

Retinol-Binding Protein 4 and Insulin Resistance

TO THE EDITOR: Graham et al. (June 15 issue)¹ report that serum levels of retinol-binding protein 4 (RBP4) correlated with the magnitude of insulin resistance in various subjects. However, RBP4 and plasma insulin levels were dissociated in subjects who did not have an improvement in insulin sensitivity after exercise.¹ Thus, we believe that the importance of RBP4 should be assessed in more diverse groups.

We assessed serum RBP4 levels in 473 subjects with normal glucose tolerance (fasting blood glucose level, <110 mg per deciliter) who were randomly selected from the Suita Study, an epidemiologic cohort study.² Serum RBP4 levels correlated with triglyceride and uric acid levels and tended to correlate with glycated hemoglobin values and levels of high-density lipoprotein (HDL) cholesterol (Table 1). However, RBP4 levels did not correlate with the fasting blood glucose level,

waist-to-hip ratio, body-mass index (BMI), systolic blood pressure, or fasting insulin level. Even in the 38 subjects with a family history of diabetes, RBP4 did not correlate with the fasting insulin level. Thus, it seems unlikely that RBP4 will be useful for assessing the risk of type 2 diabetes in Japanese people. The biologic action of RBP4 in relation to the action of insulin will need to be clarified to explain this inconsistency.

Naoyuki Takashima, M.D.

Hitonobu Tomoike, M.D., Ph.D.

Naoharu Iwai, M.D., Ph.D.

National Cardiovascular Center

Suita 565-8565, Japan

1. Graham TE, Yang Q, Blüher M, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med* 2006;354:2552-63.

2. Iwai N, Mannami T, Tomoike H, Ono K, Iwanaga Y. An acyl-CoA synthetase gene family in chromosome 16p12 may contribute to multiple risk factors. *Hypertension* 2003;41:1041-6.

Table 1. Relation of Serum RBP4 Level to Insulin Resistance and Associated Metabolic Values in 473 Subjects with Normal Glucose Tolerance.*

Characteristic	Value	Range	Correlation Coefficient with Log(RBP4)	P Value
Male sex (%)	42.28			
Age (yr)	64.84±11.09	41–90	0.7119	0.50
Waist-to-hip ratio	0.92±0.06	0.68–1.14	0.0029	0.62
BMI†	22.78±3.01	15.12–38.56	0.2703	0.34
Systolic blood pressure (mm Hg)	125.10±18.64	84–196	–0.0526	0.98
Fasting blood glucose (mg/dl)	93.86±7.73	68–109	0.5578	0.45
Glycated hemoglobin (%)	5.33±0.37	4.30–6.40	0.0673	0.06
Uric acid (mg/dl)	5.07±1.32	0.60–9.00	0.5165	<0.001
Triglycerides (mg/dl)	100.18±63.15	29.0–820.0	20.4228	<0.001
HDL cholesterol (mg/dl)	61.69±15.41	31.0–129.0	–2.7709	0.06
RBP4 (μg/ml)	47.43±24.33	6.29–208.73		
Fasting insulin (μU/ml)‡	6.45±4.69	1.00–29.70	–0.9591	0.11

* All subjects had fasting blood glucose levels of less than 110 mg per deciliter. Plus–minus values are means ±SD. Statistical analyses were performed with the use of the JMP statistical package (SAS Institute). P values were not corrected for multiple testing.

† BMI is the weight in kilograms divided by the square of the height in meters.

‡ Insulin data were available for 268 subjects.

TO THE EDITOR: Graham et al. found higher levels of RBP4 in subjects with type 2 diabetes than in lean subjects with normal glucose tolerance.

We measured plasma RBP4 levels¹ in 234 patients with type 2 diabetes, impaired glucose tolerance, or normal glucose tolerance; the groups were matched for age and sex. RBP4 levels correlated with triglyceride levels, low-density lipoprotein (LDL) cholesterol levels, HDL cholesterol levels, and age but not with values obtained with homeostatic model assessment 2² (Table 1). In an analysis of covariance adjusted for age, sex, and sex-specific BMI, the RBP4 level was lower in subjects with type 2 diabetes than in those

with normal glucose tolerance (mean ratio, 0.91; 95% confidence interval [CI], 0.85 to 0.98; $P < 0.01$).

The correlation between the RBP4 level and age suggests that the age differences between subgroups with type 2 diabetes may explain the contrasting findings. Furthermore, we did not suspend statin treatment, which may have contributed to the divergent results, since we found a strong correlation between RBP4 and LDL cholesterol levels.

The high RBP4 levels in subjects with type 2 diabetes may reflect high LDL cholesterol levels rather than insulin resistance. The RBP4-lower-

Table 1. RBP4 in Univariate and Multivariate Regression Analysis.*

Characteristic	Univariate Analysis		Multivariate Analysis	
	Regression Coefficient (95% CI)	P Value	Regression Coefficient (95% CI)	P Value
Homeostatic model assessment 2 (per factor-of-10 increase)	1.00 (0.90–1.11)	0.88	0.96 (0.85–1.09)	0.57
Trunk-to-limb fat-mass ratio (per unit increase)				
Female sex	0.50 (0.08–2.81)	0.43	0.34 (0.01–6.89)	0.48
Male sex	4.19 (1.53–11.42)	<0.01	1.09 (0.25–4.64)	0.90
BMI (per unit increase) [†]				
Female sex	1.00 (0.99–1.00)	0.70	0.99 (0.98–1.01)	0.78
Male sex	0.99 (0.98–0.99)	<0.05	0.99 (0.98–1.01)	0.92
Waist-to-hip ratio (per 0.1-unit increase)				
Female sex	1.08 (1.00–1.16)	<0.05	1.07 (1.00–1.15)	<0.05
Male sex	0.94 (0.89–0.99)	<0.05	0.95 (0.89–1.01)	0.12
Plasma CRP (per factor-of-10 increase)	0.97 (0.89–1.06)	0.56	0.99 (0.91–1.08)	0.89
Plasma albumin (per increase of 1 g/liter)	1.01 (1.00–1.02)	<0.001	1.00 (1.00–1.01)	<0.05
HDL cholesterol (per factor-of-10 increase)	1.19 (0.92–1.53)	0.17	1.67 (1.24–2.26)	<0.001
LDL cholesterol (per increase of 1 mmol/liter)	1.09 (1.05–1.12)	<0.001	1.06 (1.02–1.09)	<0.001
Triglycerides (per factor-of-10 increase)	1.28 (1.14–1.44)	<0.001	1.42 (1.24–1.63)	<0.001
Age (per 10 yr)	1.04 (1.01–1.07)	<0.01	1.02 (0.99–1.05)	0.05
Male:female sex	1.16 (1.08–1.25)	<0.001	3.02 (0.99–9.22)	0.05

* RBP4 levels were log-transformed to approximate a normal distribution. Regression coefficients and 95% CIs were back-transformed, hence estimating the factor change of RBP4 level attributable to a 1-unit change in the predictor (given in parentheses). The ratio of trunk-to-limb fat mass, BMI, and waist-to-hip ratio indicate diverging results between sexes. This difference was significant only for waist-to-hip ratio ($P < 0.05$ for homogeneity of slopes). CRP denotes C-reactive protein.

[†] BMI is the weight in kilograms divided by the square of the height in meters.

ing effect of physical training might be ascribed to the effect on blood lipids. We propose that a strategy of lowering LDL cholesterol levels might lower RBP4 levels.

Christian Erikstrup, M.D.

Ole H. Mortensen, Ph.D.

Bente K. Pedersen, M.D.

Centre of Inflammation and Metabolism at Rigshospitalet
2100 Copenhagen, Denmark
chr.erikstrup@gmail.com

1. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. *J Nutr* 2004;134:3127-32.
2. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004;27:1487-95.

TO THE EDITOR: Graham et al. showed that RBP4 is an adipokine that is elevated in the serum before the development of diabetes and appears to identify insulin resistance. Their data (in Table 2 of the article) indicate that the RBP4 and fasting insulin levels were dissociated in the group with a marginal response to exercise training. It leads me to wonder about the baseline values in certain studies. It would be particularly useful to know whether there were baseline differences between the group with a marginal response and the group with improved insulin sensitivity after exercise training in the 2-hour glucose result of the oral glucose-tolerance test, fasting insulin level, rate of glucose disposal, and RBP4 level, according to the Wilcoxon rank-sum test. The baseline data of these two groups may distinguish them up front.

Jaewon Oh

Yonsei University College of Medicine
120-752 Seoul, Republic of Korea
drjaewonoh@gmail.com

THE AUTHORS REPLY: The correlations shown by Takashima et al. support our overall finding that serum RBP4 levels predict key elements of the metabolic syndrome. Furthermore, these correlations remain despite considerable differences between the Japanese study group and ours, including older age and different genetic background, diet, and lifestyle. In our view, fasting insulin levels are not a surrogate for the measurement of insulin resistance with the use of the clamp technique, especially since limited insulin secretory

reserve is a major pathogenic factor in type 2 diabetes in Japanese subjects.^{1,2}

There were correlations between the RBP4 level and both age (groups 1 and 2) and LDL cholesterol level (group 2, $R=0.47$; $P=0.003$) in our subjects. The correlation between the RBP4 level and glucose disposal rate was independent of age in all groups. With regard to the suggestion by Erikstrup et al. that lowering serum LDL cholesterol levels might lower serum RBP4 levels, two studies showed no effect of cholesterol-lowering agents on serum RBP4 levels.^{3,4} The observation by Erikstrup et al. that the serum RBP4 level correlates positively with both triglyceride and HDL cholesterol levels is unexpected. Other differences between our data and those of Erikstrup et al. may reflect treatment with various medications or the use of plasma rather than serum samples. Studies show that the homeostasis model assessment of insulin resistance (HOMA-IR) (used by Erikstrup et al.) is not informative in subjects with diabetes.⁵ In our subjects, correlations of RBP4 levels with HOMA-IR are weaker than with fasting insulin levels or the glucose disposal rate as measured with the clamp technique.

The mean serum RBP4 levels reported by Takashima et al. appear high, reflecting the fact that most RBP4 assays were developed to detect low RBP4 levels in patients with vitamin A deficiency and have not been validated for measuring elevated RBP4 levels. We would note that different commercial RBP4 assays reported widely different values for RBP4 in identical serum samples from subjects with insulin resistance. Quantitative Western blotting of the same samples yielded RBP4 values that correlated most strongly with insulin resistance. Therefore, we recommend thorough cross-validation of assays for measurements of elevated RBP4 levels. The original RBP4 sandwich enzyme-linked immunosorbent assay we used (ALPCO Diagnostics, lot 09-14-03) is no longer available. The replacement assay (ALPCO Diagnostics, catalog 30-6110, lot 28-1-2004), which differs in reagent composition and protocol, produces values that differ from those derived by other methods, including the original assay. Since serum-collection tubes containing clot-activating agents or tubes with plasma anticoagulants may cause spurious effects, we recommend the use of additive-free glass collection tubes.

We did not report dissociation between fast-

ing insulin and RBP4 levels at baseline in the group with a marginal response, as suggested by Oh, but rather dissociation between changes in fasting insulin and RBP4 levels after exercise. Baseline values in Table 2 of our article, analyzed with the use of the Wilcoxon rank-sum test, did not differ between the two groups.

Timothy E. Graham, M.D.

Beth Israel Deaconess Medical Center
Boston, MA 02215

Ulf Smith, M.D., Ph.D.

Sahlgrenska University Hospital
S-41345 Göteborg, Sweden

Barbara B. Kahn, M.D.

Beth Israel Deaconess Medical Center
Boston, MA 02215
bkahn@bidmc.harvard.edu

1. Matsumoto K, Miyake S, Yano M, et al. Glucose tolerance, insulin secretion, and insulin sensitivity in nonobese and obese Japanese subjects. *Diabetes Care* 1997;20:1562-8.
2. Kosaka K, Kuzuya T, Yoshinaga H, Hagura R. A prospective study of health check examinees for the development of non-insulin-dependent diabetes mellitus: relationship of the incidence of diabetes with the initial insulinogenic index and degree of obesity. *Diabet Med* 1996;13:Suppl 6:S120-S126.
3. Muggeo M, Zenti MG, Travia D, et al. Serum retinol levels throughout 2 years of cholesterol-lowering therapy. *Metabolism* 1995;44:398-403.
4. Sasaki H, Iwasaki T, Kato S, Tada N. High retinol/retinol-binding protein ratio in noninsulin-dependent diabetes mellitus. *Am J Med Sci* 1995;310:177-82.
5. Tripathy D, Almgren P, Tuomi T, Groop L. Contribution of insulin-stimulated glucose uptake and basal hepatic insulin sensitivity to surrogate measures of insulin sensitivity. *Diabetes Care* 2004;27:2204-10.

Molecular Mechanisms in Melanoma

TO THE EDITOR: The review of melanoma by Miller and Mihm (July 6 issue)¹ clearly characterizes the linear progression of melanocytic lesions, from the morphologic perspective to the molecular perspective.^{2,3} However, the genetic alterations would have more relevance if they result in kinetic advantage and progression. My colleagues and I have studied a series of dysplastic nevi (92 low-grade and 31 high-grade lesions) and melanomas in situ (15 lesions) using proliferation (Ki-67 labeling), apoptosis (in situ end labeling), and cell-cycle regulators (RB1, TP53, p21WAF1, and p27Kip1).⁴ Our analysis highlighted a clear topographic heterogeneity at the early stage of melanocytic transformation: slow kinetics in the dermal compartment of low-grade melanocytic dysplasia and a higher incidence of TP53 alterations in high-grade melanocytic dysplasia than in low-grade dysplasia and melanomas. These findings suggest that melanocytic dysplasia is a marker of the risk of melanoma rather than a direct precursor.

Salvador J. Diaz-Cano, M.D., Ph.D.

King's College Hospital
London SE5 9RS, United Kingdom
salvador.diaz-cano@kcl.ac.uk

1. Miller AJ, Mihm MC Jr. Melanoma. *N Engl J Med* 2006;355:51-65.
2. Meltzer PS. Genetic diversity in melanoma. *N Engl J Med* 2005;353:2104-7.
3. Bittner M, Meltzer P, Chen Y, et al. Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature* 2000;406:536-40.

4. Pozo L, Naase M, Cerio R, Blanes A, Diaz-Cano SJ. Critical analysis of histologic criteria for grading atypical (dysplastic) melanocytic nevi. *Am J Clin Pathol* 2001;115:194-204.

TO THE EDITOR: Miller and Mihm suggest that the gene encoding the microphthalmia-associated transcription factor (MITF) is a key oncogene of the melanocytic lineage, since it was strongly amplified in 15 to 20% of metastatic melanomas.^{1,2} Furthermore, amplification of the *MITF* gene and overexpression of the MITF protein were associated with decreased overall survival among patients with metastatic melanoma. MITF overexpression was also associated with impaired sensitivity to cytotoxic agents in melanoma cell lines, suggesting that the number of copies of the *MITF* gene is a surrogate biomarker of the response to chemotherapy.² To validate these findings, we investigated *MITF* gene amplification in tumor tissues from 90 patients with metastatic melanoma before the administration of individualized, sensitivity-directed chemotherapy.³ Strong amplification of the *MITF* gene (more than four copies per cell) was found in only 4 of 90 tumors (4.4%), whereas 15 of 90 tumors (16.7%) had intermediate amplification (more than two copies per cell). We found no association between the number of copies of the *MITF* gene and chemosensitivity, the outcome of chemotherapy, or overall survival. Hence, our findings suggest that the *MITF* gene