The Relationship of Retinol Binding Protein 4 to Changes in Insulin Resistance and Cardiometabolic Risk in Overweight Black Adolescents

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Objective To assess, among overweight non-hispanic black adolescents the relationship of changes in plasma retinol binding protein 4 (RBP4) over 3 years to changes in insulin resistance (IR) and 4 associated cardiometabolic risks.

Study design Nested, retrospective study of 51 overweight, post-pubertal non-Hispanic black participants in the Princeton School District Study. Participants were in the top (worsening IR) or bottom (improved IR) quartile for 3-year change in IR. RBP4 was measured by quantitative Western blot with frozen plasma. Regression analyses adjusted for age, sex, and adiposity (baseline and change). Three measures of adiposity were assessed (waist circumference, body mass index, and weight) in separate regression models.

Results RBP4 increased in one third (n = 17). In logistic regression analyses, increased RBP4 was associated with significantly higher odds of worsening as opposed to improved IR independent of age, sex, or adiposity. Odds ratios were 5.6 (weight, $P = .024$), 6.0 (BMI, $P = .025$) and 7.4 (waist circumference, $P = .015$). Initial RBP4 ($\beta = 0.81$, $P = .005$) and change in RBP4 ($\beta = 0.56$, $P = .046$) also predicted change in triglycerides, but not change in high-density lipoprotein–cholesterol, low-density lipoprotein–cholesterol, or fibrinogen.

Conclusion This retrospective cohort study provides evidence that RBP4 may be a mechanism through which obesity influences insulin resistance and hypertriglyceridemia in overweight postpubertal black youth and suggests utility of RBP4 as a biomarker of risk. *(J Pediatr 2009;154:67-73)*

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Obesity and insulin resistance are important risk factors for serious morbidity such as type 2 diabetes, cardiovascular disease, and death.[1] To date, the physiological mechanisms that link obesity, insulin resistance, and adverse outcomes remain unknown. Retinol binding protein 4 (RBP4), a potential mediator of obesity-induced insulin resistance and metabolic risk, was described and characterized by Goodman et al[2] almost 40 years ago for its role in transporting retinol from storage sites in the liver to extrahepatic tissues.[3,4] Subsequently, RBP4 was found to also be secreted by adipocytes and to be up-regulated in adipose tissue and serum of insulin resistant human beings.[5] Elevated RBP4 levels have been found in adipose tissue but not in liver and elevated RBP4 levels in adipose tissue but not in liver have been reduced by improvements in metabolic status.[5,6] These findings suggest that adipocyte-secreted RBP4 may play an important role in systemic insulin action and metabolic homeostasis in insulin resistant states. RBP4 could play a role in obesity-induced insulin resistance, because chronic elevation of RBP4 in mice increases hepatic glucose production, down-regulates insulin-signaling in muscle, and causes systemic insulin resistance.[6] Furthermore, lowering elevated serum RBP4 levels in obese mice with a synthetic retinoid improves insulin sensitivity and normalizes glucose tolerance.[6]

The relationship of RBP4 to insulin resistance and metabolic risk in human beings has been the subject of several studies.[7-12] Genetic studies in several populations suggest a causative role for RBP4 in insulin resistance and type 2 diabetes.[13,14] Most human

BMI	Body mass index	IR	Insulin resistance
Δ	Change in	HOMA-IR	Homeostasis model of insulin resistance
HDL	High-density lipoprotein	RBP4	Retinol binding protein 4
LDL	Low-density lipoprotein		

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studies were performed in adult populations of Asian or Caucasian subjects. Few have assessed RBP4 in childhood or adolescence.[8,9,12] None have studied non-Hispanic blacks, a high-risk population.[15] Studies to date have been cross-sectional or have involved short-term interventions in clinic-based populations, making it difficult to draw conclusions regarding the long-term relationship between adiposity and RBP4.

To address these gaps in the literature, we used frozen plasma samples and data from the Princeton School District Study (2001-2004) to explore the longitudinal relationship of RBP4 to insulin resistance and metabolic risks in overweight non-Hispanic black adolescents.[16] We focused on non-Hispanic black subjects for 3 reasons: (1) data on the RBP4 insulin resistance relationship in this racial/ethnic group are lacking; (2) black subjects are acknowledged to be at increased risk for obesity and its sequelae; and (3) racial and ethnic variance in the relative contributions of insulin sensitivity and pancreatic beta cell dysfunction in the development of type 2 diabetes may potentially confound the RBP4-IR relationship, making it difficult to study the RBP4-insulin resistance relationship in racially heterogeneous groups.. We hypothesized that increased plasma RBP4 over the 3 years of follow-up would be associated with worsening as opposed to improved insulin resistance over 3 years. Secondarily, we hypothesized that initial RBP4 and change in RBP4 levels would be associated with the magnitude of 3-year change of 4 other metabolic risk factors. These risk factors included traditional cardiovascular risks (triglycerides, high-density lipoprotein–cholesterol, and low-density lipoprotein–cholesterol), and a nontraditional cardiovascular risk factor (fibrinogen) associated with risk for atherosclerotic heart disease in young people.[17]

METHODS

This study uses data that were previously collected from participants in the Princeton School District (PSD) Study during the 2001-2002 and 2004-2005 schools years. PSD Study participants were included in this study if they met the following inclusion criteria: (1) non-Hispanic black race/ethnicity, (2) overweight (BMI-for-age >85%), (3) postpubertal before enrollment in the PSD study, (4) seen at baseline (2001) and at a 3-year follow-up visit with phlebotomy at each visit, (5) availability of a frozen plasma sample for RBP4 measurement, and (6) a 3-year change in insulin resistance in the top or bottom quartile for postpubertal black youth in the PSD Study. The latter inclusion criterion was used to allow us to compare those with worsening insulin resistance (top quartile of change) to those with improved insulin resistance (bottom quartile of change), and therefore facilitate testing of our primary hypothesis in this fixed sample. Fifty-one youth (39 females, 12 males; mean age at baseline = 15.4 years, SD1.5 years, range 12.9 to 18.2 years) met the inclusion criteria. Twenty-five had worsening insulin resistance, and 26 had improved insulin resistance.

Participants in the PSD Study provided written assent, and a parent gave informed consent before entry into the study. Study visits occurred in the morning after a minimum 10-hour overnight fast, which was verified. Height, weight, waist circumference, and a venipuncture were performed by trained study personnel as previously described.[16,18,19] Body mass index (BMI, kg/m^2) was calculated from measured height and weight.[16] BMI z scores and percentiles were derived on the basis of age and sex from the CDC 2000 growth chart standards. BMI-for-age percentiles were used to determine weight status by classifying participants as normal weight (BMI-for-age <85%) or overweight (BMI-for-age ≥ 85%). Cardiometabolic risk factors assessed in the PSD Study included insulin, glucose, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides, and fibrinogen, as previously described in detail.[20] Fasting insulin and glucose were used to calculate the homeostasis model of insulin resistance (HOMA-IR).[21] HOMA-IR is a surrogate measure of insulin resistance that is frequently used in epidemiologic studies. The measure is derived using the following formula: [Glucose (mmol/L) × Insulin (mIU)]/22.5.[21] Although euglycemic clamps are the gold standard for measurement of insulin resistance, it was not feasible to do clamps in the PSD Study, which was a large school-based epidemiologic study. The validity of surrogate markers of IR, including HOMA-IR, for use in such studies of children and youth is supported by a number of studies.[22-24]

Plasma stored at −70° C from the baseline and 3-year follow-up visit was used to assay RBP4 for this study. Plasma RBP4 concentrations were calculated with a quantitative Western blotting protocol as previously described.[25] This assay allows sample concentrations to be calculated against pre-prepared RBP4 standards of 15, 30, 60, and 120 μg/mL run on the same gel as the experimental samples. In this study, baseline and follow-up assays were run in adjacent lanes within the same gel. Intraassay and interassay coefficient of variations were 9.1% and 17.2%, respectively.

Statistical Analyses

All analyses were performed with SPSS for Windows (SPSS Inc, Chicago, Illinois).[26] To test our primary hypothesis, contingency table analyses were performed between increased RBP4 and placement in the worsening versus improved insulin resistance groups. Next, we used logistic regression to assess whether the relationships between increase in RBP4 and worsening insulin resistance remained robust after adjusting for baseline age, sex, and adiposity. We tested baseline levels and change over 3 years for 3 separate measures of adiposity—waist circumference, BMI, and weight. These measures of adiposity were highly correlated (r = 0.85 for weight and BMI to r = 0.95 for waist circumference and BMI), and so could not be assessed together in a single model. Therefore each measure of adiposity modeled independently and the results of the 3 models are reported. We also assessed whether an interaction between increase in RBP4 and sex existed. This sex interaction was not found. Multiple linear regression analyses were performed to determine the relation-