Published in final edited form as:

J Pediatr Endocrinol Metab. 2011 ; 24(0): 913–919.

Retinol binding protein 4 is associated with adiposity-related co-morbidity risk factors in children

Rushika Conroy^{1,*}, Yomery Espinal¹, Ilene Fennoy¹, Siham Accacha⁴, Claudia Boucher-Berry³, Dennis E. Carey³, Sharron Close¹, Deborah DeSantis², Rishi Gupta⁴, Abeer A. Hassoun¹, Loretta Iazzetti³, Fabean J. Jacques⁴, Amy M. Jean¹, Lesly Michel⁴, Katherine Pavlovich¹, Robert Rapaport⁵, Warren Rosenfeld⁴, Elisabeth Shamoon¹, Steven Shelov², Phyllis W. Speiser³, Svetlana Ten², and Michael Rosenbaum¹

¹Pediatrics, New York Presbyterian Hospital-Columbia University Medical Center, New York, NY, USA

²Pediatrics, Maimonides Infants and Children's Hospital, Brooklyn, NY, USA

³Pediatrics, Cohen Children's Medical Center, New Hyde Park, NY, USA

⁴Pediatrics, Winthrop University Hospital, Mineola, NY, USA

⁵Pediatrics, Mount Sinai Medical Center, New York, NY, USA

Abstract

Objective—In adults, elevated levels of retinol binding protein 4 (RBP4) have been associated with biochemical markers of adiposity-related co-morbidities including insulin resistance, dyslipidemia, hypertension, and abdominal obesity. This study examined the relationship between RBP4 and risk factors for co-morbidities of adiposity in a population of ethnically diverse children in early- to mid-adolescence in the public school system of New York City.

Materials/methods—We analyzed anthropometric (body mass index, % body fat, waist circumference), metabolic (lipids, glucose), and inflammatory (TNF- α , interleukin-6, C-reactive protein, adiponectin) markers for adiposity-related co-morbidities and serum alanine aminotransferase (ALT) in 106 school children (65 males, 41 females) 11–15 years of age (mean $\pm$ SD = 13.0 $\pm$ 0.1 years) who were enrolled in the Reduce Obesity and Diabetes (ROAD) project. Insulin sensitivity was assessed by quantitative insulin sensitivity check index. Insulin secretory capacity was measured as acute insulin response and glucose disposal index.

Results—Serum RBP4 was significantly correlated directly with ALT, triglycerides, and triglyceride z-score, and inversely correlated with adiponectin. Correlations with ALT and adiponectin remained significant when corrected for % body fat, age, and gender. There were significant ethnic differences in the relationship of RBP4 to ALT, glucose disposal index and adiponectin.

*Corresponding author: Rushika Conroy, MD MS, Division of Pediatric Endocrinology, Children's Hospital of New York, Presbyterian-Columbia University Medical Center, 622 West 168th Street, PH 5E-522, New York, NY 10032, USA, Phone: +1-212-305-6559, Fax: +1-212-305-4778, rushika.conroy@baystatehealth.org.

Conflict of interest statement

Disclosures: The authors of this article have nothing to disclose.

Conclusions—In early- to mid-adolescents, circulating concentrations of RBP4 are correlated with multiple risk factors for adiposity-related co-morbidities. The observation that many associations persisted when corrected for % body fat, suggests that RBP4 can be viewed as an independent marker of adiposity-related co-morbidity risk in children.

Keywords

inflammation; insulin resistance; lipids; obesity

Introduction

The prevalence of adolescent obesity is increasing at the rate of 20%–30% per decade (1). The burden of this epidemic falls disproportionately on Native, Asian, African and Hispanic Americans (2–4). These are the same minority groups that are experiencing the greatest increase in the prevalence of adiposity-related co-morbidities (1–5). Adiposity-related co-morbidities, formerly considered to be “adult-onset”, such as metabolic syndrome, type 2 diabetes mellitus, obstructive sleep apnea, hypertension, dyslipidemia and hepatic steatosis, have now become pediatric diseases (5). Biochemical markers for risk factors for the development of these co-morbidities are being researched in both the adult and pediatric populations, with the understanding that the presence of such markers will allow physicians to target aggressive preventive measures to those patients who are at elevated risk for, but have not yet been afflicted with, these co-morbidities.

Retinol binding protein 4 (RBP4), generally known for its role in vitamin A transport and function, has recently been linked to insulin resistance (6). Increased RBP4 levels are seen in mouse models of type 2 diabetes and obesity (6, 7). In adult humans, the relationship of RBP4 to markers of other adiposity-related disease states has been studied extensively. Direct correlations to waist circumference, body mass index (BMI), blood pressure and hepatic transaminases, and inverse correlations to adiponectin levels, have been demonstrated (7–12). In addition, RBP4 has been shown to have a direct correlation to triglycerides (TG), fasting glucose and insulin and an inverse correlation to high-density lipoprotein cholesterol (HDL) (7, 11). These findings have led to the postulate that RBP4 may serve as a useful marker of a number of adiposity-related risk factors and co-morbidities in adults. In the pediatric population, studies on the relationship of RBP4 to markers of adiposity-related co-morbidities are limited, have been less consistent, and have been conducted mainly in populations of overweight and obese children (13–17).

The purpose of this study is to examine the relationship between circulating concentrations of RBP4 and risk factors for co-morbidities of adiposity in an anthropometrically and ethnically diverse population of children in early-adolescence. We hypothesized that RBP4 would serve as a useful marker of insulin resistance and increasing adiposity, as well as dyslipidemia, non-alcoholic fatty liver disease and inflammation, and would therefore serve as a valuable screen for adiposity-related illness in the peri-adolescent pediatric population.

Methods

This is a study of a multi-ethnic population of junior high school students in New York public schools that is part of a five-site research consortium called the Reduce Obesity and Diabetes (ROAD) project that has been assembled via the Academy for Medical Development and Collaboration (AMDeC, New York, NY, USA) (18). The sites are: New York Presbyterian Hospital-Columbia University Medical Center, Maimonides Infants and Children's Hospital, Mount Sinai Medical Center, Cohen Children's Medical Center, and Winthrop University Hospital. This study was approved by the Institutional Review Board of all relevant institutions as well as the New York City Board of Education and is consistent with guiding principles for research involving humans (19).

Blood samples were obtained from 106 middle school children (11–14 years of age; 61 males, 45 females) from five junior high schools in the New York Metropolitan area. To ensure a diverse multiethnic background, an initial population of 619 subjects studied on at least two occasions, and who had been identified as having a specific ethnicity, were divided into five ethnic groups: African American, East Asian American (China, Japan, Taiwan, Korea), South Asian American (India, Pakistan, Sri Lanka, Bangladesh), Caucasian, and Hispanic. Subjects were ranked from the lowest to the highest range for insulin resistance using the quantitative insulin sensitivity check index (QUICKI). Within the African American, Caucasian and Hispanic groups, subjects with the highest QUICKI in each 10th percentile rank were chosen followed by the highest glucose disposal index (GDI) in each 10th percentile rank. In the case of the East Asian American and South Asian American groups, a similar procedure was used for the highest values of QUICKI and GDI in each 5th percentile rank. This yielded 120 subjects with a relatively even distribution between ethnic backgrounds. Outliers for fasting insulin or inflammatory markers [interleukin 6 (IL-6), TNF- α , C-reactive protein (CRP)] more than 3SD from the mean were eliminated prospectively on the assumption that such subjects were probably either not truly fasting or were ill. Specifically, five subjects were excluded for insulin >40 mIU/mL, three subjects were excluded because of IL-6>4.2 pg/mL, two subjects were excluded because of TNF- α >9.6 pg/mL, and three subjects were excluded because of CRP>36.2 pg/mL. Due to overlap between these groups, a total of 12 subjects were excluded. An additional two subjects were excluded because of poor sample collection, yielding a total of 106 subjects that were studied. The ethnic distribution of subjects was as follows: 22 African American, 23 Caucasian, 30 East Asian American, 10 South Asian American, and 25 Hispanic. Written informed consent and assent were obtained from all parents and students, respectively. Subject characteristics are presented in Table 1.

Co-morbidity risk was assessed by family history, anthropometry, and biochemical evaluation of lipids, glucose homeostasis, and inflammation. A medical and family history questionnaire including age, ethnicity, gender, family history of obesity, hypertension, and diabetes, as well as the subject's current medications and health problems, was completed with the assistance of a study investigator. This information was then reviewed with the parent or legal guardian either in person or over the phone. All students underwent anthropometric measurements including weight (light clothes, no sweaters, jackets or shoes), height, and waist circumference (obtained at the level just above the iliac crest) on the

morning of the study. Body composition was analyzed by bioelectrical impedance (Body Fat Analyzer, Model HBF-306, Omron, Gays Mills, WI, USA).

Students were studied in the fasting state between 09:00 h and 10:00 h. A 21-gauge butterfly needle was placed in an antecubital or dorsal hand vein and blood was drawn for fasting glucose, insulin, TNF- α , CRP, IL-6, total cholesterol, HDL, low density lipoprotein-cholesterol (LDL), and TG levels, as well as alanine aminotransferase (ALT), adiponectin, and RBP4. Students then received a standard intravenous glucose load (0.5 g/kg, maximum 25 g) over 3–4 min and blood was drawn for insulin at 1, 3 and 5 min after glucose administration. Following the intravenous glucose tolerance test, students were given breakfast and then returned to their regularly scheduled classes.

Fat mass was calculated from the weight and % body fat using the following equation: [fat mass=(weight in kg) $\times$ (% body fat)/100]. The homeostatic model of insulin resistance (HOMA-IR) was used as an index of insulin sensitivity and was calculated as [(fasting glucose in mg/dL) $\times$ (fasting insulin in mU/L)/405]. Insulin sensitivity was measured as QUICKI [$1/(\log \text{ fasting insulin} \times \log \text{ fasting glucose})$] (20). First phase insulin release was examined using the acute insulin response (AIR), which was calculated as the mean rise in circulating concentrations of insulin 3 and 5 min following administration of intravenous dextrose. Because insulin sensitivity and insulin release are significantly correlated, the GDI was calculated as $\log_{10} (\text{AIR} \times \text{fasting glucose concentration} / \text{fasting insulin concentration})$ to examine insulin secretory capacity once corrected for insulin sensitivity (21–24).

Serum RBP4 was measured by ELISA (ALPCO, Salem, NH, USA), with intra-assay precision of 5.0% and interassay precision of 9.7%–9.8%. Glucose was measured by the hexokinase (HK) method (glucose/HK; Roche Molecular Biochemicals, Werk Penzberg, Germany). Plasma insulin was measured by solid phase ^{125}I -RIA (Coat-a-count; DPC, Los Angeles, CA, USA). Lipid profiles were measured colorimetrically using an automated Hitachi 704 spectrometer (Roche Diagnostics; Indianapolis, IN, USA). CRP and TNF- α were measured by ELISA. ALT was measured enzymatically (Olympus, Diagnostica, GmBH, Lismeehan, Ireland), with intra-assay coefficient of variation of <2% and interassay coefficient of variation of <3%. IL-6 was measured by RIA (24).

Statistics and calculations

Data are presented as mean $\pm$ SD unless otherwise indicated. Inflammatory cytokine data more than 3SD above the mean, were excluded from the analysis against the possibility that they were indicative of ongoing inflammatory processes and did not reflect the true status for those subjects. Fasting insulin concentrations, and all data derived from them, were excluded in subjects with a fasting insulin concentration greater than 3SD from the mean, against the possibility that they had not fasted on the day of testing.

Whenever possible, data were converted into z-scores to control for the effects of age and gender. This was done for BMI and waist circumference as indices of adiposity, and for total cholesterol, TG, HDL, and LDL for lipids. In addition, we calculated a lipid risk score as the z-scores for LDL and TG minus the z-score for HDL. Further analyses of individual components of the lipid panel were conducted, only if there were significant between ethnic

group differences in the lipid risk score, or if there was a significant correlation of RBP4 with the lipid risk score. Multiple linear regression analyses were run and partial correlation coefficients calculated using the entire data set with ethnicity and RBP4 as independent variables and measures of glucose homeostasis, lipids, and inflammation as individual dependent variables. Similar analyses were conducted using BMI z-score as an independent variable and RBP4 as a dependent variable. When race was a significant covariate, further analyses comparing individual ethnic groups were conducted. Using this technique significantly reduced the number of comparisons being made. The initial hypotheses are that RBP4 is significantly correlated with fat (BMI, BMI z-score), fat distribution (waist circumference, waist circumference z-score, % body fat), insulin sensitivity (HOMA), insulin secretory capacity (GDI), lipids (HDL, LDL, total cholesterol, TG, lipid risk score), and inflammatory markers (CRP, IL-6, adiponectin, TNF- α). Because these dependent variables are all inter-related, post-hoc adjustments, which assume that the tests are independent, were not made (25–27).

Results

Between group comparisons

There were no significant gender differences in circulating concentrations of RBP4. Males were significantly taller (160.0 ± 7.0 cm vs. 156.5 ± 5.9 cm, $p < 0.05$), had significantly higher total cholesterol (172 ± 35 mg/dL vs. 159 ± 27 mg/dL, $p < 0.05$), total cholesterol z-scores (0.33 ± 1.01 vs. -0.04 ± 0.76 , $p < 0.05$), ALT (12.7 ± 5.5 U/L vs. 10.6 ± 3.8 U/L, $p < 0.05$) and lower adiponectin (13.7 ± 4.8 μ g/mL vs. 11.1 ± 4.7 μ g/mL, $p < 0.01$) than females (data not shown). As shown in Table 2, BMI z-score was significantly higher in Caucasians than in East Asian Americans and fat mass was significantly greater in African American, Caucasian, and Hispanic children compared to East Asian American children ($p < 0.05$). ALT was significantly higher in South Asian Americans than in African Americans, Caucasians, and East Asian Americans, and, if BMI z-score was included as a covariate, than in Hispanics as well ($p < 0.05$).

Body composition

No significant correlations were noted between serum levels of RBP4 and any measures of body composition in the group as a whole or in any ethnic group (data not shown).

Glucose homeostasis

No significant correlations were noted between serum levels of RBP4 and any indices of insulin sensitivity (data not shown). Insulin secretory capacity, as assessed by GDI, was significantly negatively correlated with RBP4 in South Asian Americans ($p < 0.05$) and nearly significantly negatively correlated with RBP4 in East Asian Americans ($p = 0.09$).

Lipids

Serum levels of RBP4 were significantly correlated with overall lipid risk score and more specifically with TG and TG z-scores in the overall group (all $p < 0.05$) but these correlations were not statistically significant within any single ethnic group (Figure 1).

Inflammation

Serum levels of RBP4 were significantly negatively correlated with adiponectin ($p<0.0001$) in the group as a whole and in African Americans ($p<0.01$, Figure 2). It was nearly significantly negatively correlated with adiponectin in Caucasians ($p=0.06$) and East Asian Americans ($p=0.08$). These correlations persisted when corrected for % body fat, age and gender. No significant correlations of serum levels of RBP4 with any of the pro-inflammatory cytokines (IL-6, CRP, or TNF- α) were noted (data not shown).

Other

RBP4 was positively correlated with ALT over all subjects ($p<0.0001$), as well as in East Asian Americans ($p<0.01$) and Hispanics ($p=0.05$, Figure 2). These correlations persisted when corrected for % body fat, age and gender.

Discussion

RBP4 has been identified as an important marker of insulin resistance, obesity and other co-morbidities of adiposity in a number of subject populations. The major findings of this study are that RBP4 exhibits direct correlations to TG, TG z-score, ALT and lipid risk score, as well as indirect correlations to adiponectin, in a multi-ethnic group of middle school aged children. Correlations with ALT and adiponectin persist when corrected for % body fat, age and gender, and a number of correlations varied within ethnic groups.

The pediatric literature is not consistent regarding the relationships of RBP4 to adiposity-related co-morbidities. This may reflect possible ethnic-specific differences or confounding effects of other variables, such as insulin sensitivity. As in this study, Balagopal et al. (14) did not observe a significant correlation between RBP4 and BMI in lean and obese adolescents. Yeste et al. (13) reported no differences in circulating RBP4 concentrations between lean and obese peri-adolescent children with normal glucose tolerance, but found that obese children with abnormal glucose tolerance had significantly higher circulating RBP4 levels than obese or lean children with normal glucose tolerance, suggesting an independent effect of insulin sensitivity on RBP4, but little independent effect of adiposity. Similarly, Lee et al. (16) studied obese and non-obese Korean adolescents and observed that the relationship between BMI and RBP4, while significant in the study population as a whole, did not persist when the obese group and the non-obese group were analyzed separately. Correlations of RBP4 and BMI were also not significant once corrected for other co-variables, such as gender and insulin sensitivity, suggesting that the significant correlation in the overall group may have been due to a non-normal distribution of RBP4. Unlike the present study, some studies in lean, overweight, and obese children and adolescents have demonstrated a significant correlation between RBP4 and BMI (13, 15). Reinehr et al. (15) reported a significant correlation of RBP4 with BMI (or BMI z-score) in obese and non-obese children, but did not examine the possibility that the correlation of RBP4 with body fatness was not independent of insulin sensitivity.

Both pediatric and adult studies examining the relationship between circulating concentrations of RBP4 and lipids, have reported significant correlations between RBP4 and

TG (7, 11–17), in agreement with our findings. More specifically, Lee et al. (16), Aeberli et al. (17) and Rhie et al. (28) reported significant correlations of RBP4 and TG in mixed obese and non-obese pediatric populations. Yeste et al. (13) also noted these findings in obese children. The physiologic mechanisms behind this relationship may, in part, be due to the role of RBP4 in fatty acid oxidation. RBP4 decreases fatty acid oxidation in the liver, thereby increasing accumulation of fatty acids that are stored as TG. Increasing levels of RBP4 would therefore lead to increasing levels of TG. The lipid risk score (TG z-score plus LDL z-score minus HDL z-score) provides an assessment for future cardiovascular risk. This score is similar to other cardiovascular risk scores, such as the Framingham score, which take into account not only lipid levels, but also blood pressure, age, diabetes status and smoking status (29). Our findings indicate that increasing levels of RBP4 are correlated with a worsening lipid risk score. The correlation of RBP4 with both lipid risk score and TG emphasizes the importance of diets that are low in cholesterol, total and saturated fat, not only for cardiovascular health, but for prevention of insulin resistance as well.

This study is the first, to our knowledge, to assess the relationship of RBP4 to the risk of non-alcoholic fatty liver disease in healthy children. We found statistically significant correlations between RBP4 and ALT in the entire group as well as in East Asian American and Hispanic children. Nobili et al. (30) studied RBP4 levels in children with non-alcoholic fatty liver disease and found that elevated levels were associated with healthier livers. Similar to our findings, Seo et al. (10) noted direct correlations of RBP4 with ALT, while Schina et al. (31) and Alkhouri et al. (32) found decreased levels of RBP4 in association with worsening liver disease in adults. While RBP4 is stored in and released from adipose tissue, the primary source is the liver. With progressive disease of the liver, such as steatosis, steatohepatitis and nonalcoholic fatty liver disease, production of RBP4 can decrease. Levels may increase, however, before declining, similar to the rise in hepatic transaminases during early stages of liver disease, with a fall accompanying later stages. The rise in RBP4 may be a useful indicator of the ensuing risk of liver disease in adolescents who do not yet have overt hepatic dysfunction.

The link between RBP4 and insulin resistance was initially established in mouse models (6, 7), with compatible findings noted in both adults and children (7, 9, 13–17). Rhie et al. (28) found significant correlations between RBP4 and HOMA-IR in a group of lean and obese children. Correlations of RBP4 to HOMA-IR were described in a study by Lee et al. (16) in obese and non-obese Korean adolescents when analyzed as a whole group as well as in the non-obese group, but was not found in the obese group. In Aeberli et al.'s study (17) of obese children, no correlation was noted between RBP4 and HOMA-IR, while Reinehr et al. (15) did note that changes in RBP4 with weight loss were correlated with changes in HOMA-IR in obese children. Our findings are in agreement with many of the pediatric studies, in that they did not confirm a significant association between RBP4 and HOMA-IR in early adolescent non-obese children. The degree of overweight or obesity appears to contribute to the presence or absence of significance in the relationship of RBP4 to insulin resistance in the pediatric population.

South Asians have been noted to have increased visceral fat as well as increased markers of inflammation, cardiovascular risk and insulin dynamics when compared to Caucasians of

similar BMI and age (33, 34). The South Asian American group in our study was the only one to exhibit a statistically significant correlation between RBP4 and a marker of insulin secretion (GDI). The group also had a higher (albeit not statistically significant) mean fasting insulin level compared to the other ethnic groups studied. These findings may indicate that, even at young ages, South Asian Americans are already starting to display an increasing risk for pathologic changes in insulin dynamics, and that RBP4 may be a useful indicator of this risk in this more vulnerable population.

Adiponectin is produced in adipose tissue and is decreased in states of insulin resistance and obesity (35). Adiponectin infusion to hyperinsulinemic mice resulted in an improvement in insulin sensitivity, suggesting that decreased levels may contribute to the insulin resistant state (36). This study is the first to assess the relationship of RBP4 to adiponectin among children of varying ethnic backgrounds. Rhie et al. (28) studied lean, overweight and obese adolescents of Korean descent and noted an inverse correlation between RBP4 and adiponectin, which did not persist, however, after multiple linear regression analysis. Balagopal et al. (14) also noted a correlation between adiponectin and RBP4 in a group of lean and obese children. Our study is consistent with these findings and also noted significant correlations between the two in African American children. African Americans have been noted to have increased insulin resistance compared to Caucasians (37). We show correlations between decreased adiponectin and increasing RBP4 in African American periadolescents. In East Asian American and Caucasian children, p-values approached statistical significance. Qi et al. (11) observed a correlation between RBP4 and adiponectin in older Chinese men and women. The study by Qi et al., however, had over 3000 subjects, while our study had only 28 East Asian Americans. Increasing the number of East Asian Americans in our study may lead to a significant correlation in this ethnic group.

This study is unique, in that our subjects were anthropometrically and ethnically diverse, which allowed us to explore the relationship of RBP4 to markers of adiposity-related co-morbidities in a non-high risk population of children. The significance of our findings underscores the need to target children with preventive therapy prior to the development of obesity, or the manifestation of adiposity-related co-morbidities, since correlations exist in those who are not overweight or obese in addition to those who are. Limitations of this study include the inability to utilize gold standard methodologies to assess insulin resistance and body composition (hyperinsulinemic euglycemic clamp and dual energy X-ray absorptiometry, respectively), potentially providing a different outcome. However, performing such tests on a large number of students would involve long hospital visits and missed school days, which would have been a detriment to the students and likely made the number of participants decrease immensely. In addition, we did not determine pubertal status in our study subjects. Adams et al. (33) reported worsening insulin sensitivity with pubertal progression, and Moran et al. (37) noted a worsening lipid profile and insulin resistance in males throughout puberty. The pattern of RBP4 levels, and whether or not its relationship to adiposity-related co-morbidities changes with pubertal progression, could not be determined, and may have been a confounding factor in our results.

In summary, we show that circulating concentrations of RBP4 are positively associated with TG, TG z-score, ALT and negatively associated with adiponectin. Many of these

associations between RBP4 and other risk factors for adiposity-related co-morbidities persist when corrected for % body fat, age, and sex. In addition, ethnic variability was observed with respect to some of these relationships. These data suggest that RBP4 is an independent marker of adiposity-related co-morbidity risk in children. Further establishment of RBP4 as such a marker may offer health care providers a tool to begin aggressive preventive measures in those who are at increasing risk for co-morbidities, but have not yet been afflicted with them.

Acknowledgments

We would like to gratefully acknowledge the invaluable participation of all of the students, teachers, and school administrators, as well as the New York City Board of Health and Department of Education.

Funding: AMDeC (Academy for Medical Development and Collaboration) by the Starr Foundation, NIH grant numbers UL1RR0023568 and UL1RR0024156, and T32 grant number 5T32-DK06552-07.

References

1. Rosenbaum M. Epidemiology of pediatric obesity. *Pediatr Annals*. 2007; 36:89–95.
2. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WM. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988–1994. *Arch Pediatr Adolesc Med*. 2003; 157:821–7. [PubMed: 12912790]
3. Dablea D, Pettitt D, Jones K, Arslanian S. Type 2 diabetes mellitus in minority children and adolescents. *Endocrinol Metab Clin N Am*. 1999; 28:709–29.
4. Caprio S, Tamborlane W. The metabolic impact of obesity in childhood. *Endocrinol Metab Clin N Am*. 1999; 28:731–47.
5. Lee YS. Consequences of childhood obesity. *Annals Acad Med*. 2009; 38:75–81.
6. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature*. 2005; 436:356–62. [PubMed: 16034410]
7. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, et al. Retinol-binding protein 4 and insulin resistance in lean, obese and diabetic subjects. *N Engl J Med*. 2006; 354:2552–63. [PubMed: 16775236]
8. von Eynatten M, Lepper PM, Liu D, Lang K, Baumann M, et al. Retinol-binding protein 4 is associated with components of the metabolic syndrome, but not with insulin resistance, in men with type 2 diabetes or coronary artery disease. *Diabetologia*. 2007; 50:1930–7. [PubMed: 17639305]
9. Broch M, Vendrell J, Ricart W, Richart C, Fernandez-Real JM. Circulating retinol-binding protein-4, insulin sensitivity, insulin secretion, and insulin disposition index in obese and non-obese subjects. *Diabetes Care*. 2007; 30:1802–6. [PubMed: 17416795]
10. Seo JA, Kim NH, Park SY, Kim HY, Ryu OH, et al. Serum retinol-binding protein 4 levels are elevated in non-alcoholic fatty liver disease. *Clin Endocrinol*. 2008; 68:555–60.
11. Qi Q, Yu Z, Xingwang Y, Zhao F, Huang P, et al. Elevated retinol-binding protein 4 levels are associated with metabolic syndrome in Chinese people. *J Clin Endocrinol Metab*. 2007; 92:4827–34. [PubMed: 17878249]
12. Kowalska I, Strackowski M, Adamska A, Nikolajuk A, Karczewska-Kupczewska M, et al. Serum retinol binding protein 4 is related to insulin resistance and non-oxidative glucose metabolism in lean and obese women with normal glucose tolerance. *J Clin Endocrinol Metab*. 2008; 93:2786–9. [PubMed: 18430770]
13. Yeste D, Vendrell J, Tomasini R, Gallart LL, Clemente M, et al. Retinol-binding protein 4 levels in obese children and adolescents with glucose intolerance. *Horm Res Paediatr*. 2010; 73:335–40. [PubMed: 20389103]

14. Balagopal P, Graham TE, Kahn BB, Altomare A, Funanage V, et al. Reduction of elevated serum retinol binding protein in obese children by lifestyle intervention: association with subclinical inflammation. *J Clin Endocrinol Metab.* 2007; 92:1971–4. [PubMed: 17341558]
15. Reinehr T, Stoffel-Wagner B, Roth CL. Retinol-binding protein 4 and its relation to insulin resistance in obese children before and after weight loss. *J Clin Endocrinol Metab.* 2008; 93:2287–93. [PubMed: 18397979]
16. Lee DC, Lee JW, Im JA. Association of serum retinol binding protein 4 and insulin resistance in apparently healthy adolescents. *Metabolism.* 2007; 56:327–31. [PubMed: 17292720]
17. Aeberli I, Biebinger R, Lehmann R, l'Allemand D, Spinass GA, et al. Serum retinol-binding protein 4 concentration and its ratio to serum retinol are associated with obesity and metabolic syndrome components in children. *J Clin Endocrinol Metab.* 2007; 92:4359–65. [PubMed: 17726085]
18. Rosenbaum M, Accacha S, Altshuler L, Carey DE, Fennoy I, et al. The reduce obesity and diabetes (ROAD) project: design and methodological considerations. *Child Obes.* 2011; 7:223–34.
19. World Medical Association, American Physiological Society. Guiding principles for research involving animals and human beings. *Am J Physiol Regul Integr Comp Physiol.* 2002; 283:R281–3. [PubMed: 12121837]
20. Katz A, Nambi S, Mather K, Baron AD, Follmann DA, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab.* 2000; 85:2402–10. [PubMed: 10902785]
21. Zethelius B, Byberg L, Hales C, Lithell H, Berne C. Proinsulin and acute insulin response independently predict type 2 diabetes mellitus in men—report from 27 years of follow-up study. *Diabetologia.* 2003; 46:20–6. [PubMed: 12637978]
22. Kahn S. The relative contributions of insulin resistance and β -cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia.* 2003; 46:3–19. [PubMed: 12637977]
23. Rosenbaum M, Nonas C, Horlick M, Fennoy I, Vargas I, et al. The EI Camino diabetes prevention group. Beta-cell function and insulin sensitivity in early adolescence: association with body fatness and family history of type 2 diabetes mellitus. *J Clin Endocrinol Metab.* 2004; 89:5469–76. [PubMed: 15531499]
24. Rosenbaum M, Nonas C, Weil R, Horlick M, Fennoy I, et al. The EI Camino diabetes prevention group. School-based intervention acutely improves insulin sensitivity and decreases inflammatory markers and body fatness in junior high school students. *J Clin Endocrinol Metab.* 2007; 92:504–8. [PubMed: 17090635]
25. Rothman K. No adjustments are needed for multiple comparisons. *Epidemiol.* 1990; 1:43–6.
26. Perneger T. What's wrong with Bonferroni adjustments. *Br Med J.* 1998; 316:1236–8. [PubMed: 9553006]
27. Thomas D, Siemiatycki J, Dewar R, Robins J, Goldberg M, et al. The problem of multiple inference in studies designed to generate hypotheses. *Am J Epidemiol.* 1985; 122:1080–95. [PubMed: 4061442]
28. Rhie YJ, Choi BM, Eun SH, Son CS, Park SH, et al. Association of serum retinol binding protein 4 with adiposity and pubertal development in Korean children and adolescents. *J Korean Med Sci.* 2011; 26:797–802. [PubMed: 21655067]
29. Wilson PW, D'Agostino RB, Levy D, Belanger A, Silbershatz H, et al. Prediction of coronary heart disease using risk factor categories. *Circulation.* 1998; 97:1837–47. [PubMed: 9603539]
30. Nobili V, Alkhouri N, Alisi A, Ottino S, Lopez R, et al. Retinol-binding protein 4: a promising circulating marker of liver damage in pediatric non-alcoholic fatty liver disease. *Clin Gastroenterol Hepatol.* 2009; 7:575–9. [PubMed: 19268270]
31. Schina M, Koskinas J, Tiniakos D, Hadziyannis E, Savvas S, et al. Circulating and liver tissue levels of retinol-binding protein-4 in non-alcoholic fatty liver disease. *Hepatol Res.* 2009; 39:972–8. [PubMed: 19624771]
32. Alkhouri N, Lopez R, Berk M, Feldstein AE. Serum retinol-binding protein 4 levels in patients with non-alcoholic fatty liver disease. *J Clin Gastroenterol.* 2009; 43:985–9. [PubMed: 19525859]
33. Adams TC, Hasson RE, Lane CJ, Davis JN, Weigensberg MJ, et al. Fasting indicators of insulin sensitivity: effects of ethnicity and pubertal status. *Diab Care.* 2011; 34:994–9.

34. Annaswamy R, Seely EW, Arky RA, Simonson DC. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. *J Clin Endocrinol Metab.* 2001; 86:5366–71. [PubMed: 11701707]
35. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol.* 2010; 316:129–39. [PubMed: 19723556]
36. Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends Endocrinol Metab.* 2002; 13:84–9. [PubMed: 11854024]
37. Moran A, Jacobs DR Jr, Steinberger J, Steffen LM, Pankow JS, et al. Changes in insulin resistance and cardiovascular risk during adolescence. *Circulation.* 2008; 117:2361–8. [PubMed: 18427135]

Figure 1. Correlations of RBP4 to TG, TG z-score and lipid risk score for all study subjects and divided by ethnic background. * $p < 0.01$, † $p < 0.05$.

Figure 2. Correlations of RBP4 to adiponectin and ALT for all study subjects and divided by ethnic background. *p<0.01.

Table 1

Mean (SD) anthropometric data for all study subjects.

Variables	African American	Caucasian	East Asian American	Hispanic	South Asian American	All subjects ^a
Gender	12 Male 10 Female	10 Male 11 Female	19 Male 9 Female	13 Male 9 Female	6 Male 4 Female	61 Male 45 Female
Age, years	12.9 (0.8)	12.7 (1.0)	12.4 (0.8)	13.3 (0.9)	13.1 (1.1)	12.6 (1.0)
Height, cm	159.9 (7.9)	156.5 (6.8)	155.7 (8.5)	161.6 (11.6)	160.5 (9.1)	158.3 (9.2)
Weight, kg	60.9 (14.7)	62.2 (15.2)	50.7 (16.2)	63.0 (17.2)	56.2 (11.5)	58.2 (15.9)
BMI, kg/m ²	23.7 (5.1)	24.9 (5.6)	20.6 (5.1)	23.9 (5.2)	21.7 (3.3)	22.9 (5.3)
BMI z-score	1.08 (0.98)	1.32 (0.85) ^b	0.44 (1.08)	0.95 (0.97)	0.87 (0.59)	0.88 (0.98)
Waist, cm	78.3 (11.9)	80.4 (13.7)	72.5 (13.9)	81.0 (13.7)	78.8 (12.4)	77.7 (13.4)
Waist z-score	0.70 (0.90)	0.86 (1.07)	0.37 (1.12)	0.98 (1.22)	0.66 (0.91)	0.67 (1.04)
Body fat, %	29.0 (9.1) ^b	29.7 (8.8) ^b	25.5 (6.9)	28.4 (8.3) ^b	29.1 (7.7)	28.0 (8.1)

^a Includes three subjects (one male, two female) classified as "other" because ethnicity could not be ascertained.^b p<0.05 compared to East Asian Americans.

Table 2

Mean (SD) laboratory values for all study subjects.

Variables	African American	Caucasian	East Asian American	Hispanic	South Asian American	All subjects ^a
n	22	21	28	22	10	106
SBP ^a , mmHg	118.9 (4.7)	120.4 (4.0)	121.1 (4.0)	119.8 (5.1)	122.5 (3.3)	120.3 (4.3)
ALT, U/L	11.0 (4.0)	12.2 (4.4)	10.1 (6.2)	13.0 (4.3)	15.8 (4.1) ^b	11.8 (5.0)
Cholesterol, mg/dL	174 (43)	165 (28)	168 (22)	151 (24)	169 (30)	164 (31)
Cholesterol z-score	0.40 (1.25)	0.17 (0.84)	0.12 (0.74)	-0.22 (0.66)	0.30 (0.82)	0.12 (0.89)
Triglycerides, mg/dL	60 (22)	80 (30)	78 (50)	83 (36)	85 (42)	76 (37)
HDL, mg/dL	55 (11)	50 (10)	59 (13)	47 (12)	50 (10)	53 (12)
LDL, mg/dL	107 (38)	100 (25)	93 (21)	87 (21)	102 (23)	96 (27)
Lipid risk score ^c	-0.40 (1.19)	0.20 (1.12)	-0.56 (1.64)	0.20 (1.46)	0.29 (1.45)	-0.14 (1.40)
Glucose, mg/dL	90 (8)	93 (7)	95 (6)	92 (7)	96 (5)	93 (7)
Insulin, mU/mL	10.2 (6.6)	9.0 (4.4)	9.7 (9.5)	10.0 (5.4)	14.1 (7.9)	10.1 (7.0)
GDI	2.99 (0.27)	2.76 (0.24)	3.03 (0.30)	2.82 (0.19)	2.75 (0.280)	2.89 (0.32)
Adiponectin, µg/mL	13.7 (7.2)	12.4 (4.3)	12.2 (4.9)	11.3 (3.4)	11.4 (3.3)	12.2 (4.9)

^a Included three subjects (one male, two female) classified as "other" because ethnicity could not be ascertained

^b p<0.05 compared to African Americans, Caucasians and East Asian Americans

^c calculated as the sum of the LDL and triglyceride z-scores minus the HDL z-score.