Serum Levels of Adipokine Retinol-Binding Protein-4 in Relation to Renal Function

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OBJECTIVE — Retinol-binding protein (RBP)-4 was recently identified as an adipokine that induces insulin resistance. In the current study, we investigated RBP-4 serum levels in diabetic and nondiabetic patients on chronic hemodialysis (CD) compared with control subjects with a glomerular filtration rate >50 ml/min. The majority of the diabetic subjects used oral hypoglycemic agents or insulin.

RESEARCH DESIGN AND METHODS — RBP-4 was determined by enzyme-linked immunosorbent assay in control subjects ($n = 59$) and CD patients ($n = 58$) and correlated with clinical and biochemical measures of renal function, glucose and lipid metabolism, and inflammation in both groups.

RESULTS — Mean serum RBP-4 levels were almost fourfold higher in CD patients (102 ± 30 mg/l) compared with control subjects (28 ± 8 mg/l). Furthermore, serum creatinine independently predicted RBP-4 concentrations in multiple regression analyses in both control subjects and CD patients. In addition, C-reactive protein and systolic blood pressure independently and negatively correlated with RBP-4 serum concentrations in CD patients but not control subjects. In contrast, markers of glucose and lipid metabolism were not independently related to serum RBP-4 in control subjects or CD patients.

CONCLUSIONS — We show that markers of renal function are independently related to serum RBP-4 levels.

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The incidence of obesity and related disorders, such as insulin resistance, has been growing rapidly and reaching global epidemic proportions. In recent years, it has been shown that adipocyte-secreted factors, called adipokines, are novel mediators contributing to insulin resistance when body weight is gained (1–3).

Retinol-binding protein (RBP)-4 was reported in 2005 by Yang et al. (4) as an adipokine that impairs insulin sensitivity. Thus, RBP-4 knockout mice showed improved insulin sensitivity (4). Furthermore, transgenic overexpression of RBP-4 or injection of recombinant RBP-4 in normal mice induced insulin resistance (4). Mechanistic studies have suggested that RBP-4 impaired insulin sensitivity by inhibition of insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activation in muscle and by induction of glucose production in liver via PEPCK stimulation (4).

Several studies have determined the influence of components of the metabolic syndrome on human RBP-4 concentrations. In the initial report, RBP-4 levels were elevated not only in obese and diabetic mice but also in overweight humans (4). Increased serum RBP-4 levels were associated with BMI, waist-to-hip ratio (WHR), serum triglycerides, and systolic blood pressure in another study (5). Furthermore, concentrations of this adipokine were increased in human subjects with impaired glucose tolerance (IGT) and type 2 diabetes compared with probands with normal glucose tolerance (6). Recently, it has been shown that RBP-4 levels decreased in morbidly obese patients 6 months after gastric banding (7). In contrast to these studies, differences in RBP-4 levels were not observed in normal-weight, overweight, and obese women (8).

Whereas the connection of RBP-4 with several metabolic parameters has been studied in detail, little is known about the relation of this adipokine to renal function, especially in patients with a mild to moderate decrease in glomerular filtration rate (GFR). Therefore, we determined RBP-4 serum levels in 58 chronic hemodialysis (CD) patients (32 diabetic and 26 nondiabetic subjects) and 59 control subjects (29 diabetic and 30 nondiabetic subjects) with a GFR >50 ml/min and correlated RBP-4 to clinical and biochemical measures of renal function, glucose and lipid metabolism, and inflammation in both groups.

RESEARCH DESIGN AND METHODS

— We recruited 117 Caucasian men ($n = 61$) and women ($n = 56$), with 59 patients having a GFR >50 ml/min (control subjects) as assessed by the Cockroft-Gault formula and 58 patients being on CD. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist and hip circumferences were determined, and WHR was calculated. The age ranged from 32 to 85 years and BMI from 18.7 to 46.1 kg/m^2. A total of 29 patients in the control group and 32 patients in the CD group had type 2 diabetes. Diabetes was defined as fasting blood glucose ≥126 mg/dl or use of insulin or oral hypoglycemic agents. Furthermore, diabetes was excluded in the control group by perform-

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Abbreviations: CD, chronic hemodialysis; CRP, C-reactive protein; FFA, free fatty acid; GFR, glomerular filtration rate; HOMA-IR, homeostasis model assessment of insulin resistance; IGT, impaired glucose tolerance; RBP, retinol-binding protein; WHR, waist-to-hip ratio.

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

ing 75-g oral glucose tolerance tests. However, six nondiabetic control patients presented with untreated IGT. Homeostasis model assessment of insulin resistance (HOMA-IR) was determined as previously described (9). Patients with severe conditions such as generalized inflammation or end-stage malignant diseases were excluded from the study. The study was approved by the local ethics committee, and all subjects gave written informed consent before taking part in the study.

Assays

After an overnight fast, blood samples were taken. Serum insulin was measured with a two-site chemiluminescent enzyme immunometric assay for the Immulite automated analyzer (Diagnostic Products, Los Angeles, CA). Leptin (Mediagnost, Reutlingen, Germany), adiponectin (Mediagnost), and RBP-4 (Adipogen, Seoul, South Korea) serum levels were determined with commercially available enzyme-linked immunosorbent assays according to the manufacturers' instructions. Serum creatinine, urea, parathyroid hormone, free fatty acids (FFAs), cholesterol, triglycerides, and C-reactive protein (CRP) were measured by standard laboratory methods in a certified laboratory.

Statistical analysis

SPSS statistical software version 11.5 (SPSS, Chicago, IL) was used for all analyses. Distribution was tested for normality using the Shapiro-Wilk *W* test. For all analyses, nonnormally distributed parameters were logarithmically transformed to approximate a normal distribution. Differences in RBP-4 serum levels between control subjects and CD patients were assessed by unpaired Student's *t* test and one-way ANOVA with Bonferroni post hoc analysis, as indicated in the table headings and figure legend. Correlations were performed using the Pearson's correlation method. To adjust the effects of covariates and identify independent relationships, multivariate linear regression analyses were performed. A *P* value of <0.05 was considered as statistically significant in all analyses.

RESULTS

RBP-4 serum levels are increased in CD patients compared with control subjects

Mean ± SD serum RBP-4 was 65 ± 43 mg/l (range 15–163) in the total sample. Clinical characteristics of the subgroups studied (control and CD) are shown in Table 1. Furthermore, the clinical characteristics of the subgroups further divided into nondiabetic and diabetic subjects are presented in Table 2. Mean serum RBP-4 levels were significantly different between control subjects (28 ± 8 mg/l) and subjects treated with CD (102 ± 30 mg/l) ($P < 0.01$) (Table 1). In contrast, a significant difference in serum RBP-4 levels could not be demonstrated depending on diabetes (diabetic subjects: 63 ± 40 mg/l; nondiabetic subjects: 66 ± 47 mg/l) and sex (female subjects: 59 ± 42 mg/l; male subjects: 69 ± 44 mg/l). Since mean RBP-4 serum levels were significantly different in control subjects compared with CD patients, all subsequent analyses were performed in the two subgroups separately.

Table 1—*Baseline characteristics of the study population*

	Control subjects	CD patients
n	59	58
RBP-4 (mg/l)	28 ± 8	102 ± 30*
Age (years)	62 ± 10	64 ± 12
Sex (male/female)	26/33	35/23
Diabetic/nondiabetic	29/30	32/26
BMI (kg/m^2)	30 ± 5	27 ± 5*
WHR	0.91 ± 0.09	0.96 ± 0.10*
Systolic blood pressure (mmHg)	127 ± 14	123 ± 22
Diastolic blood pressure (mmHg)	74 ± 10	71 ± 11
Creatinine (μmol/l)	76 ± 16	776 ± 261*
Urea (mmol/l)	5.5 ± 1.5	19.9 ± 7.1*
GFR (ml/min)	99 ± 35	10 ± 4*
Parathyroid hormone (pmol/l)	4.1 ± 1.6	22.5 ± 20.4*
Fasting glucose (mmol/l)	6.51 ± 2.32	5.52 ± 2.19*
Fasting insulin (pmol/l)	60 ± 71	76 ± 110
HOMA-IR	2.51 ± 2.39	3.39 ± 7.38
FFAs (mmol/l)	0.56 ± 0.23	0.64 ± 0.37
Cholesterol (mmol/l)	5.20 ± 1.04	4.41 ± 1.05*
Triglycerides (mmol/l)	1.46 ± 0.81	2.05 ± 1.33*
Leptin (μg/l)	23 ± 23	52 ± 72
Adiponectin (mg/l)	6.61 ± 3.82	16.34 ± 11.26*
CRP (mg/l)	3.63 ± 2.94	13.75 ± 20.79*

Data are means ± SD. *$P < 0.01$ vs. control, as assessed by unpaired Student's *t* test.

Univariate and multivariate correlations

In control subjects, serum RBP-4 levels positively correlated with creatinine and urea ($P < 0.05$) (data not shown) and negatively with GFR (Fig. 1). In contrast, RBP-4 was not correlated with markers of insulin sensitivity (BMI, WHR, fasting glucose, fasting insulin, HOMA-IR, and adiponectin), lipid metabolism (FFAs, cholesterol, and triglycerides), and inflammation (CRP) in this subgroup (data not shown). In CD patients, serum RBP-4 concentrations were positively correlated with creatinine, urea, and cholesterol ($P < 0.05$) (data not shown). Furthermore, RBP-4 was negatively correlated with GFR (Fig. 1), WHR, systolic blood pressure, FFAs, and CRP ($P < 0.05$) (data not shown).

Multiple regression analysis revealed that serum creatinine remained independently associated with RBP-4 levels after adjustment for CRP, WHR, systolic blood pressure, and FFAs in the control group ($P < 0.05$) (Table 3). In CD patients, creatinine also independently predicted serum RBP-4 in multiple regression analysis ($P < 0.05$) (Table 3). Furthermore, CRP and systolic blood pressure independently and negatively correlated with RBP-4 serum concentrations in this subgroup ($P < 0.05$) (Table 3). In contrast, markers of glucose metabolism (BMI, WHR, fasting glucose, fasting insulin, HOMA-IR, and adiponectin) and lipid metabolism (FFAs, cholesterol, and triglycerides) were not independently related to serum RBP-4 in both subgroups (Table 3 and data not shown).

CONCLUSIONS — The major novel finding of the current study is that serum creatinine independently predicts RBP-4

Table 2—*Baseline characteristics of the study population further divided into control subjects without diabetes (Con-ND) or with diabetes (Con-D) and CD patients without diabetes (CD-ND) or with diabetes (CD-D)*

	Con-ND	Con-D	CD-ND	CD-D
n	30	29	26	32
RBP-4 (mg/l)	28 ± 7	28 ± 8	109 ± 33*†	96 ± 28*†
Age (years)	61 ± 11	63 ± 10	60 ± 14	67 ± 10
Sex (m/f)	11/19	15/14	17/9	18/14
BMI (kg/m^2)	30 ± 6	30 ± 5	26 ± 5*†	28 ± 5
WHR	0.88 ± 0.08	0.94 ± 0.08	0.93 ± 0.10	0.99 ± 0.09*
Systolic blood pressure (mmHg)	126 ± 16	128 ± 12	124 ± 23	123 ± 21
Diastolic blood pressure (mmHg)	75 ± 11	72 ± 8	73 ± 12	69 ± 9
Creatinine (μmol/l)	77 ± 15	75 ± 18	815 ± 246*†	743 ± 271*†
Urea (mmol/l)	5.4 ± 1.3	5.6 ± 1.6	20.0 ± 7.0*†	19.7 ± 7.2*†
GFR (ml/min)	94 ± 32	105 ± 38	9 ± 4*†	10 ± 4*†
Parathyroid hormone (pmol/l)	4.4 ± 1.7	3.8 ± 1.4	25.2 ± 26.6*†	20.4 ± 13.6*†
Fasting glucose (mmol/l)	5.2 ± 0.77	7.89 ± 2.57*	4.67 ± 0.89†	6.21 ± 2.67†‡
Fasting insulin (pmol/l)	46 ± 26	75 ± 95	60 ± 84	89 ± 126
HOMA-IR	1.55 ± 0.96	3.49 ± 2.97	1.98 ± 3.17†	4.54 ± 9.43
FFAs (mmol/l)	0.52 ± 0.19	0.60 ± 0.27	0.56 ± 0.32	0.70 ± 0.39
Cholesterol (mmol/l)	5.47 ± 0.84	4.92 ± 1.36	4.52 ± 1.00*	4.31 ± 1.10*
Triglycerides (mmol/l)	1.22 ± 0.47	1.71 ± 1.00	1.66 ± 0.53*	2.37 ± 1.68*
Leptin (μg/l)	24 ± 21	22 ± 26	34 ± 55	66 ± 81†‡
Adiponectin (mg/l)	7.55 ± 4.13	5.52 ± 3.26	17.42 ± 11.18*†	15.46 ± 11.42*†
CRP (mg/l)	3.93 ± 3.27	3.32 ± 2.57	8.66 ± 13.19	17.88 ± 24.80*†

Data are means ± SD. For comparisons between groups, one-way ANOVA was applied followed by Bonferroni post hoc analysis. *$P < 0.05$ vs. Con-ND; †vs. Con-D; ‡vs. CD-ND.

concentrations in multiple regression analyses in control subjects with a GFR >50 ml/min. These findings indicate that markers of renal function should be included in studies concerning RBP-4 physiology. Furthermore, we show that serum RBP-4 levels are almost fourfold higher in CD patients compared with control subjects. These data confirm prior work (10,11) suggesting that RBP-4 concentrations are significantly increased in end-stage renal disease and that renal excretion is a primary pathway for RBP-4 clearance. Interestingly, similar mechanisms of elimination have been proposed for other adipokines. Thus, plasma levels of insulin-sensitizing adiponectin are 2.5-fold higher in CD patients compared with healthy subjects (12). This result is comparable with the data obtained in the present study, where adiponectin concentrations in CD patients and control subjects are 16.34 and 6.61 mg/l, respectively ($P < 0.05$). Furthermore, plasma leptin concentrations are increased about twofold in CD patients (13). In accordance with this finding, mean leptin serum concentrations are more than twofold higher in CD patients compared with control subjects; in our hands, however, this difference does not reach statistical significance.

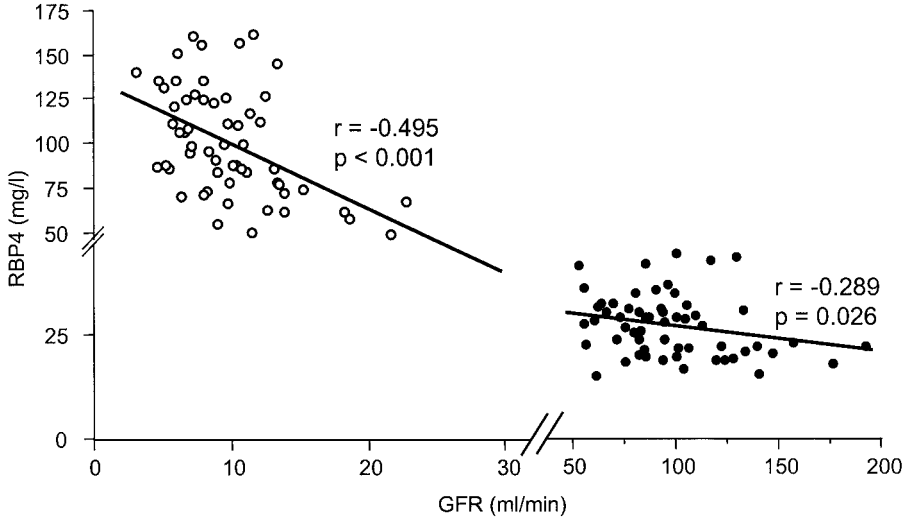

Figure 1—*Univariate correlation between RBP-4 serum levels and GFR in control (●) and CD (○) patients using Pearson's correlation method.*

Interestingly, urinary RBP-4 excretion is increased in early diabetic nephropathy and might even be a marker of early renal damage preceding microalbuminuria (14–16). One study by Abahusain et al. (15) demonstrates that both urinary and serum RBP-4 concentrations are increased in diabetic patients compared with control subjects. These results indicate that increased RBP-4 serum concentrations are not necessarily accompanied by decreased urinary RBP-4 excretion. Therefore, it needs to be tested in further studies how urinary RBP-4 excretion is influenced by renal function and how it is related to serum RBP-4 levels in the control subjects.

The physiological significance of increased RBP-4 serum concentrations in renal failure remains to be elucidated. It is interesting to note in this context that increased renal excretion of RBP-4 induced

Table 3—*Multivariate linear regression analyses between RBP-4 (dependent variable) and serum creatinine concentrations adjusted for CRP (model 1), WHR (model 2), CRP and WHR (model 3), and CRP, WHR, systolic blood pressure, and FFAs (model 4)*

Model	Control	CD
Model 1		
Independent variable		
Creatinine	0.280/0.032*	0.524/0.000*
CRP	−0.148/0.250	−0.435/0.000*
Model 2		
Independent variable		
Creatinine	0.320/0.022*	0.572/0.000*
WHR	−0.046/0.732	−0.216/0.045*
Model 3		
Independent variable		
Creatinine	0.292/0.038*	0.523/0.000*
CRP	−0.146/0.264	−0.413/0.000*
WHR	−0.034/0.800	−0.058/0.568
Model 4		
Independent variable		
Creatinine	0.284/0.046*	0.554/0.000*
CRP	−0.166/0.216	−0.361/0.001*
WHR	−0.015/0.914	−0.031/0.757
Systolic blood pressure	−0.050/0.720	−0.237/0.014*
FFAs	0.128/0.366	−0.021/0.829

Data are β-coefficient/*P* value. Dependent variable: RBP-4. *Significant correlation.

by fenretinide not only normalizes serum RBP-4 levels but also improves insulin sensitivity in obese mice (4). Here, studies in humans are awaited that will determine whether a decrease in RBP-4 serum levels decreases insulin resistance in a similar manner. In the present study, CRP and systolic blood pressure negatively correlate with serum RBP-4 concentrations independent of renal function in CD patients but not control subjects. It needs to be elucidated in future studies whether inflammatory status and blood pressure directly modulate RBP-4 levels in patients with renal failure.

We find significant correlations between RBP-4 and WHR, FFAs, and cholesterol in univariate analyses in CD patients but not control subjects. However, these significant correlations are all lost after adjustment for serum creatinine. Furthermore, other markers of glucose and lipid metabolism, including BMI, fasting glucose, fasting insulin, HOMA-IR, triglycerides, and adiponectin, do not correlate with RBP-4 serum concentrations in both groups studied. In accordance with our data, RBP-4 levels are not different among normal-weight, overweight, and obese women (8). In contrast, RBP-4 levels are increased in overweight women with polycystic ovary syndrome (17), in obese adolescents (18), and in obese children (19) compared with their respective control subjects. Furthermore, RBP-4 serum concentrations are reduced in obese children after physical activity–based lifestyle intervention (19). A positive correlation between RBP-4 levels and BMI is found in several studies (5,18,19). In contrast, serum RBP-4 concentrations positively correlate with visceral adiposity but not with BMI in another study (20). Similarly, RBP-4 is not positively correlated with BMI in 58 Japanese adult volunteers (21). Stefan et al. (22) convincingly demonstrated that circulating RBP-4 positively correlates with HOMA-IR in healthy subjects. A positive association between RBP-4 levels and insulin resistance is also shown in obese and nonobese adolescents (18); in subjects with obesity, IGT, or type 2 diabetes; in nonobese, nondiabetic subjects with a strong family history of type 2 diabetes (5); and in nonobese individuals without a family history or diagnosis of diabetes (23). In contrast, no correlation between RBP-4 and insulin resistance is found in Aboriginal Canadian women and white women (24). Cho et al. (6) found significantly different RBP-4 levels between patients with normal glucose tolerance on one hand and IGT and diabetes on the other hand. A similar increase in serum RBP-4 concentrations in diabetic subjects is observed in an independent study (25). Different patient characteristics might explain the differences observed in the association between RBP-4 and metabolic parameters. Thus, prior reports showing an association between RBP-4 and glucose metabolism have been performed with subjects either not taking glucose-lowering drugs or undergoing an extensive withdrawal period from their medications (5,22). In contrast, the majority of the diabetic patients in our study use insulin or oral hypoglycemic medications. Therefore, the results of these studies cannot be easily compared.

Dilution experiments (data not shown) suggest that the RBP-4 enzyme-linked immunosorbent assay kit used in the current study does not have a lack of linearity like other commercially available enzyme-linked immunosorbent assays (26). However, our enzyme-linked immunosorbent assay kit exhibits greater reactivity for urinary proteolyzed RBP-4 compared with full-length nonproteolyzed RBP-4 at all concentrations tested (data not shown), in accordance with a recent report (26). It needs to be determined in further experiments whether a preferential accumulation of proteolyzed RBP-4 in end-stage renal disease (10) might lead to an overestimation of RBP-4 serum concentrations in the CD patients. Here, quantitative Western blotting appears as the gold standard to measure RBP-4 in human serum or plasma (26).

Taken together, we present evidence that serum RBP-4 levels may be closely related to renal function, even in subjects with only mild to moderate renal impairment. Our findings reinforce prior observations that renal filtration is an important route of RBP-4 elimination and emphasize that renal function should be considered in studies regarding the relationship between RBP-4 and metabolic disease.

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References

1. Trujillo ME, Scherer PE: Adipose tissue-derived factors: impact on health and disease. *Endocr Rev* 27:762–778, 2006
2. Semple RK, Chatterjee VK, O'Rahilly S: PPAR gamma and human metabolic dis-

ease. *J Clin Invest* 116:581–589, 2006
3. Fasshauer M, Paschke R: Regulation of adipocytokines and insulin resistance. *Diabetologia* 46:1594–1603, 2003
4. 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
5. Graham TE, Yang Q, Bluher 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
6. Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH, Lee HK, Park KS: Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. *Diabetes Care* 29:2457–2461, 2006
7. Haider DG, Schindler K, Prager G, Bohdjalian A, Luger A, Wolzt M, Ludvik B: Serum retinol-binding protein-4 is reduced after weight loss in morbidly obese subjects. *J Clin Endocrinol Metab* 92:1168–1171, 2007
8. Janke J, Engeli S, Boschmann M, Adams F, Bohnke J, Luft FC, Sharma AM, Jordan J: Retinol-binding protein 4 in human obesity. *Diabetes* 55:2805–2810, 2006
9. Bluher M, Engeli S, Kloting N, Berndt J, Fasshauer M, Batkai S, Pacher P, Schon MR, Jordan J, Stumvoll M: Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. *Diabetes* 55:3053–3060, 2006
10. Jaconi S, Rose K, Hughes GJ, Saurat JH, Siegenthaler G: Characterization of two post-translationally processed forms of human serum retinol-binding protein: altered ratios in chronic renal failure. *J Lipid Res* 36:1247–1253, 1995
11. Jaconi S, Saurat JH, Siegenthaler G: Analysis of normal and truncated holo- and apo-retinol-binding protein (RBP) in human serum: altered ratios in chronic renal failure. *Eur J Endocrinol* 134:576–582, 1996
12. Zoccali C, Mallamaci F, Tripepi G, Benedetto FA, Cutrupi S, Parlongo S, Malatino LS, Bonanno G, Seminara G, Rapisarda F, Fatuzzo P, Buemi M, Nicocia G, Tanaka S, Ouchi N, Kihara S, Funahashi T, Matsuzawa Y: Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. *J Am Soc Nephrol* 13:134–141, 2002
13. Merabet E, Dagogo-Jack S, Coyne DW, Klein S, Santiago JV, Hmiel SP, Landt M: Increased plasma leptin concentration in end-stage renal disease. *J Clin Endocrinol Metab* 82:847–850, 1997
14. Hong CY, Chia KS, Ling SL: Urinary protein excretion in type 2 diabetes with complications. *J Diabetes Complications* 14:259–265, 2000
15. Abahusain MA, Wright J, Dickerson JW, de Vol EB: Retinol, alpha-tocopherol and carotenoids in diabetes. *Eur J Clin Nutr* 53:630–635, 1999
16. Galanti LM, Jamart J, Dell'omo J, Donckier J: Comparison of urinary excretion of albumin, alpha 1-microglobulin and retinol-binding protein in diabetic patients. *Diabetes Metab* 22:324–330, 1996
17. Tan BK, Chen J, Lehnert H, Kennedy R, Randeva HS: Raised serum, adipocyte, and adipose tissue retinol-binding protein 4 in overweight women with polycystic ovary syndrome: effects of gonadal and adrenal steroids. *J Clin Endocrinol Metab* 92:2764–2772, 2007
18. Lee DC, Lee JW, Im JA: Association of serum retinol binding protein 4 and insulin resistance in apparently healthy adolescents. *Metabolism* 56:327–331, 2007
19. Balagopal P, Graham TE, Kahn BB, Altomare A, Funanage V, George D: Reduction of elevated serum retinol binding protein in obese children by lifestyle intervention: association with subclinical inflammation. *J Clin Endocrinol Metab* 92:1971–1974, 2007
20. Jia W, Wu H, Bao Y, Wang C, Lu J, Zhu J, Xiang K: Association of serum retinol binding protein 4 and visceral adiposity in Chinese subjects with and without type 2 diabetes. *J Clin Endocrinol Metab* 2007 (Epub ahead of print)
21. Yoshida A, Matsutani Y, Fukuchi Y, Saito K, Naito M: Analysis of the factors contributing to serum retinol binding protein and transthyretin levels in Japanese adults. *J Atheroscler Thromb* 13:209–215, 2006
22. Stefan N, Hennige AM, Staiger H, Machann J, Schick F, Schleicher E, Fritsche A, Haring HU: High circulating retinol-binding protein 4 is associated with elevated liver fat but not with total, subcutaneous, visceral, or intramyocellular fat in humans. *Diabetes Care* 30:1173–1178, 2007
23. Gavi S, Stuart LM, Kelly P, Melendez MM, Mynarcik DC, Gelato MC, McNurlan MA: Retinol-binding protein 4 is associated with insulin resistance and body fat distribution in nonobese subjects without type 2 diabetes. *J Clin Endocrinol Metab* 92:1886–1890, 2007
24. Silha JV, Nyomba BL, Leslie WD, Murphy LJ: Ethnicity, insulin resistance, and inflammatory adipokines in women at high and low risk for vascular disease. *Diabetes Care* 30:286–291, 2007
25. Takebayashi K, Suetsugu M, Wakabayashi S, Aso Y, Inukai T: Retinol binding protein-4 levels and clinical features of type 2 diabetes patients. *J Clin Endocrinol Metab* 92:2712–2719, 2007
26. Graham TE, Wason CJ, Bluher M, Kahn BB: Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. *Diabetologia* 50:814–823, 2007