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Retinol-binding protein 4 correlates with triglycerides but not insulin resistance in prepubertal children with and without premature adrenarche

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Abstract

Background—Retinol-binding protein 4 (RBP4) has been proposed as an early marker for insulin resistance (IR), but no prior studies have addressed RBP4 in an exclusively prepubertal population. Children with premature adrenarche (PA) are at increased risk for IR and metabolic syndrome (MeS), thus finding an appropriate early marker for IR in this population would allow for early intervention and prevention of morbidity related to IR and MeS.

Objective—To determine whether prepubertal children with PA have higher levels of RBP4 than controls and whether RBP4 correlates with comorbidities of metabolic disease in prepubertal children.

Subjects—This study comprised 49 prepubertal children (24 with PA and 25 control subjects), 20 boys and 29 girls, who were between the ages of 5 and 9 years.

Methods—This was a cross-sectional, case-control study conducted in a subspecialty ambulatory clinic based in a quaternary care center. RBP4 levels, hormonal values, lipids and response to an oral glucose tolerance test were evaluated in children with PA and controls, and body composition measures were obtained in a subset of patients (n=18).

Results—RBP4 correlated with triglycerides ($r = 0.57$, $P < 0.0001$) but did not correlate with IR in a body mass index z-score-adjusted Pearson correlation analysis. There was no difference in RBP4 levels between the PA and control groups.

Conclusions—These findings suggest that RBP4 may be an early marker of dyslipidemia, which may herald future onset of hepatic IR, polycystic ovary syndrome and MeS.

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Keywords

premature adrenarche; premature pubarche; retinol-binding protein 4; insulin resistance

Introduction

Precocious pubarche is the appearance of sexual hair before 8 years in girls and 9 years in boys, without signs of true puberty. Premature adrenarche (PA) describes individuals with precocious pubarche whose adrenal androgens are elevated for age, but are within range for Tanner stage for pubic hair, and without evidence of enzymatic defects of steroidogenesis, precocious puberty, or adrenal or gonadal malignancy[1]. PA is caused by early maturation of the adrenal zona reticularis or increased peripheral sensitivity to adrenal hormones[1] and is associated with obesity[2,3]. Until recently, PA was thought to be a benign process, but studies demonstrate that children with PA have increased future risk of metabolic syndrome (MeS), and affected girls are more likely to develop polycystic ovary syndrome (PCOS), even in the absence of obesity[4]. It would therefore be beneficial to have early markers to screen children with PA for risk of developing metabolic disease.

Retinol-binding protein 4 (RBP4) is an adipokine secreted primarily by the liver and, to a lesser extent, adipose tissue and may be an early marker of insulin resistance (IR) or involved in its pathogenesis[5]. An elevation in circulating RBP4 is associated with GLUT4 dysregulation[6]. Additionally, RBP4 may be linked to hepatic IR through its effect on the peroxisome proliferator activated receptor family (PPAR), a regulator of fatty acid metabolism, and thus may herald the onset of MeS[6,7]. Mouse models suggest that RBP4 may play a causative role in IR[5,6], yet clinical studies in adults and children have yielded conflicting results regarding this relationship[8,9,10]. The relationship of RBP4 with serum triglycerides has been more consistent[11,12].

Several studies have examined RBP4 in women with PCOS. One showed higher RBP4 in the PCOS group, whereas another demonstrated no difference compared to controls[13,14]. No study, to our knowledge, has described RBP4 in exclusively prepubertal children or in children with PA.

The goal of this study was to determine whether RBP4 may function as a marker for future metabolic risk in prepubertal children with PA and/or obesity. Our primary hypothesis was that children with PA with or without obesity will have higher RBP4 than control subjects. Our secondary hypothesis was that RBP4 will correlate with IR and triglycerides.

Materials and Methods

Subjects

The study group comprised 49 prepubertal children (29 girls, 20 boys) ages 5–9 years, 24 with PA and 25 controls (Table 1). Both groups were predominantly Hispanic (PA 79% and control 84%) and primarily from the Dominican Republic. Ethnicity was determined by self-report of black, white or Hispanic background of both parents and all four grandparents; those with backgrounds that did not fit these criteria were classified as “other.”

Subjects were recruited from the pediatric ambulatory services of Children’s Hospital of New York, Columbia University Medical Center (CUMC). Informed consent was obtained from a parent or guardian and assent was obtained from subjects older than 7 years. The protocol was approved by the Institutional Review Boards of CUMC and St. Luke’s-Roosevelt Hospital Center.

The diagnosis of precocious pubarche was made clinically. The inclusion criteria for PA were: 1) onset of pubic and/or axillary hair before age 8 years in girls or 9 years in boys; 2) no clitoromegaly in girls; 3) Tanner stage 1 breasts in girls or testicular volume ≤ 3cc in boys; and 4) no evidence of 21-hydroxylase deficiency, as documented by basal 17-hydroxyprogesterone, or of other adrenal or gonadal disorder by hormonal analysis. The inclusion criteria for controls were: 1) Tanner stage 1 pubic hair and 2) Tanner stage 1 breasts in girls or testicular volume ≤ 3cc in boys. Exclusion criteria for all subjects included: 1) gestational history complicated by gestational diabetes, multiple gestation, preterm delivery, or small for gestational age; 2) chronic medical conditions; 3) chronic glucocorticoid or other hormonal therapy.

Pubertal and clinical assessment

Pubertal and clinical assessment of subjects was performed on the day of testing at the Pediatric Outpatient Unit of the Clinical Research Resource affiliated with the Irving Institute for Clinical and Translational Research, CUMC. Breasts, male genitalia and pubic hair were assessed using the rating scales of Tanner and Marshall. Testicular volumes were estimated using the orchidometer of Prader. Height, weight, heart rate and blood pressure (BP) were measured; body mass index (BMI) and BMI z-scores were calculated using reference data[15].

Procedures

Blood samples were drawn between 0800 and 0900 h after an overnight fast for basal RBP4, glucose (G_0), insulin (I_0), dehydroepiandrosterone sulfate (DHEAS), androstenedione, T, free T, lutenizing hormone (LH), follicle stimulating hormone (FSH), hemoglobin A1c, lipids, and thyroid function tests. Glucose and insulin levels were measured at 30, 60, 90, and 120 minutes after a 1.75g/kg (maximum 75g) oral glucose tolerance load using GlutolR (Paddock Laboratories, Minneapolis, MN, USA).

Measures of insulin sensitivity and insulin secretion

Fasting glucose to insulin ratio (FGIR)[16], homeostasis model assessment (HOMA)[17], insulin sensitivity measure (SiM)[18], whole-body insulin sensitivity index (WBISI)[19], insulin area under the curve (I_{AUC}) and glucose area under the curve (G_{AUC})[20] were calculated as measures of IR.

Assays

RBP4 was measured by ELISA (ALPCO, Salem, NH, USA). The following labs were measured by Esoterix Laboratories: insulin by immunochemiluminescent assay; DHEAS by radioimmunoassay after enzymolysis; androstenedione and T by high-performance liquid chromatography with tandem mass spectrometry; free T by equilibrium dialysis, sex hormone binding globulin (SHBG), FSH, LH, and hemoglobin A1c. The following were measured in the Core Laboratory, CUMC: plasma glucose by the glucose hexokinase method; serum total cholesterol, high density lipoprotein, and triglycerides by Hitachi analyzer; low-density lipoprotein was calculated.

Body Composition

Body composition analysis was performed on a subgroup of study subjects. Truncal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were measured using contiguous-slice magnetic resonance imaging (MRI) of the thorax, abdomen, and pelvis (GE 6x Horizon 1.5T GE Healthcare, Milwaukee, WI, USA) (PA: n = 8, control: n = 7); Intramyocellular lipid content (IMCL) was measured using single-voxel ^{1}H NMR spectroscopy (GE 6x Horizon 1.5T; GE Healthcare, Milwaukee, WI, USA) of the right

tibialis anterior muscle (PA: n = 9, control: n = 7). Trunk fat (TF) and percentage body fat (%BF) were determined using whole-body dual-energy x-ray absorptiometry (DXA) (GE Lunar DPX (L) and Prodigy; GE Healthcare, Milwaukee, WI, USA) (PA: n = 11, control: n = 7).

Statistical Analysis

Comparison of groups for continuous variables were achieved by Student's t-test for age, height, weight, and BMI z-score, and by BMI z-score adjusted analysis of covariance (ANCOVA) for BP, RBP4, lipids, T, free T, DHEAS, androstenedione and OGTT-derived IR measures (n = 49) and for subgroup analyses of VAT and SAT (PA: n = 8, control: n = 7); IMCL (PA: n = 9, control: n = 7); and TF and %BF (PA: n = 11, control: n= 7).

Pearson correlations were performed on the entire group (n = 49) and on body composition subgroups. Correlations assessed the association between RBP4 and BMI Z-score, BP, lipids, T, free T, DHEAS, androstenedione, G_0, G_{AUC}, I_0, I_{AUC}, OGTT derived IR measures, VAT, SAT, IMCL, TF and %BF.

P- values of < 0.05 were considered to represent statistical significance for all analyses.

Results

Clinical Characteristics

Age, height, weight, BP, and waist circumference were similar in the PA and control groups (Table 1). BMI z-score was significantly higher in the control group compared to the PA group.

Hormonal and Metabolic Characteristics and Body Composition

DHEAS, androstenedione, T, free T, and I_{AUC} were higher and SHBG, SiM and WBISI were lower in the PA group ($P < 0.05$ for all). There were no differences in RBP4 or lipids between the groups or among VAT, SAT, IMCL, TF or % BF in the subgroup analyses (Tables 2 and 3).

Pearson correlation analysis

RBP4 correlated with serum triglycerides ($r = 0.57$, $P < 0.0001$) in the entire group. In subgroup analyses the correlations were: PA (n=24; r=0.72, p=0.0001); controls (n=25; r=0.58, p=0.007); girls (n=29; r=0.72, p<0.0001); and boys (n=20; r=0.56, p=0.009). There was no correlation between RBP4 and IR, body composition parameters in the entire group, or in any of the subgroups.

Discussion

To our knowledge, this is the first study to examine RBP4 in an exclusively prepubertal cohort, and the first to examine it in PA, a group that is at high risk for development of MeS and PCOS[3,4]. We demonstrate a significant correlation between RBP4 and serum triglycerides for the combined population of prepubertal children with and without PA.

Our results are consistent with reports showing a link between RBP4 and features of MeS in mixed populations of children and adolescents, which have demonstrated an association between RBP4 and elevated triglycerides[11,12,21,22]. Our findings suggest that RBP4 may be a marker of early MeS even in this very young population. In this study, RBP4 did not distinguish those with PA from controls; however, lipid parameters did not differ between PA and controls in this study.

Insulin sensitivity in PA subjects was reduced compared with controls by WBISI, SiM, and I_{AUC} but not by HOMA or FGIR. Glucose and insulin during the first 30 minutes of the OGTT reflect hepatic IR, while glucose disposal after 60 minutes reflects peripheral (skeletal muscle) uptake[23]. WBISI, SiM, and I_{AUC} incorporate post-60-minute values of glucose and insulin, while HOMA and FGIR utilize only fasting levels. It is possible that we do not see an increase in RBP4 in children with PA compared to controls because they have not yet developed hepatic IR, and RBP4 may be a better marker of hepatic IR than of peripheral IR[7]. Our findings are in agreement with other pediatric studies showing no correlation between RBP4 and IR[24], although Yeste et al. [22] show a correlation between RBP4 and muscle IR but not hepatic IR, and others have shown a correlation between RBP4 and IR[6,12,21,25].

We did not find significant differences between PA and control groups for body composition measures, nor did we find correlations between RBP4 and these measures. However, the sample size for this subset may have been too small to detect intergroup differences and to adjust for age, a potentially important confounding variable.

Our study limitations include the relatively small number of study subjects, the higher BMI z-score in the control group, and the limited number of participating boys with PA. Additionally, our population was primarily Caribbean Hispanic; therefore our findings are not necessarily applicable to other ethnic groups.

In summary, in this study of exclusively prepubertal children with and without PA, RBP4 correlated with serum triglyceride level. However, we did not find a correlation between RBP4 and IR. Although differences in RBP4 or serum lipids were not found between children with and without PA, children with PA had more IR than controls, suggesting that early metabolic abnormalities may be present in prepubertal children with PA. Further research is needed before RBP4 can be established as a marker for metabolic dysfunction, although its correlation with triglycerides in young children suggests that RBP4 may indicate early features of MeS. Large-scale pediatric population studies are needed to clarify the utility of RBP4 as an early marker of MeS and to establish clinically applicable cutoffs and longitudinal studies of prepubertal children are necessary to define the relationship of RBP4 with metabolic markers over time. Additionally, it will be of interest to study RBP4 in other pediatric populations at risk for MeS, including obese children and adolescents with PCOS.

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Table 1

Clinical characteristics of the study population

	PA (n=24)	Control (n=25)	P value
Age (years)	7.7 ± 1.4	7.2 ± 1.5	NS
Height (cm)	128.2 ± 9.6	126.4 ± 8.9	NS
Weight (kg)	32.8 ± 10.3	36.2 ± 12.6	NS
Waist circumference (cm)	65.3 ± 11.3	70.9 ± 13.6	NS
BMI z-score	1.2 ± 1.1	1.8 ± 1.0	0.04
Systolic blood pressure (mmHg)	97.1 ± 11.1	102.3 ± 12.7	NS
Diastolic blood pressure (mmHg)	61.1 ± 7.7	62.2 ± 6.8	NS

The results are expressed as mean ± SD; NS, not significant.

Table 2

Hormonal and metabolic characteristics of the study population[a]

	PA (n=24)	Control (n=25)	P value[b]
Retinol-binding protein 4 (mg/L)	16.7 ± 6.4	14.3 ± 5.5	NS
Testosterone (ng/dL)	6.0 ± 2.7	3.5 ± 0.8	0.0003
Free Testosterone (pg/mL)	0.48 ± 0.27	0.30 ± 0.18	0.0003
DHEAS (ug/dL)	53.4 ± 42.1	22.1 ± 18.9	0.002
SHBG (nmol/L)	89.1 ± 32.5	105.3 ± 52.0	0.02
Androstenedione (ng/dL)	46.2 ± 16.5	29.4 ± 7.3	<0.0001
Cholesterol (mg/dL)	161.0 ± 26.4	164.0 ± 32.0	NS
Triglycerides (mg/dL)	72.0 ± 26.2	77.9 ± 40.0	NS
LDL (mg/dL)	90.3 ± 23.6	97.0 ± 32.6	NS
HDL (mg/dL)	56.3 ± 9.7	51.6 ± 12.1	NS
Glucose (mg/dL, 0 min)	88.8 ± 7.0	90.1 ± 7.8	NS
Glucose (mg/dL, AUC)	233.5 ± 45.1	221.4 ± 51.1	NS
Insulin (uU/mL, 0 min)	7.2 ± 6.2	5.7 ± 2.8	NS
Insulin (uU/mL, AUC)	71.6 ± 38.3	54.3 ± 37.8	0.02
FGIR	21.7 ± 17.1	21.7 ± 17.9	NS
HOMA	1.3 ± 0.7	1.6 ± 1.4	NS
SiM	10.0 ± 11.1	13.7 ± 14.4	0.002
WBISI	10.4 ± 6.3	11.1 ± 7.6	0.04

[a]Results are expressed as mean ± SD;

[b]BMI Z-score adjusted analysis. AUC, area under the curve; NS, not significant

Table 3

Body composition in a subgroup of subjects[a]

	PA (n = 24)	Control (n = 25)	P value[b]
Visceral adipose tissue (L) [c]	0.46 ± 0.44	0.53 ± 0.41	NS
Subcutaneous adipose tissue (L) [c]	3.6 ± 2.6	3.2 ± 1.5	NS
Intramyocellular lipid/water ratio[d]	0.040 ± 0.021	0.036 ± 0.013	NS
%, Body fat percentage[e]	29.8 ± 13.8	29.3 ± 5.5	NS
Trunk fat (kg)[e]	4.7 ± 3.4	3.7 ± 2.3	NS

[a]The results are expressed as mean ± SD;

[b]BMI Z-score adjusted;

[c]7 control, 8 PA;

[d]7 control, 9 PA;

[e]7 control, 11 PA.