

# High Circulating Retinol-Binding Protein 4 Is Associated With Elevated Liver Fat but Not With Total, Subcutaneous, Visceral, or Intramyocellular Fat in Humans

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**OBJECTIVE** — Retinol-binding protein 4 (RBP4) is an adipokine that induced insulin resistance in mice, and high plasma RBP4 levels were associated with insulin-resistant states in humans. To determine which fat compartments are associated with elevated RBP4 levels in humans, we measured circulating RBP4 in 75 healthy subjects and used state-of-the-art measurements of body fat distribution.

**RESEARCH DESIGN AND METHODS** — Total body, visceral, and subcutaneous abdominal fat were determined by magnetic resonance tomography and liver fat and intramyocellular fat by localized proton magnetic resonance spectroscopy. Insulin sensitivity was measured by the euglycemic-hyperinsulinemic clamp and, together with insulin clearance, estimated from the oral glucose tolerance test (OGTT).

**RESULTS** — Adjusted circulating RBP4 correlated negatively with insulin sensitivity (clamp:  $r = -0.33$ ,  $P = 0.005$ ; OGTT:  $r = -0.36$ ,  $P = 0.002$ ) and positively with parameters in the fasting state as insulin levels ( $r = 0.35$ ,  $P = 0.003$ ) and homeostasis model assessment of insulin resistance ( $r = 0.34$ ,  $P = 0.004$ ). In addition, circulating RBP4 correlated negatively with hepatic insulin clearance ( $r = -0.25$ ,  $P = 0.04$ ). Circulating RBP4 was not associated with total body, visceral, or subcutaneous abdominal fat (all  $P \geq 0.29$ ). Plasma RBP4 levels were also not associated with intramyocellular fat or circulating adiponectin or leptin. In contrast, plasma RBP4 levels correlated positively with liver fat in cross-sectional ( $r = 0.27$ ,  $P = 0.03$ ) and longitudinal ( $r = 0.37$ ,  $P = 0.04$ ) analyses.

**CONCLUSIONS** — Circulating RBP4 is not associated with the amount of fat in the classical depots or in the ectopic depots in muscle. However, it correlates positively with liver fat. Furthermore, metabolic parameters support the close relationship between circulating RBP4 with liver fat and, presumably, hepatic insulin resistance.

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Insulin resistance is a fundamental aspect of the etiology of type 2 diabetes (1,2). While insulin action starts to decline, increased insulin secretion of the

$\beta$ -cells compensates to decrease hyperglycemia. However, a failure to do so eventually results in the manifestation of the disease (3,4). In recent years, the role

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**Abbreviations:** AUC, area under the curve; FFA, free fatty acid; HOMA-IR, homeostasis model assessment of insulin resistance; IMCL, intramyocellular lipid; MRT, magnetic resonance tomography; OGTT, oral glucose tolerance test; RBP4, retinol-binding protein 4.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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of adipose tissue as an endocrine organ capable of secreting a number of adipose tissue-specific hormones that are involved in the regulation of insulin action gained appreciation (5,6).

One of them, retinol-binding protein 4 (RBP4), was first found to be expressed in rodent adipocytes (7,8) in 1992. More recently, it was identified as an adipokine that is increased in circulation in mouse models of obesity and insulin resistance (9). Furthermore, transgenic expression and administration of RBP4 into mice induced insulin resistance. Particularly, mice lacking GLUT4 in adipocytes displayed increased RBP4 blood levels in the presence of muscular and hepatic insulin resistance (9). When this research was extended to humans, a positive correlation of circulating RBP4 with BMI and a negative correlation with whole-body insulin sensitivity was found (9,10).

There is ongoing research to identify the major sources for elevated plasma RBP4 levels that are observed in insulin-resistant states in humans. So far, it is assumed that fat is a major origin of circulating RBP4; however, it is unclear which fat compartments determine plasma RBP4 levels. Precise phenotyping methods, such as magnetic resonance tomography (MRT), to measure total body fat content and fat content in the visceral as well as in the subcutaneous depots may help to advance our understanding of RBP4 production in humans. Using such techniques, we previously investigated the relationships of body fat distribution with plasma adiponectin levels in humans (11). Applying these measurements in the present study, we determined the relationships of plasma RBP4 levels with body fat distribution and insulin sensitivity.

## RESEARCH DESIGN AND METHODS

A total of 75 Caucasians without type 2 diabetes were included in the analysis. They participated in an ongoing study on the pathophysiology of type 2 diabetes (12,13). Due to claustrophobia or metal implantations, magnetic resonance imaging and spec-

Table 1—Demographics and metabolic characteristics of the subjects

Variables	Means $\pm$ SE	Range
Sex (male/female)	36/39	
Age (years)	44 $\pm$ 1	23–64
Height (cm)	172 $\pm$ 1	154–193
Weight (kg)	87 $\pm$ 2	53–166
Body fat <sub>bioimpedance</sub> (%)	32 $\pm$ 1	15–54
Body fat <sub>MRT</sub> (kg)	25 $\pm$ 1	8.5–26.3
Visceral fat <sub>MRT</sub> (kg)	3.24 $\pm$ 0.24	0.42–9.21
Subcutaneous abdominal fat <sub>MRT</sub> (kg)	10.96 $\pm$ 0.67	3.21–34.18
Liver fat <sub>1MRS</sub> (%)	5.80 $\pm$ 0.77	0.39–29.12
IMCL <sub>tibialis anterior</sub> (arbitrary units)	3.94 $\pm$ 0.28	0.54–13.17
IMCL <sub>soleus</sub> (arbitrary units)	17.30 $\pm$ 1.04	4.94–40.27
Fasting glucose (mmol/l)	5.10 $\pm$ 0.05	4.22–6.44
2-h glucose (mmol/l)	7.05 $\pm$ 0.18	4.17–11.00
Fasting insulin (pmol/l)	59 $\pm$ 4	19–180
2-h insulin (pmol/l)	509 $\pm$ 43	78–2,132
Fasting FFA ( $\mu$ mol/l)	606 $\pm$ 26	226–1,315
2-h FFA ( $\mu$ mol/l)	91 $\pm$ 11	29–775
Insulin sensitivity <sub>clamp</sub> ( $\mu$ mol/l $\cdot$ kg <sup>-1</sup> $\cdot$ min <sup>-1</sup> per pmol/l)	0.061 $\pm$ 0.004	0.010–0.20
Insulin sensitivity <sub>OGTT</sub> (arbitrary units)	12.88 $\pm$ 0.80	3.23–32.14
HOMA-IR (arbitrary units)	2.26 $\pm$ 0.15	0.64–6.93
Insulin clearance (arbitrary units)	5.67 $\pm$ 0.22	2.75–10.93
Adiponectin ( $\mu$ g/ml)	11.96 $\pm$ 0.57	5.28–30.23
Leptin (ng/ml)	22.40 $\pm$ 2.18	3.18–103.30
RBP4 ( $\mu$ g/ml)	29.98 $\pm$ 1.81	1.40–80.17

MRS, magnetic resonance spectroscopy.

troscopy were not done in 11 subjects. A group of 33 subjects had follow-up data after they underwent dietary counseling and increased physical activity. Informed written consent was obtained from all participants, and the local medical ethics committee had approved the protocol.

### Body composition and body fat distribution

Body fat was measured by the bioelectrical impedance method (RJL, Detroit, MI). Furthermore, we measured total, visceral, and subcutaneous abdominal fat with an axial T1-weighted fast spin echo technique with a 1.5 T whole-body imager (Magnetom Sonata; Siemens Medical Solutions) (14).

### Quantitative analysis of liver fat and intramyocellular lipids

Liver fat was measured by localized proton magnetic resonance spectroscopy (13). Intramyocellular lipid (IMCL) content and lipid content interlaced between the muscle fibers (extramyocellular lipid content) of the tibialis anterior (IMCL<sub>tibialis</sub>) and soleus muscle (IMCL<sub>soleus</sub>) were determined as previously described (13).

### Oral glucose tolerance test and euglycemic-hyperinsulinemic clamp

All individuals underwent a 75-g oral glucose tolerance test (OGTT). We obtained venous plasma samples at 0, 30, 60, 90, and 120 min for determination of plasma glucose, insulin, C-peptides, and free fatty acids (FFAs). Glucose tolerance was determined according to the 1997 World Health Organization diagnostic criteria (15). With the euglycemic-hyperinsulinemic clamp, insulin sensitivity was determined with a primed insulin infusion at a rate of 40 mU/m<sup>2</sup> per min for 2 h as previously described (12,13).

### Analytical procedures

Blood glucose was determined using a bedside glucose analyzer (glucose-oxidase method; Yellow Springs Instruments, Yellow Springs, CO). Plasma insulin was determined by microparticle enzyme immunoassay (Abbott Laboratories, Tokyo, Japan), and serum FFA concentrations were measured with an enzymatic method (WAKO Chemicals, Neuss, Germany). Plasma samples were frozen immediately and stored at  $-80^{\circ}\text{C}$ . Fasting plasma levels of adiponectin and

leptin were determined with enzyme-linked immunosorbent assays (ELISA) (Linco Research, St. Charles, MO), and fasting plasma RBP4 levels were measured with ELISA (AdipoGen, Seoul, Korea). Data for longitudinal analyses derived from radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA).

### Calculations

The insulin sensitivity index ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{per pmol/l}$ ) for systemic glucose uptake was calculated as the mean infusion rate of glucose ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) necessary to maintain euglycemia during the last 40 min of the euglycemic-hyperinsulinemic clamp divided by the steady-state plasma insulin concentration. The latter was the mean insulin concentration at minute 100, 110, and 120 of the clamp (mean for all subjects:  $532 \pm 15$  pmol/l). Insulin sensitivity from the OGTT was estimated as proposed by Matsuda and DeFronzo (16):  $\text{ISI}_{\text{est}} = 10,000 / \sqrt{(\text{Ins}_{\text{mean}} \times \text{Gluc}_{\text{mean}} \times \text{Ins}_0 \times \text{Gluc}_0)}$  and with homeostasis model assessment of insulin resistance (HOMA-IR) =  $\text{Ins}_0 \times \text{Gluc}_0 / 22.5$  (17). The plasma insulin area under the curve (AUC) during the OGTT was calculated as  $0.5 \times (0.5 \times \text{Ins}_0 + \text{Ins}_{30} + \text{Ins}_{60} + 0.5 \times \text{Ins}_{120})$ , and those for plasma C-peptide and serum FFA were calculated analogously. To obtain a measure of suppression of lipolysis from the OGTT that is independent of insulin sensitivity, we adjusted FFA AUC for the insulin AUC. Insulin clearance was estimated from the OGTT as C-peptide AUC/insulin AUC.

### Statistical analyses

Unless otherwise stated, data are means  $\pm$  SE. Data that were not normally distributed (e.g., liver fat, insulin sensitivity, body fat distribution; Shapiro-Wilk *W* test) were logarithmically transformed, and a normal distribution of these parameters was achieved. To adjust the effects of covariates and identify independent relationships, we first performed forward stepwise regression analyses to identify the variables to be included in a multiple regression model. Next, we performed multivariate linear regression analyses with these variables. Differences between parameters at baseline and at follow-up were tested using the one-tail, matched, paired Student's *t* test. A *P* value  $\leq 0.05$  was considered statistically significant. The statistical software package JMP (version 4.0; SAS Institute, Cary, NC) was used.

Table 2—Univariate correlations of circulating RBP4 with distinct characteristics

Variables	<i>r</i>	<i>P</i>
Weight	0.11	0.35
Height	0.08	0.52
Body fat <sub>bioimpedance</sub>	0.04	0.71
Body fat <sub>MRT</sub>	0.13	0.30
Visceral fat <sub>MRT</sub>	0.16	0.19
Subcutaneous abdominal fat <sub>MRT</sub>	0.14	0.25
Liver fat <sub>1MRS</sub>	0.24	0.05
IMCL <sub>tibialis anterior</sub>	0.13	0.32
IMCL <sub>soleus</sub>	0.10	0.44
Fasting glucose	0.008	0.94
2-h glucose	0.09	0.41
Fasting insulin	0.25	0.03
2-h insulin	0.29	0.01
Insulin sensitivity <sub>clamp</sub>	−0.26	0.02
Fasting FFA	0.02	0.86
2-h FFA	0.04	0.76
Insulin sensitivity <sub>OGTT</sub>	−0.25	0.03
HOMA-IR	0.26	0.02
Insulin clearance	−0.15	0.21
Adiponectin	0.04	0.74
Leptin	0.08	0.47

MRS, magnetic resonance spectroscopy.

## RESULTS

### Demographics, anthropometrics, and metabolic characteristics of study subjects

As shown in Table 1, male and female subjects were evenly represented. Anthropometrics and metabolic characteristics covered a wide range that was particularly large for body fat distribution and plasma RBP4 levels.

### Relationships of plasma RBP4 levels with demographics and metabolic characteristics

Plasma RBP4 levels were not significantly associated with sex, age, weight, or height (all  $P \geq 0.19$ ). To investigate whether plasma RBP4 levels were associated with insulin resistance, we tested the relationship of circulating RBP4 with several metabolic characteristics representing insulin-resistant states. No significant associations of RBP4 levels with fasting and 2-h glycemia, FFAs, or suppression of lipolysis in univariate analyses (Table 2)—and after adjustment for age, sex, height, and body fat (all  $P \geq 0.32$ )—were detected.

In contrast, plasma RBP4 levels correlated significantly with fasting and 2-h insulinemia in univariate analyses (Table 2) as well as after adjustment for age, sex, height, and body fat ( $\text{Ins}_0$   $r = 0.35$ ,  $P =$

0.003 [Fig. 1A];  $\text{Ins}_{120}$   $r = 0.36$ ,  $P = 0.002$ ). In addition, RBP4 levels correlated significantly with insulin sensitivity determined by the clamp before and after adjustment for the above-mentioned variables (Table 2; adjusted  $r = -0.33$ ,  $P = 0.005$ ) and with insulin sensitivity estimated by the formula of Matsuda and DeFronzo (Table 2 and Fig. 1B) and the HOMA-IR (Table 2; adjusted  $r = 0.34$ ,  $P = 0.004$ ). While RBP4 levels were not significantly correlated with insulin clearance in univariate analyses (Table 2), the correlation was significant after adjustment for the determinants of insulin clearance, age, sex, height, and body fat ( $r = -0.25$ ,  $P = 0.04$ ). As impaired insulin clearance is often associated with liver diseases such as cirrhosis (18), this points to an association of plasma RBP4 levels with liver function.

### Relationships of plasma RBP4 levels with fat compartments

Plasma RBP4 levels were not significantly associated with total body fat measured by bioelectrical impedance or MRT (Table 2, Fig. 1C). Circulating RBP4 was also not associated with the amount of subcutaneous abdominal or visceral fat determined by MRT in univariate analyses (Table 2) or after adjustment for age, sex, and body fat ( $P \geq 0.29$ ).

Moreover, while the circulating adi-

pokines leptin (positively) and adiponectin (negatively), adjusted for sex and age, were correlated with total body fat (leptin  $P < 0.0001$ ; adiponectin  $P = 0.06$ ), visceral fat (leptin  $P < 0.0001$ ; adiponectin  $P = 0.0006$ ), and subcutaneous abdominal fat (leptin  $P < 0.0001$ ; adiponectin  $P = 0.07$ ), circulating RBP4 was not correlated with these adipokines before (Table 2) or after adjustment for age, sex, and body fat (both  $P \geq 0.18$ ).

### Relationships between RBP4 levels and ectopic fat

We further tested whether circulating RBP4 is correlated with ectopic fat deposition in muscle. No associations of RBP4 levels with IMCL<sub>soleus</sub> or IMCL<sub>tibialis anterior</sub> were seen in univariate analyses (Table 2) or after adjustment for age, sex, and body fat (all  $P \geq 0.38$ ).

Because RBP4 is predominantly expressed in liver in rodents and presumably in humans as well (19) and the fatty liver represents an insulin-resistant state in which circulating RBP4 was found to be elevated, we hypothesized that plasma RBP4 levels may be associated with fat accumulation in the liver. Plasma RBP4 levels correlated significantly with liver fat before (Table 2) and after adjustment for sex, height, and body fat (Fig. 1D). The variability ( $r^2$ ) in liver fat that was gradually increased to 0.40 after inclusion of these covariates was further elevated to 0.44 after additional inclusion of plasma RBP4 levels into the model. In addition to this statistically parsimonious model, inclusion of visceral fat and IMCL that were positively correlated with liver fat (visceral fat  $r = 0.63$ ,  $P < 0.0001$ ; IMCL  $r = 0.22$ ,  $P = 0.08$ ) into the model only moderately affected the contribution of plasma RBP4 levels to the variability in liver fat (Table 3, model 1).

### Associations between plasma RBP4 levels, liver fat, and insulin sensitivity

As plasma RBP4 levels correlated both with insulin sensitivity and liver fat, we further tested whether these relationships were independent of each other. Plasma RBP4 levels contributed significantly to the variability in insulin sensitivity after additional adjustment for visceral fat and IMCL, which were determinants of insulin sensitivity (visceral fat  $r = -0.39$ ,  $P = 0.001$ ; IMCL  $r = -0.39$ ,  $P = 0.001$ ) (Table 3, model 2). Additional inclusion into the model of liver fat that was significantly correlated with insulin sensitivity ( $r =$

**Table 3—Determinants of liver fat and insulin sensitivity in multivariate linear regression models**

Dependent variable	Estimate $\pm$ SE	P
Liver fat		
Model 1 ( $r^2 = 0.50$ )		
Female sex	$-0.454 \pm 0.246$	0.07
Height (cm)	$-6.280 \pm 3.267$	0.06
Body fat <sub>MRT</sub> (kg)	$0.160 \pm 0.443$	0.72
Visceral fat <sub>MRT</sub> (kg)	$0.557 \pm 0.335$	0.10
IMCL (arbitrary units)	$0.236 \pm 0.178$	0.19
Plasma RBP4 levels ( $\mu\text{g/ml}$ )	$0.013 \pm 0.006$	0.04
Insulin sensitivity <sub>OGTT</sub>		
Model 2 ( $r^2 = 0.48$ )		
Female sex	$0.440 \pm 0.138$	0.002
Age (years)	$0.415 \pm 0.241$	0.09
Height (cm)	$7.699 \pm 1.733$	<0.0001
Body fat <sub>MRT</sub> (kg)	$-0.263 \pm 0.250$	0.30
Visceral fat <sub>MRT</sub> (kg)	$0.030 \pm 0.212$	0.89
IMCL (arbitrary units)	$-0.223 \pm 0.099$	0.03
Plasma RBP4 levels ( $\mu\text{g/ml}$ )	$-0.007 \pm 0.003$	0.035

$-0.54$ ,  $P < 0.0001$ ) ultimately rendered the contribution of RBP4 levels nonsignificant ( $P = 0.13$ ), suggesting that there is a close relationship between RBP4 levels and liver fat in determining insulin sensitivity.

### Longitudinal analyses

Thirty-three subjects underwent a lifestyle intervention (14 male/19 female subjects; age  $45 \pm 2$  years, BMI  $28.7 \pm 0.9$  kg/m<sup>2</sup>) with 9 months of follow-up. At baseline, plasma RBP4 levels were positively correlated with liver fat ( $r = 0.42$ ,  $P = 0.01$ ), and a nonsignificant trend was found between plasma RBP4 levels and insulin sensitivity (OGTT  $r = -0.30$ ,  $P = 0.09$ ). Under intervention, there was a mean decrease in liver fat ( $-31\%$ ,  $P < 0.0001$ ) and plasma RBP4 levels ( $-5\%$ ,  $P = 0.02$ ), while insulin sensitivity (OGTT) increased ( $+24\%$ ,  $P = 0.004$ ). A decrease in plasma RBP4 levels was associated with a decrease in liver fat ( $r = 0.37$ ,  $P = 0.04$ ) and an increase in insulin sensitivity ( $r = 0.61$ ,  $P = 0.0003$ ), adjusted for sex and baseline values.

**CONCLUSIONS**— Data from mice and humans suggested that circulating RBP4 is elevated in insulin-resistant and obese states. Our data confirm these findings regarding the relationship between circulating RBP4 and insulin resistance. In contrast, we cannot confirm that circulating RBP4 is elevated in obesity in humans. Plasma RBP4 levels were not significantly associated with total body fat

measured by bioelectrical impedance or more precisely determined by MRT. Also, no correlations of plasma RBP4 levels with visceral fat or subcutaneous abdominal fat measured by MRT were detected. Furthermore, no correlations of plasma RBP4 levels with the circulating adipokines leptin and adiponectin, which are strongly regulated by total adiposity and particularly by the amount of visceral fat (11,20–22), were observed. To our knowledge, this is the first study investigating the relationships of circulating RBP4 with precise measurements of adiposity in humans. Our findings regarding the lack of a correlation of plasma RBP4 levels with BMI are in agreement with three recent studies; among them, one did not detect elevated RBP4 expression in adipose tissue from obese subjects (23–25).

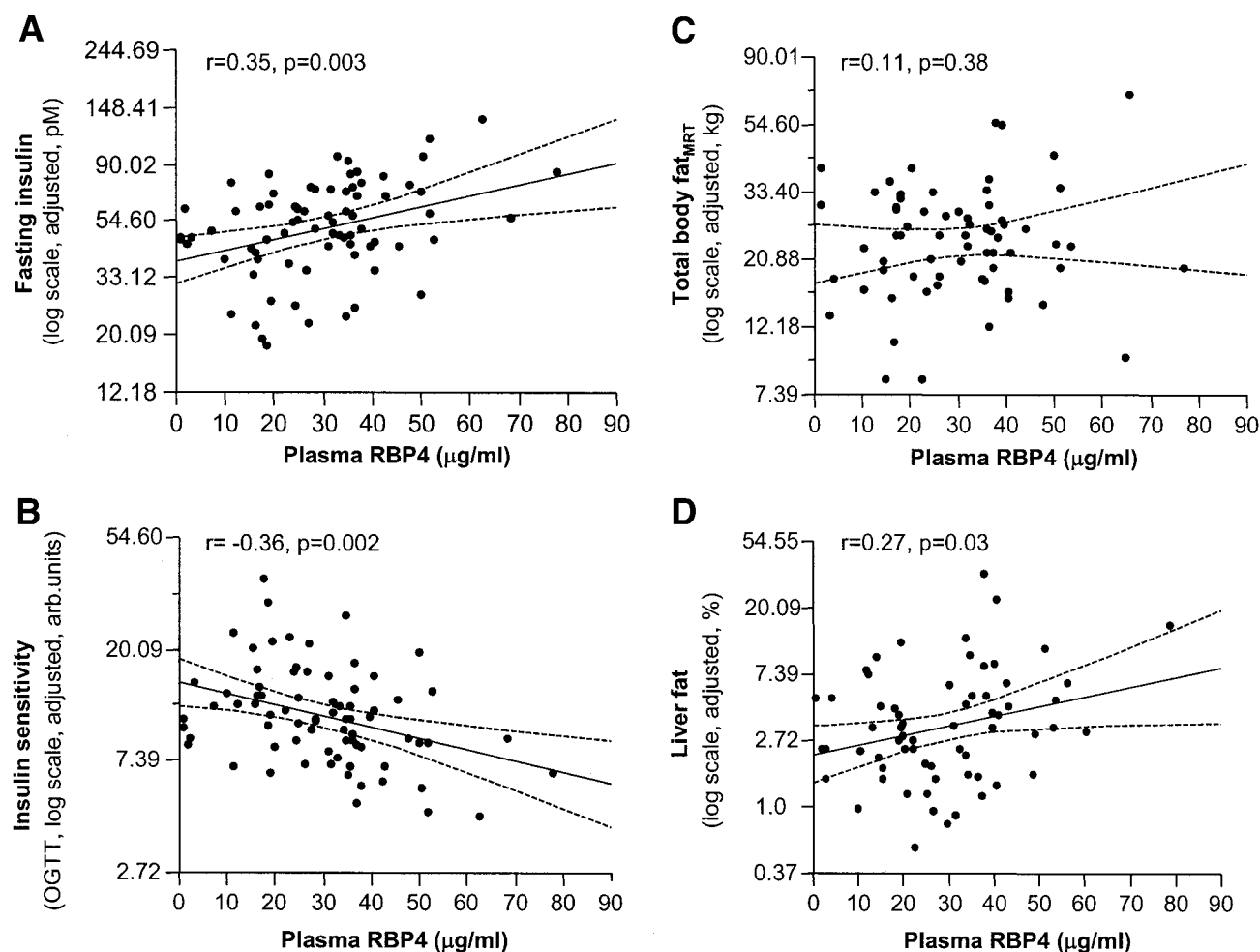
We further tested whether fat deposition in nonadipose tissue was associated with circulating RBP4. Particularly, intramyocellular fat deposition in the tibialis anterior and soleus muscle were found to be important determinants of impaired insulin-stimulated glucose disposal and type 2 diabetes (26–29). No significant relationships were found between RBP4 levels and intramyocellular lipids, suggesting that ectopic fat accumulation in these depots did not account for the elevated circulating RBP4.

Since the liver is the major source of RBP4 production in rodents and probably also in humans (19) and because ectopic fat deposition in the liver represents an insulin-resistant state (30–34), we tested

whether circulating RBP4 is elevated under increased fat accumulation in the liver. Particularly, hepatic fat accumulation was found to decrease insulin activation of glycogen synthase and increased gluconeogenesis, thus contributing to whole-body insulin resistance (35). Moreover, prevention of fatty liver (35) and decrease in hepatic fat accumulation (34) were found to largely affect insulin resistance. Indeed, we can provide novel information that plasma RBP4 levels correlated positively with fat deposition in the liver in humans. These cross-sectional data are supported by our longitudinal analyses, where a decrease in liver fat under lifestyle intervention was associated with a decrease in plasma RBP4 levels. In addition, high plasma RBP4 levels were also correlated with low insulin clearance in our study. In this aspect, it is of note that fatty liver was found to be strongly associated with low hepatic insulin clearance (36). Thus, the correlation of RBP4 with insulin clearance is compatible with the hypothesis of a close relationship between circulating RBP4 with liver function, possibly via hepatic fat accumulation.

Because of the relationship of RBP4 with liver fat and based on the fact that fatty liver is closely associated with hepatic insulin resistance, we asked whether circulating RBP4 is more strongly correlated with indexes of hepatic than whole-body insulin resistance. As expected, we found a significantly negative relationship between RBP4 levels and whole-body insulin sensitivity measured by the euglycemic-hyperinsulinemic clamp. Moreover, plasma RBP4 levels correlated strongly and more closely with indirect measurements of insulin sensitivity estimated from the OGTT and derived from fasting values such as fasting insulinemia and HOMA-IR. Whole-body insulin sensitivity measured by the clamp is a function of both insulin-stimulated glucose disposal and hepatic insulin sensitivity to suppress endogenous glucose production. In contrast, estimates of insulin sensitivity obtained from fasting values, particularly fasting insulinemia, largely represent insulin sensitivity of the liver (37–39). Thus, the close correlations of circulating RBP4 with these parameters may reflect stronger effects of RBP4 on hepatic insulin sensitivity than on insulin sensitivity of glucose disposal, possibly due to the relationship with liver fat and/or due to the stimulatory effects of RBP4 on gluconeogenesis (9).

To rule out that RBP4 reduces insulin



**Figure 1**—Relationships of RBP4 plasma levels (enzyme-linked immunosorbent assay) with fasting insulinemia (A), insulin sensitivity (16) (B), total body fat (C) determined by magnetic resonance imaging, and liver fat (D). In multivariate linear regression models, insulin sensitivity was adjusted for sex, age, body fat, and height. Total body fat was adjusted for sex and age. Liver fat was adjusted for its determinants: sex, body fat, and height (regression line and 95% CI).

action via effects on lipolysis, and thus regulation of serum FFAs, we determined its relationship with suppression of FFAs during the OGTT. Suppression of FFAs was significantly correlated with insulin sensitivity measured by the clamp; however, it was not correlated with RBP4 plasma levels. This makes it rather unlikely that RBP4 is involved in the regulation of lipolysis. Our findings regarding the relationships of circulating RBP4 with insulin sensitivity are consistent with two studies (10,22). However, other studies only detected a nonsignificant trend (24) or did not find significant relationships (25).

Our study is limited by the relatively small sample size, the fact that we have no data on the expression of RBP4 in fatty liver in humans, and the fact that we did not directly measure hepatic insulin sensitivity using tracer methods. On the other hand, we applied precise measure-

ments to get an insight into the role of RBP4 in glucose and lipid metabolism. In addition, the longitudinal analyses support the close relationships between plasma RBP4 levels, liver fat, and insulin sensitivity and imply that fatty liver may be a source of increased RBP4 production in humans. In this aspect, circulating RBP4 might serve as a biomarker of fatty liver. Alternatively, because RBP4 regulates retinoic acid action (40,41) and impaired retinoic signaling in the liver leads to hepatic steatosis (42), it cannot be excluded that high circulating RBP4 may contribute to increased fat accumulation in the liver. Further studies are warranted to test this hypothesis.

In conclusion, we show that high plasma RBP4 levels are not associated with total body, visceral, or subcutaneous abdominal fat or the ectopic fat depots in the muscle. However, they are specifically asso-

ciated with ectopic fat accumulation in the liver, low whole-body insulin sensitivity, low insulin clearance, and possibly low hepatic insulin sensitivity in humans.

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