Original Article

Retinol-Binding Protein 4 in Human Obesity

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Studies in mice suggest that adipocytes serve as glucose sensors and regulate systemic glucose metabolism through release of serum retinol-binding protein 4 (RBP4). This model has not been validated in humans. RBP4 was highly expressed in isolated mature human adipocytes and secreted by differentiating human adipocytes. In contrast to the animal data, RBP4 mRNA was downregulated in subcutaneous adipose tissue of obese women, and circulating RBP4 concentrations were similar in normal weight, overweight, and obese women (n = 74). RBP4 was positively correlated with GLUT4 expression in adipose tissue, independent of any obesity-associated variable. Five percent weight loss slightly decreased adipose RBP4 expression but did not influence circulating RBP4. In another set of experiments, we stratified patients (n = 14) by low or high basal fasting interstitial glucose concentrations, as determined by the microdialysis technique. Venous glucose concentrations were similar throughout oral glucose tolerance testing, and basal RBP4 expression in adipose tissue and serum RBP4 concentrations were similar in the groups with higher and lower interstitial glucose levels. Our findings point to profound differences between rodents and humans in the regulation of adipose or circulating RBP4 and challenge the notion that glucose uptake by adipocytes has a dominant role in the regulation of RBP4. *Diabetes* 55: 2805–2810, 2006

Studies in mice suggest that adipose tissue serves as a glucose sensor and regulates systemic glucose metabolism through release of a circulating factor in response to decreased intracellular glucose concentrations (1). Subsequent experiments led to the conclusion that adipose-derived serum retinol-binding protein 4 (RBP4) may represent such a circulating factor (2). RBP4 is upregulated in several insulin-resistant mouse models and in subjects with insulin resistance or type 2 diabetes (2–5). Furthermore, genetic manipulation of GLUT4 expression in adipose tissue led to reciprocal changes in adipose RBP4 expression and circulating RBP4 levels. Transgenic expression or injections of RBP4 caused insulin resistance in mice, whereas experimentally decreased RBP4 levels ameliorated insulin resistance in diet-induced obesity. Indeed, RBP4 augmented hepatic gluconeogenesis and attenuated insulin signaling in skeletal muscle (2). According to the animal data, impaired glucose uptake by adipocytes results in secondary systemic insulin resistance through RBP4 secretion. Thus, adipocytes may function as glucose sensors and regulators of whole-body insulin sensitivity by the secretion of adipokines. RBP4 was established as a rodent adipokine several years ago (6,7). However, the proposed model of the relationship between adipocyte GLUT4/glucose uptake and RBP4 formation has not been validated in humans. GLUT4 expression in adipocytes is known to be decreased in obesity (8,9). Thus, we established RBP4 as a human adipokine, tested the hypotheses that RBP4 is upregulated in human obesity, and examined whether GLUT4 and RBP4 gene expression are closely linked in human adipose tissue, as previously described in mice (2). Finally, we measured RBP4 gene expression in adipose tissue and circulating RBP4 in obese subjects with various degrees of adipose glucose uptake in vivo.

RESEARCH DESIGN AND METHODS

Clinical study. The institutional review board approved all studies (cross-sectional, weight-loss, microdialysis, and in vitro adipogenesis), and all subjects gave prior written informed consent. From a previously described (10) population of Caucasian menopausal women, we compared lean, overweight, and obese menopausal women in a cross-sectional study. In the weight-loss study, 30 Caucasian menopausal women started a dietary weight-reduction protocol and were counseled to reduce energy intake by 600 kcal/day, as previously described (11). Seventeen women reached the 5% weight-reduction goal after 13 weeks and were included in the analysis of adipose-tissue gene expression and serum RBP4. In both studies, no participants were diabetic or had renal or liver disease, congestive heart failure, or coronary heart disease. Hormone replacement therapy was discontinued 4 weeks before and all other medication 1 week before the studies. Participants had not changed their body weight by >1 kg in the 3 months before the study began. Anthropometric measurements and blood samples were obtained at 9:00 A.M. following an overnight fast. Periumbilical subcutaneous adipose tissue was obtained by needle biopsy as previously described (10,11).

Microdialysis study. Fourteen obese patients were recruited, and none had type 2 diabetes. All concomitant medication was withdrawn 2 weeks before testing. No lipid-lowering drugs were allowed for at least 6 months before study entry. All specimens were obtained from the patients on the same day after an overnight fast. One catheter was placed in a large antecubital vein for blood sampling. One microdialysis probe was inserted into abdominal subcutaneous adipose tissue as described elsewhere (12). After probe insertion, tissue perfusion was started with Ringer's solution (Serumwerk Bernburg, Bernburg, Germany) at a flow rate of 2 μl/min. The solution was supplemented with 50 mmol/l ethanol (Alkohol Konzentrat 95%; B. Braun Melsungen, Melsungen, Germany). CMA/60 microdialysis probes and CMA/102 microdialysis pumps (both from CMA Microdialysis, Solna, Sweden) were used. Sixty minutes were allowed for recovery of the tissues from insertion trauma and for baseline calibration. Then, volunteers ingested oral glucose (75 g glucose/300 ml solution, Dextro oral glucose tolerance test; Hoffmann-La Roche, Grenzach-Wyhlen, Germany). We obtained blood samples for the determination of glucose and insulin, and microdialysis samples were taken at baseline

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ELISA, enzyme-linked immunosorbent assay; HOMA, homeostasis model assessment; RBP4, retinol-binding protein 4.

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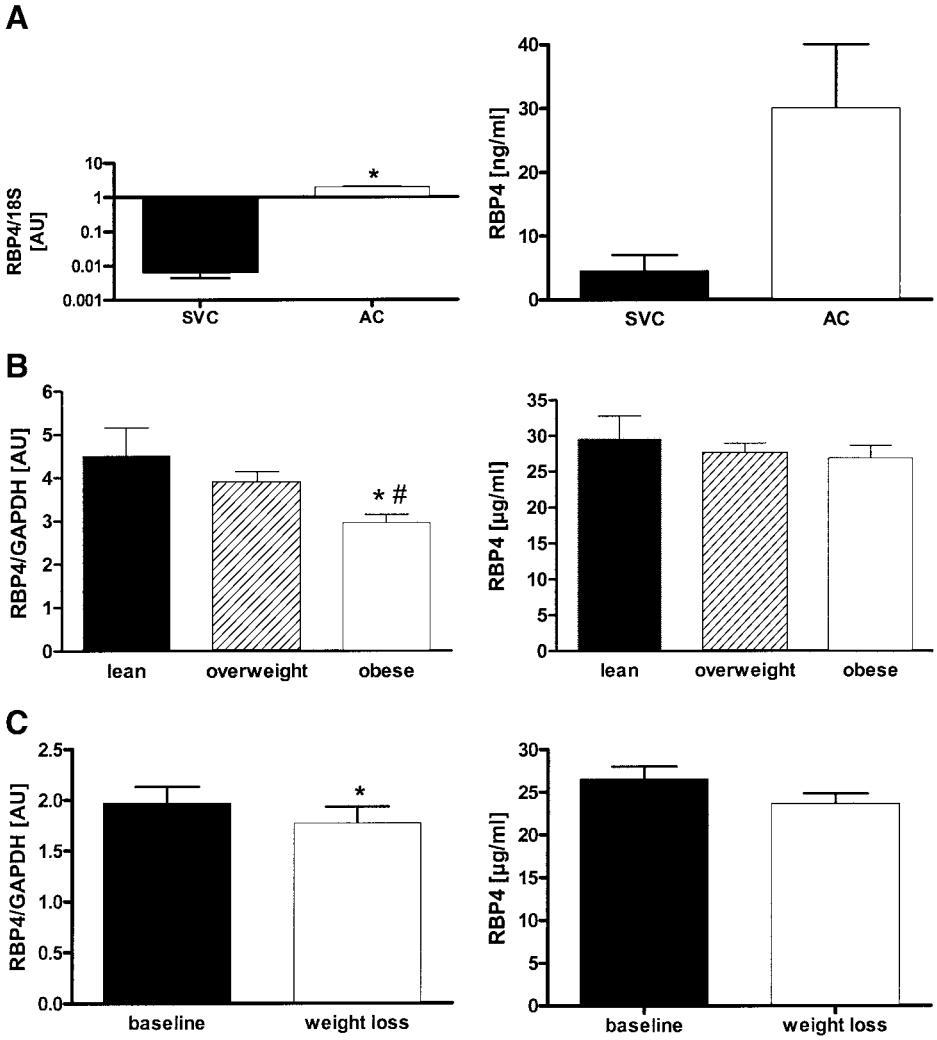

FIG. 1. RBP4 expression in adipose tissue and circulating RBP levels. *A*: Stromavascular cells (SVC) and mature human adipocytes (AC) were analyzed for RBP4 gene expression (n = 6 independent paired samples, *left*). Data are means ± SD and presented on a logarithmic scale. *P < 0.0001 by t test for paired samples. RBP4 secretion into the culture medium was compared between stromavascular cells and in vitro–differentiated adipocytes at day 12. Data are means ± SEM (n = 2). *B*: In a cross-sectional study including 74 menopausal women, RBP4 gene expression in subcutaneous abdominal adipose tissue (*left*) and RBP4 serum levels (*right*) were determined. Classification is according to BMI. Data are means ± SEM. Group comparison is by ANOVA and post hoc Bonferroni's multiple t test. *P < 0.05 vs. lean, #P < 0.05 vs. overweight. *C*: Seventeen women lost 5% of initial body weight by dietary calorie restriction. We measured RBP4 gene expression in adipose tissue (*left*) and RBP4 serum levels (*right*). Data are means ± SEM. Group comparison by t test for paired samples. *P < 0.05 vs. baseline.

and every 15 min for a 2-h period following the glucose load. One hour after the end of the glucose test, we obtained subcutaneous abdominal adipose tissue samples as previously described (12).

In vitro adipogenesis study. Mammary subcutaneous adipose tissue was obtained from healthy women (BMI 25–30 kg/m^2, aged 35–58 years) by breast reduction surgery for isolation of human adipose cells. Stromavascular cells and mature adipocytes were isolated by collagenase digestion and cultured overnight in serum containing medium as previously described (13). Total RNA was isolated from paired samples of stromavascular cells and mature adipocytes from each donor after 1 additional day of culture under serum-free conditions to compare RBP4 gene expression. Adipogenesis in stromavascular cells was induced by a cocktail of insulin, isobutylmethylxanthine, cortisol, and triiodothyronine as previously described (13). RBP4 secretion into the culture medium was compared between stromavascular cells and differentiated adipocytes at day 12 after induction of adipogenesis.

Analytical methods. Total RNA from preadipocytes, adipocytes, and adipose tissue biopsies was isolated using the Qiagen RNeasy mini kit (including the RNase-free DNase set; Qiagen, Hilden, Germany). RBP4 and GLUT4 gene expression were determined with the ABI 5700 Sequence Detection System for real-time PCR (PE Biosystems, Weiterstadt, Germany) by the standard-curve method and normalized by endogeneous controls, resulting in arbitrary units (AU) (13). Premixed Assays-on-Demand for human RBP4, GLUT4, glyceraldehyde-3 phosphate dehydrogenase (GAPDH), and 18S rRNA were used (PE Biosystems, Weiterstadt, Germany) that contain premixed primers and fluorescently labeled probes. Interassay coefficients of variation (CVs) for GAPDH (1.1%), 18S rRNA (0.9%), RBP4 (1.3%), and GLUT4 (1.4%) were determined using a standardized human adipose tissue cDNA from our laboratory.

RBP4 in serum samples was measured by competitive enzyme-linked immunosorbent assay (ELISA) (AdipoGen, Seoul, Korea) using the standard-curve method with a dilution series of a provided human RBP4 sample (R^2 = 0.99 for standard-curve linear regression). Interassay CV (measured by a control provided by the manufacturer with each plate) was 8.4%; intra-assay CV (measurement of six identical serum samples on each plate) was 5.3%.

Blood lipids, glucose, and insulin were measured by a certified laboratory. Changes in blood flow were determined using the ethanol dilution technique and Fick's principle. A decrease in the outflow-to-inflow ratio (ethanol ratio) is equivalent to an increase in blood flow and vice versa. Ethanol was measured with a standard enzymatic assay (14). Dialysate glucose and lactate concentrations were measured with the CMA/600 analyzer (CMA Microdialysis). In situ recovery for glucose and lactate in adipose tissue dialysates was ~30% (12).

Statistics. Data were analyzed by SPSS 13.0 (SPSS, Chicago, IL). Data are presented as the means ± SEM except otherwise stated. Unpaired or paired t tests or ANOVA (with Bonferroni's multiple t test for post hoc analysis) were used for group comparison as appropriate and are detailed in the figure legends. A linear regression model with stepwise exclusion of the least important contributing variables was used to identify determinants of RBP4

TABLE 1
Patient characteristics in the cross-sectional study

Variable	Lean	Overweight	Obese
n	15	26	33
Age (years)	57 ± 1	56 ± 1	58 ± 1
BMI (kg/m^2)	22.6 ± 0.5	27.6 ± 0.3*	34.9 ± 0.7*†
Waist circumference (cm)	76 ± 2	86 ± 2*	102 ± 2*†
$ABPM_{systolic}$ daytime (mmHg)	116 ± 8	125 ± 12	134 ± 14*
$ABPM_{diastolic}$ daytime (mmHg)	72 ± 5	77 ± 6	80 ± 7*
Mean daily heart rate (min^{-1})	74 ± 5	81 ± 8*	82 ± 8*
Total cholesterol (mmol/l)	5.4 ± 1.1	5.5 ± 0.7	5.5 ± 0.7
HDL cholesterol (mmol/l)	1.5 ± 0.4	1.4 ± 0.3	1.3 ± 0.3
LDL cholesterol (mmol/l)	3.5 ± 1.0	3.7 ± 0.8	3.7 ± 0.8
Triglycerides (mmol/l)	1.0 ± 0.4	1.0 ± 0.4	1.3 ± 0.6
HOMA index	0.5 ± 0.1	0.8 ± 0.1*	1.9 ± 0.2*†

Data are means ± SE. Group comparison by ANOVA and Bonferroni's multiple *t* test for post hoc analysis. *$P < 0.05$ vs. lean, †$P < 0.05$ vs. overweight. ABPM, ambulatory blood pressure measurement.

expression and serum levels in the cross-sectional study. Statistical significance was considered at $P < 0.05$.

RESULTS

We first compared the expression of the *RBP4* gene in human stromavascular cells with paired samples of mature adipocytes and found a ~300× difference. RBP4 gene expression in stromavascular cells was nearly undetectable. We then compared the secretion of RBP4 into the culture medium between stromavascular cells and in vitro–differentiated adipocytes at day 12 after induction of adipogenesis. (Sampling of the medium started at day 10 and lasted 2 days.) RBP4 secretion into the culture medium was detectable, and immature in vitro–differentiated adipocytes secreted considerably higher amounts of RBP4 than stromavascular cells (Fig. 1*A*).

Clinical data of the menopausal women in the cross-sectional study are given in Table 1. Subjects were stratified according to BMI into lean, overweight, and obese groups. RBP4 gene expression in adipose tissue was significantly reduced in obese subjects. RBP4 serum levels were not different between lean, overweight, and obese subjects (Fig. 1*B*). When we selected the 15 women with the lowest and highest RBP4 plasma levels (16.3 ± 0.5 vs. 41.6 ± 1.5 μg/ml [mean ± SEM], $P < 0.001$), we found no difference in the HOMA index of insulin resistance between both groups (1.1 ± 0.2 vs. 1.3 ± 0.3, $P = 0.57$). RBP4 adipose gene expression and RBP4 plasma concentrations were not correlated with each other in the cross-sectional study. A 5% weight reduction significantly improved the homeostasis model assessment (HOMA) index but only slightly decreased adipose RBP4 gene expression. RBP4 serum levels, however, were not influenced by this amount of weight loss (Fig. 1*C*).

GLUT4 mRNA in subcutaneous adipose tissue of obese women was significantly reduced (11.7 ± 1.6 vs. 7.2 ± 0.6 vs. 4.3 ± 0.4 AU for lean, overweight, and obese groups, respectively, $P < 0.001$). A stepwise linear multiple regression model including BMI, waist circumference, HOMA index, and GLUT4 revealed that only GLUT4 mRNA expression in adipose tissue was a significant determinant of adipose RBP4 expression ($r = 0.72$, $r^2 = 0.52$, $P < 0.001$ all variables included and $r = 0.70$, $r^2 = 0.49$, $P < 0.001$ after stepwise exclusion of all variables except GLUT4; Fig. 2). Circulating RBP4 and GLUT4 mRNA expression in subcutaneous adipose tissue were not correlated, and no significant determinants for RBP4 serum levels were identified in the cross-sectional study.

In the microdialysis study, we stratified subjects according to basal interstitial glucose concentrations into two groups of equal size. The groups were similar in age and BMI, as shown in Table 2. The groups also had similar adipose tissue blood flow and venous glucose levels, suggesting that glucose uptake is decreased in the group with higher interstitial glucose concentrations (Fig. 3). The difference in interstitial glucose remained throughout the glucose tolerance test. Dialysate lactate concentration was also increased in the group with higher glucose levels (not shown). Adipose tissue RBP4 expression and serum RBP4 levels were similar in the groups with lower and higher interstitial glucose concentrations.

DISCUSSION

Previous studies in mice suggested that RBP4 is an adipokine whose production is tightly regulated by adipose tissue glucose uptake. In an initial set of experiments, we observed that RBP4 gene expression was hardly detectable in stromavascular cells derived from human adipose tissue. Isolated mature human adipocytes featured high RBP4 mRNA levels. Indeed, we detected a steady increase in RBP4 secretion into the culture medium during adipogenesis. Remarkably, the increase in RBP4 secretion during adipocyte differention is much smaller than the increase in RBP4 gene expression. A possible explanation is that differentiating adipocytes at day 12 are not equal to mature isolated adipocytes from adipose tissue in their secretory capacity. This notion is supported by important phenotypical differences, such as adherence to the culture dish and accumulation of multiple small lipid droplets in differentiating adipocytes. Nevertheless, our findings suggest RBP4 as a human adipokine and confirm earlier reports in rodent adipocytes (6,7).

As we did not see a relationship between adipose tissue RBP4 expression and serum RBP4 levels in postmenopausal women, adipose tissue may be a less important

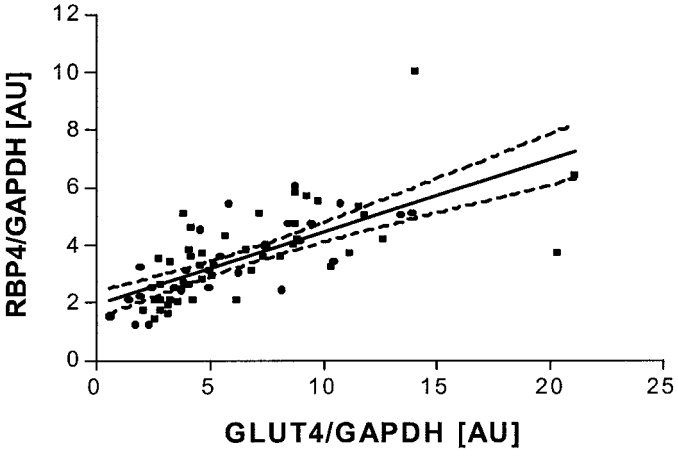

FIG. 2. Relationship between *GLUT4* and *RBP4* genes in adipose tissue. GLUT4 and RBP4 mRNA levels in subcutaneous abdominal adipose tissue of 74 menopausal women were measured, and the relationship was calculated by linear regression analysis. As no other confounding variables were identified for RBP4 gene expression; uncorrected data are shown with calculated regression line and 95% CI for the regression line ($r = 0.72$, $r^2 = 0.52$, $P < 0.001$).

TABLE 2
Patient characteristics in the microdialysis study

Variable	Lower glucose group	Higher glucose group
Basal adipose glucose (mmol/l)	0.16 ± 0.02	0.47 ± 0.03*
Women:men	3:4	4:3
Age (years)	55 ± 4	58 ± 3
BMI (kg/m^2)	35 ± 1	34 ± 1
HOMA index	1.8 ± 0.7	2.2 ± 0.6
RBP4 expression (AU)	2.0 ± 0.4	1.6 ± 0.3
Serum RBP4 (μg/ml)	28 ± 4	35 ± 4

Data are means ± SE. Group comparison by t test. *$P < 0.0001$.

source for circulating RBP4 in humans than in animals. But even in rodents, only 20% of systemic RBP4 is produced by adipocytes, and RBP4 gene expression was ~20% compared with expression in the liver (7), which is the major source for RBP4 in rodents and most likely also in humans (15). It is thus possible that the increase in systemic RBP4 concentrations in insulin-resistant subjects or subjects with type 2 diabetes (2–5) is not explained by increased RBP4 production in adipose tissue.

Animal studies suggested a tight reciprocal relationship between GLUT4 expression/glucose uptake in adipose tissue and RBP4 expression. We applied several strategies to assess the relationship in humans. First, we expected to observe an increase in adipose RBP4 expression and in circulating RBP4 concentrations with increasing adiposity, as adipocyte expression of GLUT4 is decreased in obesity (8,9). Yet, RBP4 mRNA was downregulated in subcutaneous abdominal adipose tissue of obese postmenopausal women, and circulating RBP4 concentrations were similar in the normal weight, overweight, and obese groups. Five-percent weight loss improved the HOMA index by 20% (11), while this change was associated with only a small decrease of adipose RBP4 expression and no significant change in RBP4 serum levels. In contrast, higher RBP4 serum levels were recently reported in overweight and obese subjects (2,3). Besides the use of different polyclonal antibodies and different methods for protein detection (ELISA in our study and ELISA or Western blotting in the other reports), it must be recognized that our study population is more homogeneous in terms of age of the control group, sex composition, and levels of fasting insulin.

Second, RBP4 expression and GLUT4 expression in adipose tissue were inversely correlated in mice (2). Similar to previous studies in animals and humans (8,9), we also found that adipose tissue GLUT4 expression was reduced in overweight subjects and even more so in obese subjects. We expected to find an inverse correlation between adipose GLUT4 and RBP4 expression. However, we found a robust positive correlation between RBP4 and GLUT4 expression that was completely independent of any other confounding variable (e.g., BMI). These findings suggest a role of adipose glucose uptake/GLUT4 in the regulation of human RBP4, but profound differences exist between rodents and humans.

Third, we directly assessed adipose tissue glucose metabolism using the microdialysis technique. Interstitial glucose concentrations in adipose tissue are influenced by systemic glucose concentrations, tissue blood flow, and adipose tissue glucose uptake. An impairment in adipose tissue glucose uptake causes a disproportional increase in interstitial compared with venous glucose concentrations (6). Therefore, we stratified patients into groups of equal size with low or high basal fasting interstitial glucose concentrations, as determined by the microdialysis technique. Tissue blood flow, which was estimated using the ethanol dilution technique (16), was not different between the groups. Furthermore, venous glucose concentrations were similar in both groups throughout oral glucose tolerance testing. Thus, differences in microdialysate glucose are presumably related to differences in cellular glucose uptake rather than glucose supply. We cannot explain the molecular origin of differences in adipocyte glucose uptake between the subjects. However, if decreased adipocyte glucose uptake is the mechanism to increase RBP4 expression, as demonstrated in mice, one

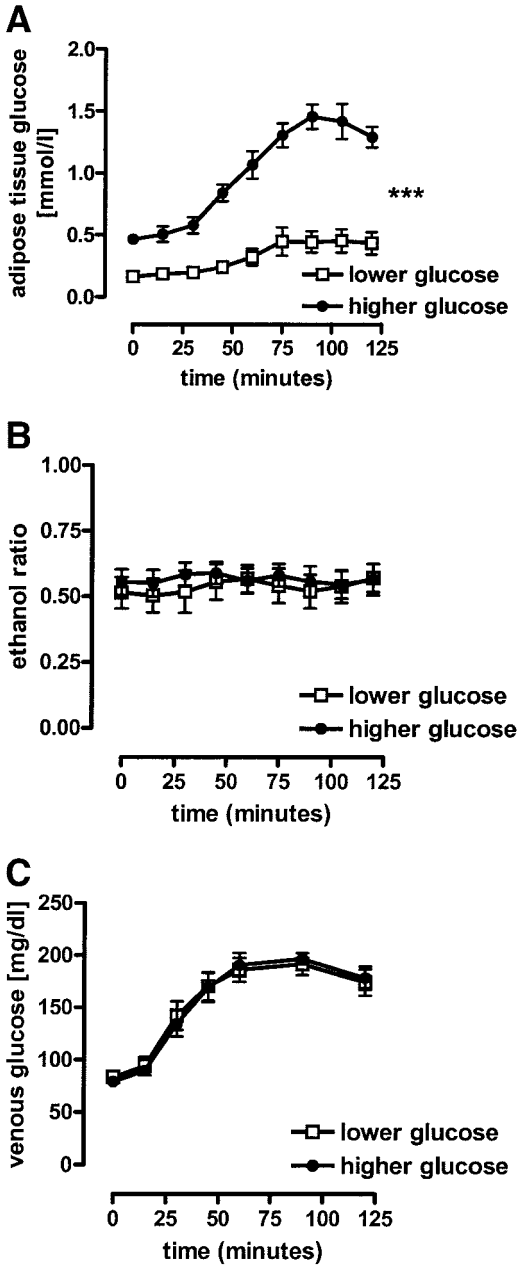

FIG. 3. Systemic and adipose tissue response to oral glucose loading. We stratified patients according to basal (fasting) interstitial glucose concentrations into groups with lower and higher interstitial glucose levels. Local blood flow (ethanol ratio) and venous glucose concentrations are shown during glucose load. ***$P < 0.0001$ by ANOVA.

would expect to observe excessive RBP4 levels in individuals with higher interstitial glucose concentrations. In any event, RBP4 expression in adipose tissue and serum RBP4 concentrations were similar in the groups with higher and lower interstitial glucose levels. Together, our findings challenge the notion that adipose glucose uptake has a dominant role in the regulation of adipose RBP4 expression or circulating RBP4 levels in humans.

An important limitation of our data is that we investigated only subcutaneous adipocytes/adipose tissue in all studies presented. We cannot rule out at present that differences exist between different adipose tissue depots (e.g., subcutaneous vs. visceral depots) in the regulation of RBP4 expression and secretion. This issue needs to be investigated in the future.

Even though RBP4 may be differentially regulated in mice and humans, RBP4 may elicit a similar physiological response. Genetic and pharmacological manipulation of RBP4 in mice led to consistent metabolic changes (2). RBP4 impaired insulin signaling in skeletal muscle at least in part through decreased phosphorylation of insulin receptor substrate 1. Furthermore, RBP4 stimulated glucose output from hepatoma cells in vitro and activated hepatic gluconeogenic enzymes in vivo including phosphoenolpyruvate kinase (2). Furthermore, in those patients that showed improved glucose handling after a period of exercise training, circulating RBP4 levels were clearly diminished, whereas those patients that did not improve by exercise training also did not show a change in circulating RBP4 (3). These findings clearly raise the question whether increased serum RBP4 in subjects with insulin resistance or type 2 diabetes is the cause or the consequence of insulin resistance.

It was not the primary purpose of our study to evaluate the relationship between RBP4 and insulin sensitivity. We did not see a relationship between the HOMA index and adipose RBP4 expression or with circulating RBP4 concentrations in our cross-sectional and weight-loss studies. Insulin resistance, determined by fasting insulin levels and the HOMA index, was not markedly decreased in our obese subjects, and fasting insulin levels in our obese subjects were clearly lower than in other study populations (2,3). We speculate that detection of RBP4-mediated changes in insulin sensitivity may require more accurate measurements of insulin sensitivity and patients with a wider range of insulin sensitivities than used in our study.

We and others (3) did not determine pharmacological effects of RBP4 but instead relied on correlational analysis. An important next step would be to test the response to exogenous RBP4 in humans. Another possibility would be to decrease circulating RBP4 levels by inhibiting the interactions between RBP4 and the binding partner transthyretin with fenretinide, a synthetic retinoid compound currently under investigation in oncology trials (17,18). Treatment with thiazolidinediones decreased RBP4 levels as well, as shown in rodents (2). However, this treatment option is not sufficiently specific to dissect out RBP4 effects from direct metabolic effects of peroxisome proliferator–activated receptor γ activation.

Secretory products from adipocytes partly link obesity with arterial hypertension, insulin resistance, inflammation, and atherosclerosis (19). Many of these adipokines have been identified in obese rodent models and confirmed in patients. Classical examples include the role of adiponectin for the development of type 2 diabetes and atherosclerosis (20,21), the role of leptin for sympathetic activation (22,23), and the role of adipose-derived angiotensinogen for blood pressure regulation (11,24). Other rodent adipokines such as resistin (25,26) or visfatin (27,28) turned out not to be human adipokines and play different roles in rodents and humans (29). Thus, careful evaluation of rodent adipokines in well-characterized human populations is crucial to define their precise role for obesity-associated diseases. Our data suggest that RBP4 is yet another adipokine with a differential regulation in animals and humans and a physiological role to define in future studies.

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REFERENCES

1. Abel ED, Peronie O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB: Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* 409:672–673, 2001
2. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB: Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 436:356–362, 2005
3. Graham TE, Yang Q, Blüher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U, Kahn BB: Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med* 354:2552–2563, 2006
4. Basualdo CG, Wein EE, Basu TK: Vitamin A (retinol) status of first nation adults with non-insulin dependent diabetes mellitus. *J Am Coll Nutr* 16:39–45, 1997
5. Abahusain MA, Wright J, Dickerson JW, de Vol EB: Retinol, alpha-tocopherol and carotenoids in diabetes. *Eur J Clin Nutr* 53:630–635, 1999
6. Zovich DC, Orologa A, Okuno M, Kong LW, Talmage DA, Piantedosi R, Goodman DS, Blaner WS: Differentiation-dependent expression of retinoid-binding proteins in BFC-1 beta adipocytes. *J Biol Chem* 267:13884–13889, 1992
7. Tsutsumi C, Okuno M, Tannous L, Piantedosi R, Allan M, Goodman DS, Blaner WS: Retinoids and retinoid-binding protein expression in rat adipocytes. *J Biol Chem* 267:1805–1810, 1992
8. Ezaki O, Fukuda N, Itakura H: Role of two types of glucose transporters in enlarged adipocytes from aged obese rats. *Diabetes* 39:1543–1549, 1990
9. Bjornholm M, Al Khalili L, Dicker A, Naslund E, Rössner S, Zierath JR, Arner P: Insulin signal transduction and glucose transport in human adipocytes: effects of obesity and low calorie diet. *Diabetologia* 45:1128–1135, 2002
10. Engeli S, Feldpausch M, Gorzelniak K, Hartwig F, Heintze U, Janke J, Möhlig M, Pfeiffer AF, Luft FC, Sharma AM: Association between adiponectin and mediators of inflammation in obese women. *Diabetes* 52:942–947, 2003
11. Engeli S, Böhnke J, Gorzelniak K, Janke J, Schling P, Bader M, Luft FC, Sharma AM: Weight loss and the renin-angiotensin-aldosterone system. *Hypertension* 45:356–362, 2005
12. Boschmann M, Engeli S, Adams F, Gorzelniak K, Franke G, Klaua S, Kreuzberg U, Lüdtke S, Kettritz R, Sharma AM, Luft FC, Jordan J: Adipose tissue metabolism and CD11b expression on monocytes in obese hypertensives. *Hypertension* 46:130–136, 2005
13. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM: Mature adipocytes inhibit in vitro differentiation of human preadipocytes via angiotensin type 1 receptors. *Diabetes* 51:1699–1707, 2002
14. Bernt E, Gutmann I: Ethanol determination with alcohol dehydrogenase and NAD. In *Methods of Enzymatic Analysis*. Bergmeyer HU, Ed. Weinheim, Germany, Verlag Chemie, 1974, p. 1499–1505
15. Blaner WS: Retinol-binding protein: the serum transport protein for vitamin A. *Endocr Rev* 10:308–316, 1989

16. Fellander G, Linde B, Bolinder J: Evaluation of the microdialysis ethanol technique for monitoring of subcutaneous adipose tissue blood flow in humans. *Int J Obes Relat Metab Disord* 20:220–226, 1996
17. Malpeli G, Folli C, Berni R: Retinoid binding to retinol-binding protein and the interference with the interaction with transthyretin. *Biochim Biophys Acta* 1294:48–54, 1996
18. Malone W, Perloff M, Crowell J, Sigman C, Higley H: Fenretinide: a prototype cancer prevention drug. *Expert Opin Investig Drugs* 12:1829–1842, 2003
19. Kershaw EE, Flier JS: Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 89:2548–2556, 2004
20. Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, Uchida S, Ito Y, Takakuwa K, Matsui J, Takata M, Eto K, Terauchi Y, Komeda K, Tsunoda M, Murakami K, Ohnishi Y, Naitoh T, Yamamura K, Ueyama Y, Froguel P, Kimura S, Nagai R, Kadowaki T: Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem* 278:2461–2468, 2003
21. Spranger J, Kroke A, Möhlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF: Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 361:226–228, 2003
22. Rahmouni K, Morgan DA, Morgan GM, Mark AL, Haynes WG: Role of selective leptin resistance in diet-induced obesity hypertension. *Diabetes* 54:2012–2018, 2005
23. Eikelis N, Schlaich M, Aggarwal A, Kaye D, Esler M: Interactions between leptin and the human sympathetic nervous system. *Hypertension* 41:1072–1079, 2003
24. Massiera F, Bloch-Faure M, Ceiler D, Murakami K, Fukamizu A, Gasc JM, Quignard-Boulange A, Negrel R, Ailhaud G, Seydoux J, Meneton P, Teboul M: Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *FASEB J* 15:2727–2729, 2001
25. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA: The hormone resistin links obesity to diabetes. *Nature* 409:307–312, 2001
26. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM: Resistin gene expression in human adipocytes is not related to insulin resistance. *Obes Res* 10:1–5, 2002
27. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I: Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 307:426–430, 2005
28. Berndt J, Kloting N, Kralisch S, Kovacs P, Fasshauer M, Schon MR, Stumvoll M, Blüher M: Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes* 54:2911–2916, 2005
29. Curat CA, Wegner V, Sengenes C, Miranville A, Tonus C, Busse R, Bouloumie A: Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. *Diabetologia* 49:744–747, 2006