentrations in nondiabetic subjects. The relationship between individual changes in RBP4 levels and abdominal visceral fat reduction indicated that RBP4 may be involved in the beneficial effect of visceral fat reduction on the improvement of insulin resistance and metabolic syndrome.

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