Serum retinol-binding protein 4 concentrations in response to short-term overfeeding in normal-weight, overweight, and obese men[1–3]

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ABSTRACT

Background: Retinol-binding protein 4 (RBP4) is a novel adipokine that induces insulin resistance in mice. Studies in humans have shown a correlation between serum RBP4 and insulin resistance in obese subjects and in subjects with type 2 diabetes. Few data are available regarding the nutritional regulation of RBP4.

Objective: The study investigated the relation of RBP4 with phenotypes of glucose and lipid metabolism at baseline and in response to a 7-d overfeeding protocol in young men.

Design: Sixty-five men participated in the study. Subjects were classified on the basis of body mass index (BMI; kg/m^2) as normal-weight ($\leq$24.9) or as overweight or obese ($\geq$25.0). Serum RBP4, interleukin-6, visfatin, glucose, insulin, total cholesterol, HDL cholesterol, and LDL cholesterol (calculated), and triacylglycerols were measured. Insulin resistance and β cell function were assessed by using the homeostasis model. Percentage body fat was measured by using dual-energy X-ray absorptiometry.

Results: No significant differences were found in serum RBP4 between the 2 groups at baseline. Likewise, no significant differences were observed in fasting serum RBP4 in response to overfeeding. Baseline RBP4 was negatively correlated with the change in insulin resistance in normal-weight subjects, independent of age and BMI. No significant correlation was found between serum RBP4 and visfatin, interleukin-6, or any other variables measured.

Conclusions: Short-term overfeeding did not induce significant changes in RBP4. Baseline RBP4 concentrations may predict insulin resistance when exposed to a positive energy challenge in normal-weight men. *Am J Clin Nutr* 2007;86:1310–5.

KEY WORDS Retinol-binding protein 4, overfeeding, adiposity status, young men, insulin resistance, lipids

INTRODUCTION

It has been well documented that an increase in adipose tissue is linked to both insulin resistance and type 2 diabetes (1). One of the major causes of these 2 conditions is impaired insulin action in adipose tissue, skeletal muscle, and liver. In fact, adipose tissue secretes many adipokines that influence insulin action in other tissues, including leptin, adiponectin, and tumor necrosis factor-α (2–4). It was recently discovered that retinol-binding protein 4 (RBP4), previously known solely as a transporter of retinol (vitamin A), is also secreted from adipose tissue, where it induces insulin resistance in the liver and skeletal muscle of mice (5). After this finding, a few studies showed a correlation between RBP4 and insulin-resistant states in humans (5–10). However, the role that adiposity status plays in this is controversial. Although some studies observed higher concentrations of RBP4 in obese subjects (5–7), others found no association between RBP4 and adiposity status (11), and others found significant correlations between elevated RBP4 and specific fat depots, including liver fat (8) and trunk fat percentage (10).

With limited data available regarding this adipokine, the mechanism through which RBP4 acts in the development of insulin resistance and type 2 diabetes in humans is still unclear. In addition, no information is available regarding the nutritional regulation of RBP4. Studies from our laboratory and others showed that changes in nutritional status, such as overfeeding, have major effects on adipose tissue metabolism (12) and influence circulating concentrations of adipokines (13) and, consequently, may influence RBP4. The subsequent responses to changes in nutritional status can provide insight regarding the role of RBP4 in the development of insulin resistance and type 2 diabetes. Furthermore, studies have shown that the response of leptin to a high-fat meal differs between lean and obese men (14), and it is therefore possible that the response of RBP4 to short-term overfeeding may also depend on adiposity status. The objectives of the present study were to further understand the role that RBP4 plays in the development of insulin resistance by investigating *1*) the correlations of RBP4 with phenotypes of insulin resistance, glucose, and lipid metabolism and other adipokines [eg, interleukin-6 (IL-6) and visfatin]; *2*) the response of RBP4 to short-term overfeeding in young men without diabetes; and *3*) the role of adiposity status in the effects of RBP4.

SUBJECTS AND METHODS

Subjects were recruited from an ongoing overfeeding study investigating the effects of a positive energy balance on endocrine factors and on glucose and lipid metabolism (13). A total of 65 young men were recruited from the city of St John's and the surrounding area in the Canadian province of Newfoundland and

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Labrador to participate in the present study. Younger men are often targeted in overfeeding studies because they tend to tolerate overfeeding better than do older subjects. Inclusion criteria were as follows: *1*) male; *2*) 19–29 y of age; *3*) at least third-generation Newfoundlander; *4*) healthy, without any serious metabolic, cardiovascular, or endocrine disease; *5*) not receiving medication for lipid metabolism; and *6*) reported weight-stable (±2.5 kg) in the previous 6 mo.

The study was approved by the Ethics Committee of the Faculty of Medicine, Memorial University, St John's, Canada. All subjects provided written informed consent.

Serum measurements

Blood samples were obtained from all subjects after they had fasted for 12 h both before and after the completion of the overfeeding study. Serum was stored at −80 °C for subsequent analyses. Serum RBP4 concentrations were measured in duplicate with the use of radioimmunoassay kits (Phoenix Pharmaceuticals Inc, Belmont, CA). Serum insulin concentrations were measured with the use of an immunoassay analyzer (Immulite; DPC, Los Angeles, CA). The homeostasis model assessment (HOMA) was used as a measure of insulin resistance [HOMA-IR = insulin (μU/mL) × glucose (mmol/L)/22.5)] and β cell function [HOMA-β = 20 × insulin (μU/mL)/(glucose − 3.5)] (15). Serum concentrations of glucose, triacylglycerols, total cholesterol, and HDL cholesterol were measured by using Synchron reagents; measurements were performed by using an Lx20 analyzer (Beckman Coulter Inc, Fullerton, CA). LDL cholesterol was calculated by using the following formula: cholesterol – HDL cholesterol – (triacylglycerols/2.2). The LDL cholesterol calculation is reliable in the absence of severe hyperlipidemia. Serum IL-6 concentrations were measured in duplicate by using Access IL-6 kits (Beckman Coulter Inc) performed on a Unicel DxI 800 Access Immunoassay system (Beckman Coulter Inc). Serum visfatin concentrations were measured in duplicate with a human visfatin (COOH-terminal) enzyme immunometric assay (Phoenix Pharmaceuticals) performed on an Alisei Quality System (SEAC Radim Group, Pomezia, Italy).

Measurement of body composition

Total body fat [percentage body fat (%BF)] was measured by using dual-energy X-ray absorptiometry (Lunar Prodigy; GE Medical Systems, Madison, WI). Measurements were performed on subjects after the removal of all metal accessories and while the subjects were in a supine position, as previously described (16). ENCORE 2002 software version 6.70 was used for analysis. All measurements were completed before and 1 d after the overfeeding.

Overfeeding protocol

Although both short- and long-term overfeeding strategies have been used to investigate biochemical and metabolic responses to a hypercaloric diet (17–19), most studies have been short, ranging from 12 h to 22 d. A 7-d overfeeding protocol was chosen for this study to ensure that the intervention would induce metabolic changes. Subjects consumed 70% more calories than their normal energy requirements, and this intake consisted of 15% protein, 35% fat, and 50% carbohydrates to mimic the common daily diet in North America.

Full details of the overfeeding protocol were previously described by us (13). Briefly, individual energy requirements were estimated for each subject before the commencement of a 70% hypercaloric diet for 7 d. Subjects were offered 3 meals/d, and energy values and macronutrient content of the food were measured by using FOOD PROCESSOR SQL software (version 9.5.0.0; ESHA Research, Salem, OR). The average baseline energy intake and the energy intake during overfeeding were 2969 kcal and 5471 kcal, respectively. On average, subjects gained 2.2 ± 0.18 kg body weight, of which 28% (0.615 kg ± 0.131) was body fat.

Statistical analysis

Data are presented as means ± SDs. Before any statistical analyses were performed, the subjects were grouped according to adiposity status. Subjects were classified on the basis of BMI as normal-weight (≤24.9) or as overweight or obese (≥25.0) according to criteria of the World Health Organization (20). Overweight and obese subjects were grouped together because of the small number of subjects in each group. Statistical analyses were also performed according to %BF as either normal-weight (<20%), overweight (20–30%), or obese (>30%) according to the criteria recommended by Bray (21). Differences in RBP4 concentrations between the 2 groups and the changes in RBP4 in response to overfeeding were analyzed by using 2-factor analysis of variance and interaction analysis software (PROC GLM, version 9.1; SAS Institute Inc, Cary, NC). Pearson's correlation analyses were performed to screen for potential factors related to fasting RBP4 concentrations and were followed by partial correlation analyses after control for age and BMI. Markers of the metabolic syndrome, which included HDL cholesterol, fasting triacylglycerols, and glucose, decline with age (22), and we therefore controlled for age within each group. Bonferroni testing was applied to correct for multiple comparisons. Fisher's *r*-to-*Z* transformation was used to test for significant differences between *r* values of normal-weight and of overweight and obese subjects. Three correlation analyses were performed: *1*) RBP4 at baseline was compared with all variables at baseline, *2*) RBP4 at baseline was compared with changes in all variables in response to overfeeding to investigate whether baseline RBP4 could predict the changes in related markers, and *3*) changes in RBP4 were compared with changes in all variables in response to overfeeding.

SPSS software (version 14.0; SPSS Inc, Chicago, IL) was used for all analyses unless otherwise stated. Statistical analyses were 2-sided, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Comparison of characteristics at baseline and in response to short-term overfeeding

Physical and biochemical characteristics of subjects at baseline are shown in **Table 1**. There were no significant differences in age and height between the 2 groups; however, the differences in BMI, body weight, and %BF were significant. There was no significant difference in fasting glucose between the 2 groups. Overweight or obese subjects had higher fasting serum insulin concentrations, greater HOMA-IR, and greater HOMA-β than did normal-weight subjects. There were no significant differences in any lipid profile markers (cholesterol, LDL cholesterol, and triacylglycerols) between the 2 groups aside from lower HDL cholesterol in the overweight and obese group. In addition,

TABLE 1
Physical and biochemical characteristics of subjects at baseline and in response to 7 d of overfeeding[1]

	Lean subjects (n = 37–40)		Overweight or obese subjects (n = 24–28)		P[2]	
Variable	Before overfeeding	After overfeeding	Before overfeeding	After overfeeding	Change from baseline	Group comparison
Age (y)	23.2 ± 3.6[3]	NA	22.0 ± 0.5	NA	NA	NA
Height (cm)	180.3 ± 6.6	NA	181.1 ± 1.3	NA	NA	NA
Weight (kg)[4,5]	72.58 ± 8.85	75.00 ± 8.83	92.26 ± 15.51	94.37 ± 15.83	<0.001	<0.001
Body fat (%)[4,5]	17.95 ± 6.64	18.51 ± 6.36	28.80 ± 7.64	28.45 ± 7.16	0.09	<0.001
BMI (kg/m^2)[5]	22.31 ± 2.16	23.09 ± 2.16	29.18 ± 4.54	29.88 ± 4.77	<0.001	<0.001
Glucose (mmol/L)	5.01 ± 0.39	4.96 ± 0.44	5.22 ± 0.67	5.12 ± 0.45	0.67	0.12
Insulin (pmol/L)[4]	55.07 ± 46.60	71.35 ± 55.68	93.72 ± 93.09	111.22 ± 71.62	0.04	0.02
Cholesterol (mmol/L)	4.47 ± 0.91	4.64 ± 0.97	4.65 ± 0.79	4.80 ± 0.57	0.031	0.52
Triacylglycerol (mmol/L)	0.99 ± 0.45	1.16 ± 0.78	1.20 ± 0.66	1.82 ± 2.10	0.031	0.08
HDL (mmol/L)[4]	1.40 ± 0.29	1.47 ± 0.28	1.22 ± 0.21	1.36 ± 0.24	<0.001	0.04
LDL (mmol/L)	2.62 ± 0.74	2.73 ± 0.73	2.89 ± 0.67	2.61 ± 1.16	0.47	0.94
HOMA-IR[4]	1.76 ± 1.71	2.22 ± 1.85	3.26 ± 3.75	3.60 ± 2.51	0.18	0.02
HOMA-β[4]	97.86 ± 55.39	143.36 ± 103.12	142.52 ± 90.07	193.54 ± 104.68	<0.001	0.03
IL-6 (pg/mL)	1.62 ± 3.86	1.29 ± 3.48	1.32 ± 1.24	1.32 ± 1.77	0.26	0.83
Visfatin (ng/mL)[5]	31.01 ± 20.98	28.33 ± 23.25	27.85 ± 19.62	18.15 ± 19.44	<0.001	0.21
RBP4 (μg/mL)	29.53 ± 6.02	30.20 ± 6.89	29.14 ± 5.30	31.57 ± 7.18	0.10	0.71

[1] RBP4, retinol-binding protein 4; IL-6, interleukin 6; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, HOMA of β cell function; NA, not available.
[2] Adiposity status and responses to overfeeding were analyzed by using 2-factor ANOVA for repeated measurements and interaction analysis software (PROC GLM, version 9.1; SAS Institute, Cary, NC).
[3] $\bar{x}$ ± SD (all such values).
[4] Significant differences between lean and overweight or obese subjects at baseline were analyzed by using Student's t test. Weight, percentage body fat, BMI, HDL, insulin, HOMA-IR, and HOMA-β were significantly different between groups, $P < 0.05$.
[5] Significant time-by-treatment interaction, $P < 0.05$.

there were no significant differences in fasting IL-6 or visfatin concentrations between groups. The average fasting serum RBP4 concentrations at baseline were 29.53 ± 6.02 and 29.14 ± 5.30 μg/mL for the normal-weight and the overweight or obese subjects, respectively, with no significant differences between the groups. RBP4 concentrations were also analyzed according to %BF criteria, and again, no significant differences were found (data not shown). Moreover, subjects were divided into 3 groups according to HOMA-IR, after control for BMI. There were also no significant differences in fasting serum RBP4 in the low-, medium-, or high-HOMA-IR groups (data not shown).

Changes in body composition and the phenotypes of glucose metabolism and lipids in response to the 7-d overfeeding period are described in Table 1. Briefly, there was a significant increase in body weight in both groups after overfeeding. A significant increase in %BF was evident in both the normal-weight and the overweight or obese groups. Cholesterol, HDL cholesterol, and triacylglycerol concentrations were significantly increased in both the normal-weight and the overweight or obese subjects. Insulin concentrations and HOMA-β also were significantly increased. There were no significant differences in fasting serum RBP4 in response to overfeeding within each group.

Correlations of RBP4 with phenotypes of glucose and lipid metabolism

Pearson's correlation analysis was used as an initial screening tool between RBP4 and phenotypes of glucose and lipid metabolism, followed by partial correlation analyses, with control for BMI and age. At baseline, RBP4 was positively correlated with baseline LDL cholesterol in the normal-weight subjects and with baseline HOMA-β in the overweight and obese subjects. When all subjects were combined, only the positive correlation with LDL cholesterol was evident. However, after multiple-comparison testing was applied, no significant results remained (**Table 2**). Correlations between baseline RBP4 and the changes in markers were also assessed. Significant negative correlations were evident between baseline RBP4 and changes in both insulin and HOMA-IR in normal-weight subjects (**Table 3**). The significant correlation between RBP4 and the change in HOMA-IR remained significant even after Bonferroni correction. Finally, we investigated correlations between the changes in RBP4 and the changes in variables measured. Although numerous significant correlations were detected between the change in RBP4 and the changes in insulin and HOMA-IR in both normal-weight and overweight or obese subjects, none of these correlations survived Bonferroni correction in any of the groups or in the entire study cohort (**Table 4**).

DISCUSSION

Type 2 diabetes is one of the fastest-growing diseases in North America and in many developing countries, and it is closely associated with both insulin resistance and obesity. Although the discovery of various adipokines has shed light on the causes of this disease, the molecular link between obesity, insulin resistance, and type 2 diabetes in humans is still largely unknown. It appears that RBP4 is a factor that acts to induce insulin resistance in the liver and skeletal muscle of rodents (5); however, the mechanism through which this adipokine acts and the role that adiposity status plays in humans are still unclear.

TABLE 2
Partial correlations of baseline variables related to baseline fasting serum retinol-binding protein 4 (RBP4), after control for BMI and age[1]

	Normal-weight subjects (n = 37–40)		Overweight or obese subjects (n = 24–28)		All subjects (n = 65)	
Variable	r	P	r	P	r	P
Glucose (mmol/L)	0.108	NS	−0.018	NS	0.021	NS
Insulin (pmol/L)	−0.139	NS	0.314	NS	0.036	NS
Cholesterol (mmol/L)	0.365	NS	0.154	NS	0.278	NS
Triacylglycerol (mmol/L)	0.286	NS	0.251	NS	0.237	NS
HDL (mmol/L)	−0.131	NS	−0.139	NS	−0.128	NS
LDL (mmol/L)	0.380	0.042[2]	0.093	NS	0.290	0.035[2]
HOMA-IR	−0.114	NS	0.275	NS	0.036	NS
HOMA-β	−0.210	NS	0.424[3]	0.049[2]	0.041	NS
IL-6 (pg/mL)	−0.093	NS	−0.098	NS	−0.076	NS
Visfatin (ng/mL)	0.028	NS	0.221	NS	0.065	NS

[1] IL-6, interleukin 6; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, HOMA of β cell function. Partial correlation analysis after control for BMI and age was used to screen for potential factors related to fasting RBP4.
[2] Not significant after Bonferroni correction to adjust for the multiple variables tested.
[3] Significant difference between normal-weight and overweight or obese subjects, $P < 0.05$ (Fisher's r-to-Z transformation).

Although RBP4 is secreted primarily by the liver (21), studies in rats showed that adipose tissue has the second-highest expression level (23), where it is expressed almost exclusively by adipocytes (24). Janke et al (11) were able to show that RBP4 is also highly expressed in mature human adipocytes and is secreted by differentiating human adipocytes. If adipose tissue is an important site contributing to circulating RBP4, it is reasonable to expect significant differences in serum RBP4 between normal-weight and obese humans because of the variations in the amount of adipose tissue. Graham et al (6) showed that concentrations of serum RBP4 were higher in obese humans without or with type 2 diabetes than in lean control subjects. Cho et al (7) found a significant difference in plasma RBP4 concentrations between subjects with normal glucose tolerance and those with type 2 diabetes. Conversely, Janke et al (11) found no significant difference in circulating concentrations of RBP4 in normal-weight, overweight, and obese women. Stefan et al (8) found a positive correlation between circulating RBP4 and liver fat but not between circulating RBP4 and total body, visceral, or subcutaneous fat. In the current study, we found no significant difference in fasting serum RBP4 concentrations between normal-weight and overweight or obese men. This was also true when subjects were classified according to %BF. Our data do not support the hypothesis that total body fat, indexed by BMI or %BF, determines circulating RBP4 concentrations in healthy young men without diabetes.

We also sought to understand the nutritional regulation of RBP4. To the best of our knowledge, the present study is the first of its kind to explore the response of RBP4 to a short-term positive energy challenge. Overfeeding studies provide a means by which the biochemical changes that would be evident with extended overeating in both adipokines and hormones can be

TABLE 3
Partial correlations of changes in variables related to baseline fasting serum retinol-binding protein 4 (RBP4), after control for BMI and age[1]

	Normal-weight subjects (n = 37–40)		Overweight or obese subjects (n = 24–28)		All subjects (n = 65)	
Variable	r	P	r	P	r	P
Glucose (mmol/L)	−0.368	NS	−0.101	NS	−0.202	NS
Insulin (pmol/L)	−0.467	0.014[2]	−0.084	NS	−0.078	NS
Cholesterol (mmol/L)	−0.067	NS	0.002	NS	−0.038	NS
Triacylglycerol (mmol/L)	−0.115	NS	0.410[3]	NS	0.130	NS
HDL (mmol/L)	0.067	NS	0.048	NS	0.059	NS
LDL (mmol/L)	−0.056	NS	−0.122	NS	−0.081	NS
HOMA-IR	−0.614	0.001[4]	−0.094[3]	NS	−0.083	NS
HOMA-β	0.050	NS	−0.061	NS	−0.019	NS
IL-6 (pg/mL)	0.265	NS	0.293	NS	−0.076	NS
Visfatin (ng/mL)	−0.089	NS	0.030	NS	−0.043	NS

[1] IL-6, interleukin 6; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, HOMA of β cell function. Partial correlation analysis after control for BMI and age was used to screen for potential factors related to fasting RBP4.
[2] Not significant after Bonferroni correction.
[3] Significant difference between normal-weight and overweight or obese subjects, $P < 0.05$ (Fisher's r-to-Z transformation).
[4] $P = 0.01$ after Bonferroni correction.

TABLE 4
Partial correlations of changes in variables related to changes in fasting serum retinol-binding protein (RBP4), after control for BMI and age[1]

	Normal-weight subjects (n = 37–40)		Overweight or obese subjects (n = 24–28)		All subjects (n = 65)	
Variable	r	P	r	P	r	P
Glucose (mmol/L)	0.024	NS	−0.328	NS	−0.123	NS
Insulin (pmol/L)	0.413	0.032[2]	−0.513	0.021[2]	−0.194	NS
Cholesterol (mmol/L)	0.018	NS	0.015	NS	0.012	NS
Triacylglycerol (mmol/L)	0.169	NS	0.077	NS	0.098	NS
HDL (mmol/L)	0.000	NS	0.085	NS	0.026	NS
LDL (mmol/L)	−0.009	NS	0.081	NS	0.015	NS
HOMA-IR	0.445	0.020[2]	−0.515	0.020[2]	−0.202	NS
HOMA-β	0.113	NS	−0.505	0.023[2]	−0.200	NS
IL-6 (pg/mL)	−0.062	NS	−0.350	NS	−0.136	NS
Visfatin (ng/mL)	−0.040	NS	0.376	NS	0.075	NS

[1] IL-6, interleukin 6; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, HOMA of β cell function. Partial correlation analysis after control for BMI and age was used to screen for potential factors related to fasting RBP4.
[2] Not significant after Bonferroni correction.

investigated. A positive energy balance is one of the major causes of obesity (25), and it triggers many hormonal responses, including an increase or decrease, or both, in the secretion of various adipokines (26, 27). In the present study, there were no significant differences in RBP4 concentrations before and after overfeeding, which suggests that RBP4 is not significantly regulated by a short-term positive energy challenge at physiologic conditions in healthy young men. It is interesting that Janke et al (11) found no significant change in circulating RBP4 in women after a 5% weight loss (11), which indicates that a change in overall energy balance, whether positive or negative, appears to have no significant effect on circulating RBP4 concentrations.

Recent studies have suggested that RBP4 is a key player in the development of insulin resistance in both healthy subjects (9–10) and subjects with type 2 diabetes (5–7). In the current study, we did not find any significant associations between RBP4 and phenotypes of insulin resistance at baseline without overfeeding. However, we found that, in normal-weight men, changes in HOMA-IR were inversely correlated with baseline serum RBP4, which suggests that RBP4 may serve as a predictor of insulin resistance when exposed to a positive energy challenge, such as the development of obesity. To further understand the relation between RBP4 and insulin resistance, we classified subjects according to HOMA-IR status as low, medium, or high to determine whether subjects with a greater degree of insulin resistance had higher concentrations of circulating RBP4. We found no significant differences in serum RBP4 among these 3 groups, which suggests that, at physiologic conditions in healthy young men, RBP4 is not a significant factor in determining the difference in insulin resistance between normal-weight and obese subjects. However, it cannot exclude the possibility that such a relation may be detected if a more sensitive method to measure insulin resistance were used, such as euglycemic clamp test and a larger sample size.

We also investigated correlations between RBP4 and phenotypes of lipid metabolism. It has been suggested that RBP4 may be linked to the metabolic syndrome. Specifically, elevated serum RBP4 was associated with higher concentrations of serum triacylglycerols and with lower HDL cholesterol (6) and smaller waist circumference (7). Although we found a weak positive correlation between baseline RBP4 and LDL cholesterol, it did not survive Bonferroni correction. Further studies are warranted to investigate the associations between RBP4 and lipid metabolism.

It has been suggested that adipokines may interact with one another in the regulation of energy balance. For example, it has been hypothesized that the stimulatory effects of ghrelin and the inhibitory effects of leptin assimilate in the regulation of energy intake and expenditure (28). Previous studies investigated the relation between RBP4 and both leptin and adiponectin (7–8); however, no significant correlations were detected in either study. In the present study, we investigated the relation between RBP4 and both IL-6 and visfatin. We did not find any association between RBP4 and these 2 adipokines.

One limitation of the present study is the homogeneous study group. By targeting men 19–29 y of age, we are limiting the population to which these findings can be applied. Further studies are warranted to investigate these issues in other age groups and in women. Another potential limitation is the short overfeeding period. Although this is the first study of its kind to explore the nutritional regulation of RBP4, future studies examining the effects of prolonged overeating are necessary.

In summary, we measured serum RBP4 in 65 men before and after a 7-d overfeeding protocol. Circulating RBP4 was similar in normal-weight and overweight or obese young men. Likewise, the changes in RBP4 in response to overfeeding were not significant within the 2 adiposity groups. RBP4 was not associated with phenotypes of insulin resistance or lipid metabolism in either the normal-weight or the overweight and obese subjects at baseline. RBP4 was inversely associated with the change in insulin resistance in normal weight men, which suggests that it may be a predictor of the changes in insulin resistance in response to a short-term positive energy challenge.

We greatly appreciate the contributions of the volunteers to the present study. We also recognize the following members who contributed to the data collection: Curtis French, Jessica Bishop, and Sammy Khalili.

The authors' responsibilities were as follows—JS: assisted in the data collection and was responsible for data analysis and the writing of the manuscript; ER: supervised the measurement of RBP4 and aided in the editing of the manuscript; PW: assisted with statistical analysis and the revision of the manuscript; SV and BR: provided excellent comments on the manuscript; GS: was responsible for the study design and the revision of the manuscript.

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