

Plasma levels and adipose tissue messenger ribonucleic acid expression of retinol-binding protein 4 are reduced during calorie restriction in obese subjects but are not related to diet-induced changes in insulin sensitivity.

Michaela Vítková, Eva Klimčáková, Michaela Kovacikova, Carine Valle, Cédric Moro, Jan Polák, Jiri Hanacek, Frédéric Capel, Nathalie Viguerie, Blanka Richterova, et al.

► **To cite this version:**

Michaela Vítková, Eva Klimčáková, Michaela Kovacikova, Carine Valle, Cédric Moro, et al.. Plasma levels and adipose tissue messenger ribonucleic acid expression of retinol-binding protein 4 are reduced during calorie restriction in obese subjects but are not related to diet-induced changes in insulin sensitivity.. *Journal of Clinical Endocrinology and Metabolism, Endocrine Society*, 2007, 92 (6), pp.2330-5. <10.1210/jc.2006-2668>. <inserm-00409823>

HAL Id: inserm-00409823

<http://www.hal.inserm.fr/inserm-00409823>

Submitted on 8 Aug 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Plasma Levels and Adipose Tissue Messenger Ribonucleic Acid Expression of Retinol-Binding Protein 4 Are Reduced during Calorie Restriction in Obese Subjects but Are Not Related to Diet-Induced Changes in Insulin Sensitivity

Michaela Vitkova,* Eva Klimcakova,* Michaela Kovacicova, Carine Valle, Cédric Moro, Jan Polak, Jiri Hanacek, Frédéric Capel, Nathalie Viguerie, Blanka Richterova, Magda Bajzova, Jindra Hejnova, Vladimir Stich, and Dominique Langin

Institut National de la Santé et de la Recherche Médicale (M.V., E.K., M.K., C.V., C.M., J.P., F.C., N.V., B.R., M.B., J.He., V.S., D.L.), Franco-Czech Laboratory for Clinical Research on Obesity, Prague, CZ-10100 Czech Republic; Department of Sports Medicine (M.V., E.K., M.K., J.P., B.R., M.B., J.He., V.S.), and Division of Cell and Molecular Biology (M.K.), 3rd Faculty of Medicine, Charles University, Prague, CZ-100 00 Czech Republic; Institut National de la Santé et de la Recherche Médicale (M.V., E.K., C.V., C.M., F.C., N.V., D.L.), U858, Obesity Research Laboratory, Toulouse, F-31432 France; Institute for Mother and Child Care (J.Ha.), Prague, Czech Republic; Paul Sabatier University (E.K., C.V., C.M., F.C., N.V., D.L.), Louis Bugnard Institute, IFR31, Toulouse, F-31432 France; and Centre Hospitalier Universitaire de Toulouse (D.L.), Biochemistry Laboratory, Biology Institute of Purpan, Toulouse, F-31059 France

Context: Retinol-binding protein 4 (RBP4) may play a role in the development of insulin resistance.

Objective: We investigated whether RBP4 adipose tissue mRNA expression and plasma level are related to insulin sensitivity during a diet-induced weight loss.

Design, Setting, Patients, and Intervention: Obese women followed a dietary intervention composed of a 4-wk very low-calorie diet (VLCD), a 2-month low-calorie diet, and 3–4 months of a weight maintenance (WM) phase.

Main Outcome Measures: Clinical investigation was performed before and at the end of each phase. Insulin sensitivity was assessed with the euglycemic hyperinsulinemic clamp. Adipose tissue mRNA and plasma levels of RBP4 were determined using reverse transcription-quantitative PCR and ELISA, respectively.

Results: Weight and fat mass decreased during VLCD and were stabilized during WM. Glucose disposal rate increased during VLCD and remained elevated thereafter. Plasma levels of RBP4 decreased after VLCD and, although increasing at subsequent phases, remained lower than prediet values. Adipose tissue mRNA levels were diminished after VLCD, and increased during low-calorie diet and WM to reach basal values. Basal RBP4 levels or diet-induced variations of RBP4 were not different in lean women and two groups of obese women with high- and low-insulin sensitivity.

Conclusions: Severe calorie restriction promotes a reduction in adipose tissue and plasma levels of RBP4. The study does not bring evidence for a role for RBP4 in the regulation of diet-induced changes in insulin sensitivity. (*J Clin Endocrinol Metab* 92: 2330–2335, 2007)

TYPE 2 DIABETES is characterized by insulin resistance and relative insulin deficiency. The resistance to insulin action occurs in multiple tissues, including the liver with an increase in glucose production and skeletal muscles with a decrease in glucose use. Insulin resistance independent of overt diabetes is an important causative factor of the metabolic syndrome and constitutes an important risk factor for cardiovascular disease. Obesity is one of the principal causes

for insulin resistance and risk factors for type 2 diabetes. The excess of fat mass is associated with release of multiple molecules with paracrine or endocrine action by adipose tissue that may contribute to the development of insulin resistance (1). A recently characterized potential candidate is retinol-binding protein 4 (RBP4) (2). Adipose tissue RBP4 expression and circulating levels are increased in several mouse models of insulin resistance. Genetic knockout of the insulin-stimulated glucose transporter 4 (Glut4) selectively in adipocytes results in impaired whole-body insulin sensitivity (3). Because adipocytes contribute little to whole-body glucose disposal, the existence of a factor released by the adipocytes and acting on the liver and skeletal muscle was predicted. RBP4 has been identified as such a factor (2). Overexpression of RBP4 or injection of recombinant RBP4 induced insulin resistance in mice, whereas pharmacologically decreased serum levels of RBP4 improved insulin sensitivity in high-fat

First Published Online April 3, 2007

* M.V. and E.K. contributed equally to the work.

Abbreviations: BMI, Body mass index; Glut4, glucose transporter 4; LCD, low-calorie diet; PPAR, peroxisome proliferator-activated receptor; RBP4, retinol-binding protein 4; VLCD, very low-calorie diet; WM, weight maintenance.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

diet-fed mice. Indeed, RBP4 impairs insulin signaling in skeletal muscle and affects glucose output in the liver.

RBP4 has been used clinically as a rapid turnover protein for assessing the short-term fluctuation of nutritional states. RBP4 is the only specific transport protein for retinol (vitamin A) in the circulation (4). It is produced by hepatocytes, which are believed to contribute to a large part of circulating RBP4, although adipocytes have the second-highest expression level (5). Elevated RBP4 levels have been reported in subjects with insulin resistance and type 2 diabetes (6–9). Correlations have been observed between serum RBP4 levels and the magnitude of insulin resistance in different groups of subjects (8). However, the cross-sectional design of most of the clinical studies performed so far does not allow for a determination of a putative causal role of RBP4 in the pathogenesis of insulin resistance and type 2 diabetes in humans (10). Here, we studied RBP4 in obese subjects enrolled in a multiple-phase weight reduction program based on a 4-wk very low-calorie diet (VLCD), followed by a 2-month low-calorie diet (LCD), and 3–4 months of a weight maintenance (WM) phase. RBP4 mRNA expression in sc adipose tissue and plasma RBP4 levels were determined before and at the end of each phase, and related to anthropometric and biological parameters, including glucose disposal rate assessed by the euglycemic hyperinsulinemic clamp.

Subjects and Methods

In vitro adipose tissue studies

Subcutaneous abdominal adipose tissue obtained from overweight women undergoing plastic surgery was digested with collagenase. Mature adipocytes were separated from the stromavascular fraction by mild centrifugation. Isolation of different cell types (endothelial cells, preadipocytes, and macrophages) in the stromavascular fraction was performed using surface antigen-coupled magnetic microbeads (11). Human preadipocytes in primary culture were differentiated as described (12). At d 13, 60–80% of cells were differentiated into lipid droplet-containing adipocytes. For culture of human adipose tissue explants, sc abdominal adipose tissue was cut into small pieces weighing approximately 10 mg or less. After washing steps, explants were cultured in DMEM F12 medium (Cambrex Corp., East Rutherford, NJ) containing 33 $\mu\text{mol/liter}$ biotin, 17 $\mu\text{mol/liter}$ pantothenate, and 50 $\mu\text{g/ml}$ gentamycin supplemented with 10% of fetal calf serum. Explants were preincubated overnight to allow for removal of soluble factors and cellular debris released by cells broken during the preparation of the small pieces of adipose tissue. On d 2, explants were washed three times with PBS. Explants (200 mg/ml) were then incubated for 24 h in fresh medium. Aliquots of the medium were stored at -80°C for protein measurements. These studies were in agreement with the French National Institute of Health and Medical Research (Inserm) and the Toulouse University Hospital ethics regulation.

Subjects

Participants in the study were recruited at the Third Faculty of Medicine of Charles University and at the Institute for Mother and Child Care in Prague, Czech Republic. The clinical investigation was performed at the Department of Sports Medicine of the Third Faculty of Medicine. A group of 24 obese premenopausal women was included in the study. Exclusion criteria were weight changes of more than 3 kg within the 3 months before the start of the study, hypertension, diabetes, or hyperlipidemia treated by drugs, drug-treated obesity, pregnancy, participation in other trials, and alcohol or drug abuse. A control group of 12 lean women [age 38 ± 10 yr; body mass index (BMI) 21 ± 2 kg/m^2] was also investigated. The studies were approved by the Ethical Committee of the Third Faculty of Medicine. Volunteers were informed of the study, and written consent was obtained before study participation.

Dietary intervention

During the first 4 wk of the dietary intervention program, the obese subjects received a 800 kcal/d VLCD (liquid formula diet; Redita, Promil, Czech Republic). During the next 2 months, a LCD was designed to provide 600 kcal/d less than the individually estimated energy requirement based on an initial resting metabolic rate multiplied by 1.3, the coefficient of correction for physical activity level. The final period was a WM phase of 3–4 months, during which the patients were instructed to keep on a weight-maintaining diet. Patients consulted a dietitian once a week during the first 3 months of the program and once a month during the WM phase. They provided a written 3-d dietary record at each dietary consultation.

Clinical investigation

A complete clinical investigation was realized before and at the end of each phase in the morning. Anthropometric and resting metabolic rate measurements were performed as previously reported (13). Body composition was determined with multifrequency bioimpedance (Bodystat QuadScan 4000; Bodystat Ltd., Isle of Man, British Isles). Blood samples were drawn from an indwelling catheter in the antecubital vein. Needle microbiopsy of sc adipose tissue was performed under local anesthesia (1% Xylocaine; AstraZeneca PLC, London, UK) from the abdominal region (14–20-cm lateral to the umbilicus) (14). The euglycemic hyperinsulinemic clamp was performed according to the DeFronzo method (15). Priming plus continuous infusion of crystalline human insulin (Actrapid Human; Novo, A/S, Bagsvaerd, Denmark), 40 mU/m² body area·min, was given for 210 min. Euglycemia (the fasting blood glucose concentration) was maintained by a variable 20% glucose infusion. The infusion rate was determined by measuring arterialized plasma glucose every 5 min (Beckman Glucose Analyzer; Beckman Coulter, Inc., Fullerton, CA). Glucose consumption was calculated from the exogenous glucose infusion rates during the last 30 min of the clamp and corrected for kilogram of body weight ($\text{mg}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) or kilogram of fat-free mass ($\text{mg}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ fat-free mass).

RNA analysis

Total RNA was extracted from adipose tissue biopsy samples, explants, and cells using the RNeasy Mini kit (QIAGEN, Inc., Valencia, CA). RT was performed with 500-ng total RNA using random hexamers (Promega Corp., Madison, WI) and Superscript II Reverse Transcriptase (Invitrogen Corp., Carlsbad, CA). Real-time quantitative PCR was performed with TaqMan probe-based gene expression assays for RBP4, Glut4, peroxisome proliferator-activated receptor (PPAR) γ , and CD68, and a SYBR Green-based assay for adiponectin using ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA). An 18S ribosomal RNA was used as control to normalize gene expression (Ribosomal RNA Control TaqMan Assay kit; Applied Biosystems). Each sample was performed in duplicate, and 10-ng cDNA was used as a template for real-time PCR. When the difference between the duplicates was above 0.5 Ct (threshold cycle), real-time PCR was performed again. Results are expressed as $2^{-\Delta\text{Ct}}$ values.

Determination of culture medium and plasma levels

RBP4 levels were measured using an ELISA kit (Immundiagnostik AG, Bensheim, Germany). Plasma samples were diluted so that the absorbance was in the middle of the range of linearity for the assay. Within-run coefficient of variation for RBP4 was 2.7%. Plasma glucose was determined by the glucose hexokinase technique (Konelab 60i; Labsystems CLD, Konelab, Finland). Plasma insulin was measured using chemiluminescent immunometric assay (Immulite 2000 Insulin; DPC Czech sro, Brno, Czech Republic). Nonesterified fatty acid levels were determined using an enzymatic procedure (Wako; Unipath Ltd., Bedford, UK). Leptin and adiponectin levels were determined using ELISA kits (BioVendor Laboratory Medicine, Brno, Czech Republic). Plasma β -hydroxybutyrate was measured by an enzymatic “Liqui-Color” kit (Stanbio Laboratory, Boerne, TX). Plasma levels of other parameters were determined using standard clinical biochemistry methods. To determine cell damage in adipose tissue explant culture, mea-

surement of adenylate kinase activity was performed in the culture medium using a bioluminescent assay (Cambrex, Corp.).

Statistical analysis

Data were analyzed using the SPSS software (SPSS, Inc., Chicago, IL). Nonparametric Wilcoxon signed rank or Mann-Whitney *U* tests were used for comparison of paired and unpaired values, respectively. Correlations were analyzed by Spearman's nonparametric test. The level of significance was set at $P < 0.05$.

Results

Expression of RBP4 in human adipose tissue

Comparison of mRNA expression in mature adipocytes and stromavascular cells from human sc adipose tissue revealed that RBP4 is expressed almost exclusively in mature adipocytes (Fig. 1A). As a control of cell isolation, Glut4 and

adiponectin mRNA were detected mostly in adipocytes and CD68 mRNA, mostly in the stromavascular fraction. Direct detection on isolated endothelial cells, macrophages, and preadipocyte-like cells confirmed that the level of RBP4 expression was very low compared with that in mature adipocytes (data not shown). RBP4 gene expression was strongly induced during the conversion of human preadipocytes into adipocytes along with markers of adipocyte differentiation, such as PPAR γ and adiponectin (Fig. 1B). We also wished to determine whether RBP4 was produced by human sc adipose tissue. Primary culture of adipose tissue explants showed that the production rate of RBP4 *in vitro* was comparable to that of adiponectin, a factor secreted at high levels by adipocytes (Fig. 1C). The release of adenylate kinase from the explants was very low, indicating that cell damage was limited in our culture conditions (data not shown).

Clinical parameters of obese subjects during a weight reduction program

Obese women followed a 6-month weight reduction program composed of three successive phases: a 4-wk 800 kcal/d VLCD, a 2-month LCD with 600 kcal less than the calculated daily energy requirements, and a 3–4 month WM diet. Anthropometric and plasma parameters were determined before and at the end of each dietary phase (Table 1). The subjects' body weight and BMI decreased during the VLCD and LCD phases. The body weight was stabilized during WM. The loss of weight was chiefly due to a decrease in fat mass. Evolution of waist circumference followed the same pattern as body weight. Regarding plasma parameters, there was a decrease in insulin, leptin, triglyceride, glycerol, and cortisol levels at all the time points. Glycemia was decreased only after VLCD, and the nonesterified fatty acid level was decreased at the end of the protocol. Circulating β -hydroxybutyrate was elevated during VLCD and then decreased during subsequent phases to reach basal levels at the end of the program. Plasma adiponectin level was not changed during the dietary intervention. To evaluate insulin sensitivity, euglycemic hyperinsulinemic clamps were performed at each phase (Table 1). The glucose disposal rate increased during VLCD, and remained elevated throughout the phases of LCD and WM.

Adipose tissue mRNA expression of RBP4 and Glut4 during a weight reduction program

Subcutaneous abdominal adipose tissue biopsies were performed before and at the end of each dietary phase. RBP4 mRNA expression decreased during VLCD (Fig. 2A). During LCD, there was an increase in RBP4 mRNA levels ($P < 0.01$) so that at the end of the LCD phase and at the end of WM, RBP4 mRNA values were not different from basal values. The profile of Glut4 mRNA expression was similar to that of RBP4 (Fig. 2B).

Plasma levels of RBP4 during a weight reduction program

Plasma RBP4 levels were decreased during VLCD (Fig. 2C). The LCD and WM phases were characterized by a gradual increase in RBP4 levels. At the end of the dietary intervention, plasma RBP4 levels were higher than VLCD values ($P < 0.01$).

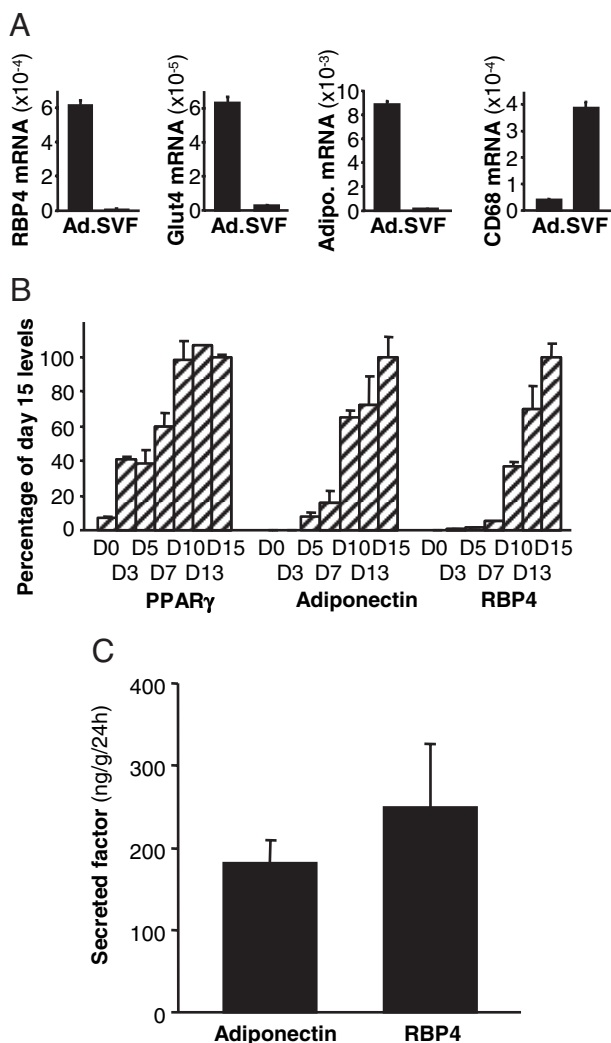


FIG. 1. RBP4 expression and production in human sc adipose tissue. A, RBP4, Glut4, adiponectin (Adipo), and CD68 mRNA expression was determined in mature adipocytes (Ad) and stromavascular cells (SVF) ($n = 6$). B, Time course of RBP4, adiponectin, and PPAR γ mRNA expression during differentiation of human preadipocytes into adipocytes from d 0 (D0) to d 15 (D15) ($n = 4$). C, Production of RBP4 and adiponectin by human adipose tissue explants ($n = 6$). The levels of secreted products were normalized to the mass of adipose tissue. Values are means \pm SEM.

TABLE 1. Clinical parameters of 24 obese women before and at the end of different phases of a weight reduction program

Parameter	Basal	VLCD 4 wk	LCD 8 wk	WM 12–16 wk
Weight (kg)	97 ± 16	90 ± 15 ^c	87 ± 15 ^c	87 ± 15 ^c
BMI (kg/m ²)	35 ± 5	33 ± 5 ^c	32 ± 4 ^c	32 ± 5 ^c
Fat mass (kg)	40 ± 12	35 ± 11 ^c	31 ± 9 ^c	32 ± 11 ^c
Fat-free mass (kg)	58 ± 6	55 ± 7 ^b	56 ± 8 ^b	55 ± 7 ^c
Waist (cm)	104 ± 13	99 ± 13 ^c	96 ± 13 ^c	96 ± 13 ^c
Glucose (mmol/liter)	5.6 ± 0.4	5.3 ± 0.6 ^a	5.4 ± 0.7	5.3 ± 0.8
Insulin (mU/liter)	13.6 ± 8.3	6.8 ± 3.7 ^c	5.8 ± 2.6 ^c	6.8 ± 2.8 ^c
Glycerol (μmol/liter)	217 ± 77	160 ± 39 ^b	145 ± 59 ^c	150 ± 43 ^c
Nonesterified fatty acid (μmol/liter)	693 ± 144	741 ± 126	589 ± 170 ^a	564 ± 171 ^a
β-hydroxybutyrate (mmol/liter)	0.16 ± 0.09	0.65 ± 0.36 ^c	0.27 ± 0.16 ^c	0.19 ± 0.17
Total cholesterol (mmol/liter)	4.82 ± 0.66	3.90 ± 0.76 ^c	4.29 ± 0.69 ^c	4.56 ± 0.66 ^a
Triglycerides (mmol/liter)	1.43 ± 0.69	1.06 ± 0.35 ^c	1.06 ± 0.37 ^b	1.03 ± 0.29 ^b
Leptin (ng/ml)	41 ± 15	20 ± 13 ^c	24 ± 14 ^c	27 ± 15 ^c
Adiponectin (μg/ml)	8.4 ± 3.9	8.4 ± 3.3	8.3 ± 3.3	9.2 ± 3.6
Cortisol (nmol/liter)	205 ± 96	157 ± 67 ^b	146 ± 61 ^b	177 ± 102 ^a
Glucose disposal rate (mg/kg·min)	2.98 ± 1.56	3.61 ± 1.65 ^b	4.05 ± 1.66 ^c	4.19 ± 1.74 ^c
Glucose disposal rate (mg/kg fat-free mass·min)	4.88 ± 2.29	5.75 ± 2.43 ^a	6.15 ± 2.31 ^c	6.48 ± 2.51 ^c

Values are means ± SD.

^a $P < 0.05$.

^b $P < 0.01$.

^c $P < 0.001$ compared to basal values.

Nevertheless, throughout the dietary protocol, plasma RBP4 levels remained lower than the levels at the beginning of the program. The evolution of RBP4 levels was different from that of the glucose disposal rate that shows steadily higher values at the different time points of the dietary intervention than at the beginning of the program (Fig. 2D).

Relationship between plasma RBP4 levels and insulin resistance

No correlations were found between RBP4 level and glucose disposal rate before the diet ($r = -0.31$; $P > 0.1$). Similarly, no correlations were found between the diet-induced changes of RBP4 and glucose disposal rate when considering either the VLCD phase or the whole dietary program ($r = -0.22$, $P > 0.3$; $r = -0.14$, $P > 0.5$, respectively). A similar conclusion was reached when the glucose disposal rate was corrected for fat-free mass. To investigate whether the lack of relationship between RBP4 and glucose disposal rate is dependent on the level of baseline insulin sensitivity, the 24 obese subjects were stratified into two groups according to prediet glucose disposal rate (Table 2). No differences in either basal plasma RBP4 levels or in the diet-induced decreases of plasma levels were observed between the two groups. Similarly, if the subjects were stratified into two groups according to the magnitude of the changes in glucose disposal rate during VLCD and the entire program, there was no difference in the diet-induced variation of plasma RBP4 levels between the groups (data not shown). No correlations were found between plasma RBP4 and plasma triglyceride, high-density lipoprotein cholesterol, or waist circumference when considering the diet-induced responses of the variables (data not shown). To investigate further the relationship between plasma RBP4 levels and insulin resistance, a group of control lean women (BMI 21 ± 2 kg/m²) was investigated and compared with the obese group. As expected, the glucose disposal rate was higher in the lean group than in the obese group (6.43 ± 1.61 vs. 2.98 ± 1.56 mg/kg/min, $P < 0.001$; and 8.27 ± 1.73 vs. 4.88 ± 2.29 mg/kg fat free mass/min, $P <$

0.001). However, plasma RBP4 levels did not differ between the two groups (26.8 ± 8.4 vs. 27.4 ± 7.4 mg/ml; $P > 0.8$).

Discussion

This study shows that RBP4 is strongly expressed in human adipocytes, as shown earlier in rat adipocytes (5). Negligible expression was detected in the stromavascular fraction of adipose tissue, as recently reported (16). During adipogenesis of human preadipocytes, there was a very strong induction of RBP4, which is typical of a marker of adipocyte differentiation (17). RBP4 is steadily secreted by human adipose tissue, as shown in experiments on adipose tissue explants. Therefore, RBP4 expression profile in human adipose tissue is similar to the profile described in rodents.

Studies in mice suggest that RBP4 is a factor produced by adipose tissue that induces insulin resistance in the liver and skeletal muscle (2). Plasma RBP4 was reported to be elevated in subjects with insulin resistance and type 2 diabetes, although the relationship between RBP4 and insulin resistance was not found in other cross-sectional studies on subjects with normal glucose tolerance or mildly insulin-resistant obese patients (6–8, 16, 18). Similarly, we did not find differences in plasma RBP4 levels between lean and obese women despite a lower glucose disposal rate in the latter group. To gain further insight into the relationship between insulin sensitivity and RBP4, we investigated, in a prospective study, nondiabetic obese women during different time points of a multiple-phase weight reduction program. Insulin sensitivity was assessed by the gold standard technique, the euglycemic hyperinsulinemic clamp. The subjects had a marked decrease in body weight and fat mass during VLCD, a further moderate diminution during LCD and a stabilization during the WM phase. The glucose disposal rate was increased during VLCD and remained elevated during the subsequent phases. The plasma levels of RBP4 were markedly diminished during VLCD and subsequently increased during the later phases while remaining lower than basal

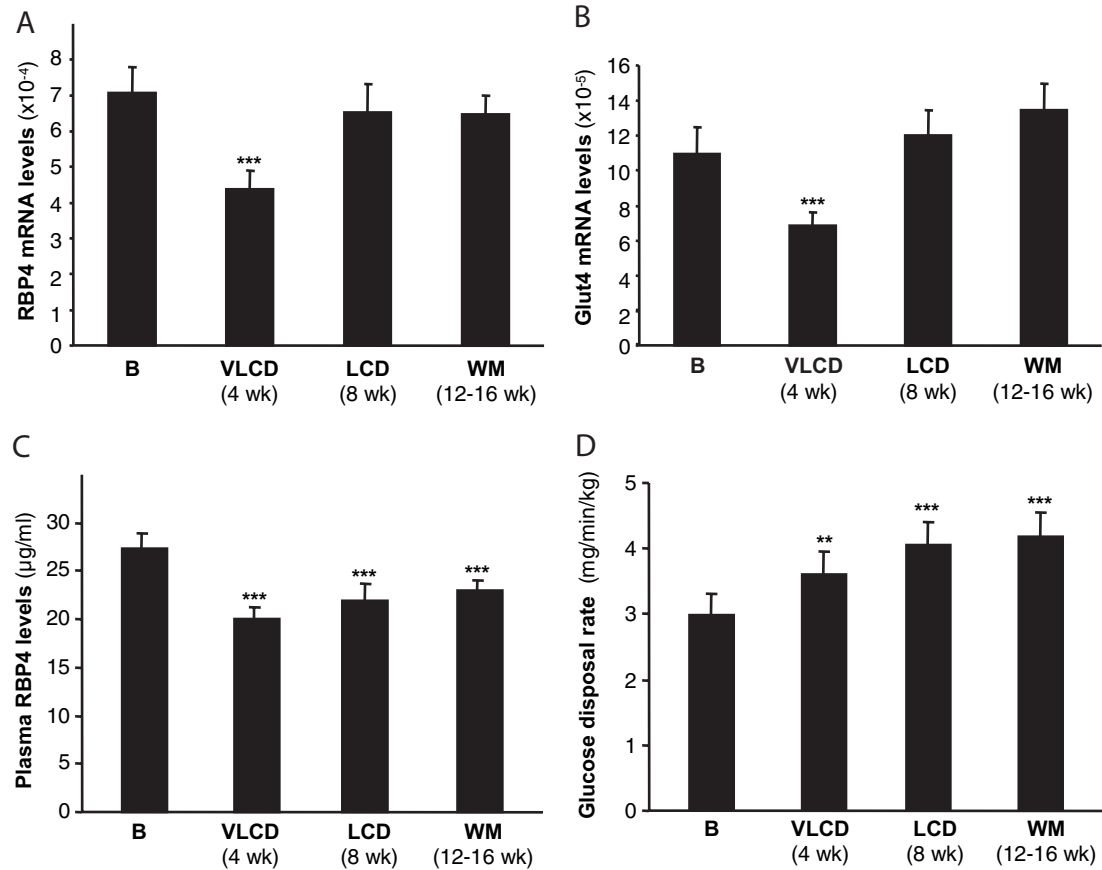


FIG. 2. Subcutaneous adipose tissue mRNA expression of RBP4 and Glut4, plasma level of RBP4, and glucose disposal rate during a weight reduction program in obese women ($n = 24$). A, Adipose tissue RBP4 mRNA levels. B, Glut4 mRNA levels. C, RBP4 plasma levels. D, Glucose disposal rate. Values are means \pm SEM. **, $P < 0.01$. ***, $P < 0.001$ compared with basal values. B, Basal conditions; LCD, end of the LCD; VLCD, end of the VLCD; WM, end of the WM phase.

values. Therefore, the profile of the plasma RBP4 time course was not superimposable to the variations in insulin sensitivity indices. Moreover, no correlations were found between RBP4 and glucose disposal rate. It was pointed out that the variability of the RBP4 response to an intervention might be influenced by the baseline insulin sensitivity of subjects (19). However, stratification of the population of obese women into a high and low-insulin sensitivity group was associated

TABLE 2. Diet-induced changes in plasma levels of RBP4 in two subgroups with high and low initial insulin sensitivity

	Obese subjects with low-insulin sensitivity ($n = 12$)	Obese subjects with high-insulin sensitivity ($n = 12$)
Glucose disposal rate (mg/min/kg)	1.77 ± 0.12	4.18 ± 0.35^a
Basal plasma RBP4 levels ($\mu\text{g/ml}$)	29 ± 3	26 ± 1
Decrease of plasma RBP4 levels during VLCD (%)	23 ± 8	26 ± 4
Decrease of plasma RBP4 levels during WM (%)	17 ± 5	13 ± 4

Values are means \pm SEM.

^a $P < 0.001$.

neither with a significant difference in plasma RBP4 levels nor with different diet-induced responses of plasma levels of RBP4. When considering stratification according to the variations in insulin sensitivity induced by the dietary program, a similar conclusion was reached. Therefore, this kinetic study reveals that RBP4 is regulated by calorie restriction and weight loss but is not associated with insulin sensitivity in this population.

Plasma RBP4 levels are increased in mice with genetic ablation of Glut4 in adipocytes (2). Glut4 mRNA and protein levels are reduced in fat cells in several insulin-resistant states and in type 2 diabetes (20). In nonobese subjects with at least one first-degree relative with type 2 diabetes, a negative correlation was found between adipocyte Glut4 and plasma RBP4 levels (8). During the dietary intervention, the profile of variation of Glut4 mRNA levels was quite similar to that of RBP4 mRNA. Accordingly, a positive relationship between adipose tissue RBP4 and Glut4 gene expression was reported in adipose tissue from obese subjects (16). Therefore, the expected inverse relationship between adipose tissue Glut4 and RBP4 mRNA is not found in obese subjects.

Concentrations of serum proteins are used in assessing the

clinical status of patients with moderate-to-severe malnutrition. RBP4 is a clinically useful marker because it has a short half-life. In that respect, the decrease of plasma RBP4 levels during VLCD is in full agreement with earlier studies (21, 22). Moreover, this study shows that RBP4 gene expression in adipose tissue is subject to regulation by VLCD. Comparison of the evolution of adipose tissue mRNA and plasma levels suggests that the decrease of RBP4 levels during severe calorie restriction may be at least partly due to decreased adipocyte production. The down-regulation during VLCD may be related to ketosis because plasma β -hydroxybutyrate levels were increased specifically during this period. During LCD and WM, adipose tissue RBP4 mRNA levels returned to basal values, whereas the increase in plasma levels was more moderate and did not reach the prediet levels. Therefore, it may be hypothesized that, during these phases, the lower RBP4 levels reflect the reduction in fat mass. The magnitude of the decrease in fat mass during LCD and WM (22–23%) is indeed coherent with the decrease in RBP4 levels (15–20%). Another possibility is that the reduced plasma levels observed during long-term weight loss result from altered production by the liver, the probable major source of RBP4 in humans, but to date evidence is lacking for such a regulation (23). Finally, it may be noted that the pattern of changes in RBP4 and leptin levels were similar, raising the possibility of coregulatory mechanisms between the two adipokines.

To conclude, although RBP4 adipose tissue gene expression and plasma levels are reduced during severe calorie restriction, no relationship was observed between RBP4 and the improvement of insulin sensitivity induced during a weight reduction program in obese women. RBP4 is a marker of nutritional deficit but does not appear as a marker of insulin resistance during dietary intervention.

Acknowledgments

We thank the laboratory of Dr. Anne Bouloumié for the gift of isolated human adipose tissue cell types.

Received December 5, 2006. Accepted March 27, 2007.

Address all correspondence and requests for reprints to: Dominique Langin, Institut National de la Santé et de la Recherche Médicale Université Paul Sabatier U858, IFR31, BP 84225, 31432 Toulouse Cedex 4, France. E-mail: langin@toulouse.inserm.fr.

The authors' work is supported by Institut National de la Santé et de la Recherche Médicale, Grant GACR 303/04/0158 of the Grant Agency of the Czech Republic, research project of the Ministry of Education of Czech Republic MSM 0021620814, RIOMA project of the Agence Nationale de la Recherche and the project "Hepatic and adipose tissue and functions in the metabolic syndrome" (HEPADIP, see <http://www.hepadip.org/>), which is supported by the European Commission as an Integrated Project under the 6th Framework Programme (Contract LSHM-CT-2005-018734).

Disclosure Statement: The authors have nothing to disclose.

References

- Arner P 2003 The adipocyte in insulin resistance: key molecules and the impact of the thiazolidinediones. *Trends Endocrinol Metab* 14:137–145
- Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB 2005 Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 436:356–362
- Abel ED, Peroni O, Kim JK, Kim Y-B, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB 2001 Adipose-selective targeting of the Glut4 gene impairs insulin action in muscle and liver. *Nature* 409:729–733
- Quadro L, Blaner WS, Salchow DJ, Vogel S, Piantadosi R, Gouras P, Freeman S, Cosma MP, Colantuoni V, Gottesman ME 1999 Impaired retinal function and vitamin A availability in mice lacking retinol-binding protein. *EMBO J* 18:4633–4644
- Tsutsumi C, Okuno M, Tannous L, Piantadosi R, Allan M, Witt SGD, Blaner WS 1992 Retinoids and retinoid-binding protein expression in rat adipocytes. *J Biol Chem* 267:1805–1810
- Basualdo CG, Wein EE, Basu TK 1997 Vitamin A (retinol) status of first nation adults with non-insulin-dependent diabetes mellitus. *J Am Coll Nutr* 16:39–45
- Abahusain MA, Wright J, Dickerson JW, de Vol EB 1999 Retinol, alpha-tocopherol and carotenoids in diabetes. *Eur J Clin Nutr* 53:630–635
- Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U, Kahn BB 2006 Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med* 354:2552–2563
- Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH, Lee HK, Park KS 2006 Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. *Diabetes Care* 29:2457–2461
- Polonsky KS 2006 Retinol-binding protein 4, insulin resistance, and type 2 diabetes. *N Engl J Med* 354:2596–2598
- Curat CA, Miranville A, Sengenès C, Diehl M, Tonus C, Busse R, Bouloumié A 2004 From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. *Diabetes* 53:1285–1292
- Tiraby C, Tavernier G, Lefort C, Larrouy D, Bouillaud F, Ricquier D, Langin D 2003 Acquisition of brown fat cell features by human white adipocytes. *J Biol Chem* 278:33370–33376
- Petersen M, Taylor MA, Saris WH, Verdich C, Toubro S, MacDonald I, Rossner S, Stich V, Guy-Grand B, Langin D, Martínez JA, Perdersen O, Holst C, Sorensen TI, Astrup A 2006 Randomised, multi-center trial of two hypo-energetic diets in obese subjects: high- versus low-fat content. *Int J Obes* 30:552–560
- Klimcakova E, Polak J, Moro C, Hejnova J, Majercik M, Viguier N, Berlan M, Langin D, Stich V 2006 Dynamic strength training improves insulin sensitivity without altering plasma levels and gene expression of adipokines in subcutaneous adipose tissue in obese men. *J Clin Endocrinol Metab* 91:5107–5112
- DeFronzo RA, Tobin JD, Andres R 1979 Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223
- Janke J, Engeli S, Boschmann M, Adams F, Bohnke J, Luft FC, Sharma AM, Jordan J 2006 Retinol-binding protein 4 in human obesity. *Diabetes* 55:2805–2810
- Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM 2000 Transcriptional regulation of adipogenesis. *Genes Dev* 14:1293–1307
- Takashima N, Tomoike H, Iwai N 2006 Retinol-binding protein 4 and insulin resistance. *N Engl J Med* 355:1392
- Oh J 2006 Retinol-binding protein 4 and insulin resistance. *N Engl J Med* 355:1394
- Shepherd PR, Kahn BB 1999 Glucose transporters and insulin action—implications for insulin resistance and diabetes mellitus. *N Engl J Med* 341:248–257
- Shetty PS, Watrasiewicz KE, Jung RT, James WP 1979 Rapid-turnover transport proteins: an index of subclinical protein-energy malnutrition. *Lancet* 2:230–232
- Scalfi L, Laviano A, Reed LA, Borrelli R, Contaldo F 1990 Albumin and labile-protein serum concentrations during very-low-calorie diets with different compositions. *Am J Clin Nutr* 51:338–342
- Blaner WS 1989 Retinol-binding protein: the serum transport protein for vitamin A. *Endocr Rev* 10:308–316