Research Communication

Retinol binding protein-4 predicts incident diabetes in Asian Indian men with prediabetes

Ram Jagannathan[1,2]
Chamukuttan Snehalatha[1,2]
Sundaram Selvam[1,2]
Arun Nanditha[1,2]
Ananth Samith Shetty[1,2]
Ian F. Godsland[3]
Desmond G. Johnston[3]
Ambady Ramachandran[1,2]*

[1]*India Diabetes Research Foundation, Chennai 600008, Tamil Nadu, India*

[2]*Dr. A. Ramachandran's Diabetes Hospitals, Chennai 600008, Tamil Nadu, India*

[3]*Faculties of Medicine and Engineering, Imperial College, London, United Kingdom*

Abstract

The association of retinol binding protein-4 (RBP4) with incident type 2 diabetes (T2DM) in Asian Indian middle-aged men with impaired glucose tolerance (IGT) was studied. This was an ancillary analysis of a subsample from a cohort of participants with IGT in a 2 year prospective diabetes prevention program in India. For this analysis, 71 incident T2DM and 76 non-diabetic cases (non-progressors) based on the final glycemic outcome were selected. Baseline serum RBP4 was measured using competitive enzyme immunoassay. Correlations of RBP4 with relevant anthropometric and biochemical variables and also its association with diabetes were assessed using appropriate statistical analyses. Participants who developed T2DM had higher levels of serum RBP4 (21.3 [IQR: 17.7–24.9] μg/mL) compared with non-progressors (17.3 [IQR: 13.1–21.0] μg/mL; $P = 0.001$). Levels of RBP4 were lower than in Caucasians. Stepwise linear regression analysis showed that body mass index (BMI), systolic blood pressure, triglycerides, and HbA1c had independent associations with RBP4 levels. Multiple logistic regression analyses showed that RBP4 was independently associated with incident diabetes (odds ratio [OR] [95%confidence interval (CI)]: 1.69 [1.18–2.41]; $P = 0.004$). Adjustment for study group, age, BMI, waist circumference, 2 H plasma glucose, triglycerides, gamma glutamyl transferase, and insulin resistance weakened the significance of its association (OR [95%CI]: 1.65 [1.03–2.66]; $P = 0.038$).The results of this preliminary analyses showed that baseline serum RBP4 levels were independently associated with incident diabetes in Asian Indian men with IGT. It may be used as an additional predictor of future diabetes. © 2015 BioFactors, 41(3):160–165, 2015

Keywords: *incident diabetes; retinol binding protein 4; gamma glutamyl transferase; prediabetes*

Abbreviations: *BMI, body mass index; GGT, gamma glutamyl transferase; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment-estimated insulin resistance; IGT, impaired glucose tolerance; NGT, normoglycemia; RBP4, retinol binding protein; TG, triglycerides; T2DM, type 2 diabetes; WHO, World Health Organization*

Volume 41, Number 3, May/June 2015, Pages 160–165

**Address for correspondence: A. Ramachandran, MD; India Diabetes Research Foundation & Chairman & Managing Director, Dr. A. Ramachandran's Diabetes Hospitals 28, Marshalls Road, Egmore, Chennai, 600 008, India. Tel.: +91 44 28582003; Fax : +91 44 42146652; E-mail: ramachandran@vsnl.com.*
Authors Contributions: Ram J - Researched data, Contributed to discussion, Wrote manuscript, Reviewed/edited manuscript; Snehalatha C - Researched data, Contributed to discussion, Wrote manuscript, Reviewed/edited manuscript; Selvam S - Researched data, Reviewed/edited manuscript; Nanditha A - Contributed to discussion, Reviewed/edited manuscript; Samith A Shetty - Contributed to discussion, Reviewed/edited manuscript; Ian F Godsland - Contributed to discussion, Reviewed/edited manuscript; DG Johnston - Researched data, Contributed to discussion, Wrote manuscript, Reviewed/edited manuscript; Ramachandran A - Researched data, Contributed to discussion, Wrote manuscript, Reviewed/edited manuscript
Conflict of Interest: None to declare.
Received 3 February 2015; accepted 4 March 2015
DOI 10.1002/biof.1209
Published online 23 March 2015 in Wiley Online Library
(wileyonlinelibrary.com)

1. Introduction

The ability to assess a person's risk type 2 diabetes (T2DM) before the onset of the disease would provide a rationale for implementing preventive lifestyle or pharmacologic interventions. Presence of either insulin resistance and/or beta cell dysfunction even in the absence of hyperglycemia is an early and strong predictor of incident diabetes [1]. It must be stressed that measurement of glycemic measures (fasting and 2 H plasma glucose and HbA1c) alone cannot reflect the pathophysiology of the disease. Hence, identification of clinically convenient tools that measure a broader scope of the pathophysiology of diabetes such as insulin resistance and beta cell function may provide improved strategies for diabetes prevention and management.

Retinol binding protein 4 (RBP4), a 21 kDa protein that belongs to the lipocalin family was initially known as a specific carrier for the delivery of retinol in circulation [2]. It is secreted mainly by hepatocytes (80%) and adipose tissue (20%), and may contribute to systemic insulin resistance and impaired glucose regulation [3]. There exists an association between RBP4 and pathophysiology of diabetes. Several studies in Chinese [4–6], Japanese [7], and white [8–12] populations showed possible association between RBP4 and adiposity, hypertriglyceridemia, insulin resistance, T2DM, and certain components of the metabolic syndrome. However, in several other clinical studies, associations and/or causality of observed changes in RBP4 with presence of insulin resistance or impaired glucose regulation could not be observed [13–15]. The present analyses were aimed to elucidate the predictive role of RBP4 with incident diabetes in Asian Indian men with prediabetes, in whom high rates of insulin resistance were observed.

2. Materials and Methods

2.1. Study Participants

This ancillary analysis used data in a cohort of men with impaired glucose tolerance (IGT) who were followed-up for 2 years in a previously published, randomized controlled trial on primary prevention of diabetes in India (ClinicalTrial.Gov no: NCT00819455) [16]. A total of 537 men were randomized into two groups: (*a*) control group ($n = 266$), which received standard care advice only at baseline; and (*b*) an intervention group ($n = 271$), which received automated, customized text messages (SMS) about healthy lifestyle habits in addition to standard care advice. The participants were followed-up every 6-month for 2 years to ascertain their glycemic status. The primary outcome was the development of diabetes as classified by the World Health Organization (WHO) recommendations [17]—a fasting plasma glucose of 7.0 mmol/L or higher and /or 11.1 mmol/L or higher 2 H after a 75-g oral glucose load. The study showed for the first time that motivation through text messaging could help to reduce the incidence of diabetes. At the end of the study, among the 517 (96.3%) responders, 179 (34.6%) had reverted to normoglycemia (NGT), 224 (43.3%) had remained IGT, and 123 (23.8%) had developed diabetes. The study protocol was approved by the Ethical Review Committee of the India Diabetes Research Foundation, Chennai, India. The study participants gave written informed consent prior to enrolment in the study. For this subanalysis, we had randomly chosen 71 newly detected diabetes cases and 76 randomly selected controls consisting of 43 NGT and 33 IGT.

2.2. Analytical Procedures

Height, weight (body mass index [BMI] calculated), and waist circumference were measured by standard procedures. Body fat percentage was measured using bioimpedence method (OMRON, Karadia Scan, Japan). Blood pressure (average of two readings) was measured using a standard mercury sphygmomanometer after at least a 5-Min' rest. A standard oral glucose tolerance test (OGTT) was performed after a 10–12 H fast with venous plasma sampling in the fasting state. The samples were frozen at -20°C until the laboratory assays were performed. Plasma glucose (hexokinase method, CV <3%; detection range: 0.11–25 mmol/L) was measured in on autoanalyzer (Roche/Hitachi 911 auto analyzer). HbA1c was measured using a COBAS INTERGRA 400 plus analyzer (turbidimetric inhibition immuno-assay; Tina-quant® HbA1c; CV <1.5%; detection limit: Hb: 4–40 g/dL [2.48–24.8 mmol/L]; HbA1c: 0.186–1.61 mmol/L (0.3–2.6 g/dL]). Triglycerides, (GPO-PAP method, CV <2.0%; detection range: 0.05–11.3 mmol/L), total cholesterol (CHOD-PAP method, CV <2.0%; detection range: 0.08–20.7 mmol/L) and HDL cholesterol (HDL plus-third generation; CV <3.0%; detection range: 0.08–3.10 mmol/L), and gamma-glutamyl transferase (GGT-2, CV <2.0%; detection limit: 3–1,200 U/L) were measured on the Roche/Hitachi 911 clinical analyzer. OGTT plasma insulin was measured at fasting and at 30 and 120 Min using electrochemiluminescence immunoassay (Roche diagnostics, Mannheim, Germany; CV <3%; detection range: 1.39–6,945 pmol/L) on the Elecsys® analyzer. Homeostasis model assessment of insulin resistance (HOMA-IR) [18] was calculated using the formula: ([fasting insulin × fasting glucose]/22.5). The insulinogenic index ($\Delta I/G$) was calculated using the difference in the values of 30-Min and fasting plasma insulin (picomoles per liter) divided by the 30-Min glucose value (millimoles per liter) [19]. Serum RBP4 levels were measured by competitive enzyme immuno assay (EIA) (RayBiotech, Norcross, The United States) according to manufacturer instructions. The sensitivity was 460 pg/mL with an assay range of 0.1–1,000 ng/mL. The intra-and inter assay coefficients of variation were less than 10% and less than 15%, respectively.

2.3. Statistical Analysis

The sample size was calculated based on the data from a previous cross-sectional study in China [4] which reported that the mean levels of RBP4 were higher in T2DM participants than in the normoglycemic group (T2DM: 30 ± 11 μg/mL; normal: 24 ± 7 μg/mL). With a minimum of 51 participants in each group, with the expected difference in the mean and

TABLE 1 ***Baseline characteristics of participants***

Variables	*Non-converters* n = *76*	*Converters* n = *71*	P *value*
Age (years)	47.7 ± 4.8	46.7 ± 4.6	0.217
BMI (kg/m^2)	25.9 ± 2.7	26.2 ± 3.5	0.491
Waist circumference (cm)	92.6 ± 7.1	93.5 ± 7.4	0.465
Total body fat (%)	26.8 ± 5.5	27.2 ± 5.2	0.683
Blood pressure (mm Hg)			
Systolic	122.8 ± 16.2	124.5 ± 14.6	0.519
Diastolic	79.2 ± 10.1	82.4 ± 8.6	0.046
Plasma glucose (mmol/l)			
Fasting	5.39 ± 0.50	5.82 ± 0.58	<0.0001
2 H	8.42 ± 0.65	9.28 ± 0.88	<0.0001
HbA1c (%)	6.0 ± 0.3	6.3 ± 0.4	<0.0001
Lipid profile (mmol/l)			
Triglycerides[a]	1.49 (1.09–2.05)	1.84 (1.32–2.33)	0.022
Cholesterol	5.01 ± 0.90	4.93 ± 0.92	0.604
HDL-C	0.91 ± 0.26	0.87 ± 0.23	0.335
GGT, IUL^{-1}[a]	22.0 (17.2–35.8)	34.1 (24.7–56.0)	<0.0001
HOMA-IR	3.0 ± 1.3	3.7 ± 1.3	<0.0001
Insulinogenic index[a]	62.7 (43.5–97.0)	34.5 (23.7–52.0)	<0.0001
RBP4 (μg/mL)[a]	17.3 (13.1–21.0)	21.3 (17.7–24.9)	0.010

The data are expressed as mean ± SD for normally distributed variables—P *value computed by two-tailed* t*-test.*
[a]*Data expressed as median (interquartile range)*—P *value computed by Mann–Whitney test.*

standard deviations, respectively, the levels can be demonstrated as significant at 5% significance and 90% power.

Comparisons between groups were done using the χ^2 test for qualitative variables, two-tailed "t" test for the normally distributed variables and the non-parametric Mann–Whitney U test for highly skewed distribution. The factors associated with circulating RBP4 levels was assessed using multiple linear regression analyses. Association of RBP4 with diabetes was assessed using multiple logistic regression analyses in three models: (*a*) unadjusted model; (*b*) model-1 adjusted for study group, baseline age, waist circumference, BMI, 2 H plasma glucose, and triglycerides; and (*c*) model-2 adjusted for model-1 + GGT and HOMA-IR. RBP4 levels were centered so that odds ratio (OR) for a 1-SD change was computed. In order to ascertain the mechanistic link between GGT and RBP4 with diabetes, the additive effect of both these variables (divided in medians) with diabetes was computed using multiple logistic regression analysis. All analyses were performed using SPSS software (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

3. Results

Baseline clinical characteristics of study participants according to the glycemic outcomes at the end of the study period are shown in Table 1. Participants who progressed to diabetes had higher baseline fasting and 2 H plasma glucose and HbA1c as compared with non-progressors ($P < 0.0001$). Progressors were more insulin resistant ($P < 0.0001$) and had decreased beta cell function ($P < 0.0001$) at baseline as compared with non-progressors. Serum concentrations of RBP4 were higher in the

TABLE 2 *Pearson correlations between serum RBP4 level and various clinical and metabolic parameters*

Variables	r	P *value*
BMI (kg/m^2)	0.303	<0.0001
Waist circumference (cm)	0.272	0.001
Blood pressure (mm Hg)		
Systolic	0.169	0.030
Diastolic	0.164	0.047
Fasting plasma glucose (mmol/L)	0.245	0.003
HbA1c (%)	0.256	0.002
Triglycerides (mmol/l)[a]	0.314	<0.0001
Fasting plasma insulin (pmol/L)[a]	0.190	0.023
HOMA-IR[a]	0.242	0.004
GGT[a]	0.234	0.004

[a]*These were log-transformed before the analysis.*

diabetic group than in the non-diabetic group (T2DM: 21.3 [17.7–24.9] μg/mL vs. non-diabetic: 17.3 (13.1–21.0) μg/mL; $P = 0.001$).

The correlation analysis demonstrated the strongest association between RBP4 and metabolic syndrome components (Table 2). Serum RBP4 concentration was correlated positively with BMI, waist circumference, systolic and diastolic blood pressure, fasting plasma glucose, HbA1c, fasting concentrations of insulin and triglycerides, HOMA-IR in all participants (Table 2). Serum RBP4 and GGT levels were positively correlated ($r = 0.234$; $P = 0.004$). Stepwise linear regression analysis revealed that BMI (β [SE]: 0.034 [0.01]; $P = 0.01$), systolic blood pressure (β [SE]: 0.005 [0.002]; $P = 0.020$), triglycerides (β [SE]: 0.299 [0.064]; $P < 0.0001$), and HbA1c (β [SE]: 0.277 [0.077]; $P < 0.0001$) were independent predictors of serum RBP4 levels.

Multiple logistic regression analysis showed that baseline RBP4 levels were significantly associated with diabetes (OR [95%CI]: 1.69 [1.18–2.41]; $P = 0.004$). The association was slightly weakened, but remained significant even after the adjusting for study group, age, BMI, waist circumference, 2 H plasma glucose, triglycerides (OR [95%CI]: 1.73 [1.08–2.75]; $P = 0.022$), and also for GGT and HOMA-IR (OR [95%CI]: 1.65 [1.03–2.66]; $P = 0.038$) (Table 3). The inclusion of HbA1c into the model resulted in the abolition of RBP4 effect. We did not observe additive effect between GGT and RBP4 with diabetes (OR [95%CI]: 0.193 [0.021–1.783]; $P = 0.147$) in this cohort.

4. Discussion

In this nested case–control study in middle-aged Asian Indian men with prediabetes, baseline serum RBP4 levels was independently associated with diabetes after adjusting for known metabolic covariates such as GGT, triglycerides, BMI, and insulin resistance. Although, cross-sectional studies [6,8,10,11,20] and a prospective study [12] had shown an association of RBP4 with abnormal glucose tolerance, the present analysis provides a new evidence about the association between RBP4 and diabetes in Asian Indian men with prediabetes in a prospective setting. In this study, the levels of RBP4 were relatively lower than in White populations [8–10,12]. It matched closely with the results observed in other Asian populations such as Chinese [6], Japanese [7], and Korean [20] adults. Ethnic variation in the levels of RBP4 may be possible. Another possibility is that the assay used in this study might have underestimated the RBP4 level, compared with methods such as quantitative western blotting and this could account for the varied results reported by different laboratories [21]. More studies are needed to investigate ethnic variations of RBP4 among different populations.

Serum RBP4 levels were positively associated with cardiometabolic risk factors, especially with levels of triglycerides in this cohort. Similar findings were reported in previous studies in Caucasians and Chinese populations [6,7,11]. Recently a cross-sectional study showed the independent association of RBP4 with coronary artery diseases [22]. Therefore, this adipokine not

TABLE 3 *The risk of diabetes associated with serum RBP4 level (dependent variable: diabetes vs. control [NGT + IGT])*

Model	*Adjustment*	*OR [95%CI]*	P *value*
Model-1	Unadjusted	1.69 [1.18–2.41]	0.004
Model-2	Adjusted for study group, age, BMI, waist circumference, 2 H PG, triglycerides	1.73 [1.08–2.754]	0.022
Model-3	Further adjusted for GGT and HOMA-IR on Model-2	1.65 [1.03–2.66]	0.038

For the risk of diabetes, we defined participants with NGT and IGT at the end of follow-up as referent (coded as "0"; n = *76) and participants who developed diabetes as cases (coded as "1";* n = *71).*

only serves as a marker of future diabetes, but also as a marker for diabetes-related complications. As far as we know this is one of the preliminary studies to demonstrate a stronger association of this adipokine with raised triglycerides in Asian Indian men. Plausible mechanistic link could be due to the production of triglycerides in the liver and release of very low-density lipoprotein into the circulation lead to an insulin-resistant state, and this may also partially explain the close association between RBP4, triglycerides, and insulin resistance [5,7,10,11,23–25]. However, a few studies failed to show an association between RBP4 and insulin resistance [13,15]. It is possible that unlike in other populations liver could be the primary source of circulating RBP4 in Asian Indians.

To our knowledge this is the first report on a positive correlation between GGT and RBP4 in Asian Indian men with prediabetes. The positive association of RBP4 and liver enzymes were demonstrated in participants with non-alcoholic fatty liver disease [25,26]. It is suggested that both GGT and RBP4 might be closely associated with hepatic insulin resistance and ectopic fat accumulation. However, we did not observe any additive effect of RBP4 and GGT with diabetes.

Our study has a few limitations. Firstly, the association between RBP4 with diabetes observed in this study was derived from a small sample size. However, the study design was adequately powered to detect the association. Secondly, we included only men in our study. The pathogenesis and variations in the levels of RBP4 with diabetes needs to be ascertained in women as the levels of RBP4 differ by sex [20]. Thirdly, we did not estimate the circulating levels of vitamin A (retinol). The expression of RBP4 is directly determined by the retinol. We did not assess the prevalence of non-alcoholic fatty liver disease in this cohort to ascertain the casual link between RBP4 and fatty liver. Nevertheless, this preliminary analysis demonstrates a possible association of RBP4 and diabetes.

In conclusion, the present study demonstrates that the serum level of RBP4 is a predictor of future development of T2DM. The etiological association of pathogenesis might be through hepatic insulin resistance. It could be used as an additional marker for early detection of participants predisposed to T2DM enabling an early intervention.

Acknowledgements

The authors acknowledge the statistical help and secretarial assistance rendered by Ms. L. Vijaya. This work was funded by the UK India Education and Research Initiative (IND/CONT/06-07/187E). We also acknowledge the partial funding given by the World Diabetes Foundation (WDF) for the study (WDF 08 – 406).

References

[1] DeFronzo, R. A. and Abdul-Ghani, M. (2011) Type 2 diabetes can be prevented with early pharmacological intervention. Diabetes Care 34 Suppl 2, S202–S209.

[2] Flower, D. R. (1996) The lipocalin protein family: structure and function. Biochem. J. 318(Pt 1), 1–14.

[3] Kloting, N., Graham, T. E., Berndt, J., Kralisch, S., Kovacs, P., et al. (2007) Serum retinol-binding protein is more highly expressed in visceral than in subcutaneous adipose tissue and is a marker of intra-abdominal fat mass. Cell Metab. 6, 79–87.

[4] Jia, W., Wu, H., Bao, Y., Wang, C., Lu, J., et al. (2007) Association of serum retinol-binding protein 4 and visceral adiposity in Chinese subjects with and without type 2 diabetes. J. Clin. Endocrinol. Metab. 92, 3224–3229.

[5] Qi, Q., Yu, Z., Ye, X., Zhao, F., Huang, P., et al. (2007) Elevated retinol-binding protein 4 levels are associated with metabolic syndrome in Chinese people. J. Clin. Endocrinol. Metab. 92, 4827–4834.

[6] Xu, M., Li, X. Y., Wang, J. G., Wang, X. J., Huang, Y., et al. (2009) Retinol-binding protein 4 is associated with impaired glucose regulation and microalbuminuria in a Chinese population. Diabetologia 52, 1511–1519.

[7] Takebayashi, K., Suetsugu, M., Wakabayashi, S., Aso, Y., and Inukai, T. (2007) Retinol binding protein-4 levels and clinical features of type 2 diabetes patients. J. Clin. Endocrinol. Metab. 92, 2712–2719.

[8] Graham, T. E., Yang, Q., Bluher, M., Hammarstedt, A., Ciaraldi, T. P., et al. (2006) Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N. Engl. J. Med. 354, 2552–2563.

[9] Craig, R. L., Chu, W. S., and Elbein, S. C. (2007) Retinol binding protein 4 as a candidate gene for type 2 diabetes and prediabetic intermediate traits. Mol. Genet. Metab. 90, 338–344.

[10] Kovacs, P., Geyer, M., Berndt, J., Kloting, N., Graham, T. E., et al. (2007) Effects of genetic variation in the human retinol binding protein-4 gene (RBP4) on insulin resistance and fat depot-specific mRNA expression. Diabetes 56, 3095–3100.

[11] Meisinger, C., Ruckert, I. M., Rathmann, W., Doring, A., Thorand, B., et al. (2011) Retinol-binding protein 4 is associated with prediabetes in adults from the general population: the Cooperative Health Research in the Region of Augsburg (KORA) F4 Study. Diabetes Care 34, 1648–1650.

[12] Luft, V. C., Pereira, M., Pankow, J. S., Ballantyne, C., Couper, D., et al. (2013) Retinol binding protein 4 and incident diabetes - the Atherosclerosis Risk in Communities Study (ARIC Study). Braz. J. Epidemiol. 16, 388–397.

[13] Janke, J., Engeli, S., Boschmann, M., Adams, F., Bohnke, J., et al. (2006) Retinol-binding protein 4 in human obesity. Diabetes 55, 2805–2810.

[14] Yao-Borengasser, A., Varma, V., Bodles, A. M., Rasouli, N., Phanavanh, B., et al. (2007) Retinol binding protein 4 expression in humans: relationship to insulin resistance, inflammation, and response to pioglitazone. J. Clin. Endocrinol. Metab. 92, 2590–2597.

[15] Chavez, A. O., Coletta, D. K., Kamath, S., Cromack, D. T., Monroy, A., et al. (2009) Retinol-binding protein 4 is associated with impaired glucose tolerance but not with whole body or hepatic insulin resistance in Mexican Americans. Am. J. Physiol. Endocrinol. Metab. 296, E758–E764.

[16] Ramachandran, A., Snehalatha, C., Ram, J., Selvam, S., Simon, M., et al. (2013) Effectiveness of mobile phone messaging in prevention of type 2 diabetes by lifestyle modification in men in India: a prospective, parallel-group, randomised controlled trial. Lancet Diabetes Endocrinol. 1, 191–198.

[17] World Health Organization (WHO) (1999) *Report of a WHO consultation. Definition, diagnosis and classification of diabetes mellitus and its complications. 1. Diagnosis and classification of diabetes mellitus. WHO/NCD/NCS/99.2*. WHO, Geneva.

[18] Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., et al. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412–419.

[19] Snehalatha, C., Mary, S., Selvam, S., Sathish Kumar, C. K., Shetty, S. B., et al. (2009) Changes in insulin secretion and insulin sensitivity in relation to the glycemic outcomes in subjects with impaired glucose tolerance in the Indian Diabetes Prevention Programme-1 (IDPP-1). Diabetes Care 32, 1796–1801.

[20] Cho, Y. M., Youn, B. S., Lee, H., Lee, N., Min, S. S., et al. (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.

[21] Graham, T. E., Wason, C. J., Bluher, M., and Kahn, B. B. (2007) Shortcomings in methodology complicate measurements of serum retinol binding

protein (RBP4) in insulin-resistant human subjects. Diabetologia 50, 814–823.
[22] Lambadiari, V., Kadoglou, N. P., Stasinos, V., Maratou, E., Antoniadis, A., et al. (2014) Serum levels of retinol-binding protein-4 are associated with the presence and severity of coronary artery disease. Cardiovasc. Diabetol. 13, 121.
[23] Stefan, N., Hennige, A. M., Staiger, H., Machann, J., Schick, F., et al. (2007) 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.
[24] Yamaaki, N., Yagi, K., Kobayashi, J., Nohara, A., Ito, N., et al. (2013) Impact of serum retinol-binding protein 4 levels on regulation of remnant-like particles triglyceride in type 2 diabetes mellitus. J. Diabetes Res. 2013, 143515.
[25] Seo, J. A., Park, S. Y., Kim, H. Y., Ryu, O. H., Lee, K. W., et al. (2008) Serum retinol-binding protein 4 levels are elevated in non-alcoholic fatty liver disease. Clin. Endocrinol. (Oxf.) 68, 555–560.
[26] Shin, J. Y., Shin, Y. G., Seo, K. S., and Chung, C. H. (2009) Elevated serum gamma-glutamyltransferase levels are independently associated with insulin resistance in non-diabetic subjects. Diabetes Res. Clin. Pract. 84, 152–157.