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ORIGINAL PAPER

Retinol binding protein 4, low birth weight-related insulin resistance and hormonal contraception

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Abstract It has been recently reported that increased serum levels of retinol binding protein 4 (RBP4), a molecule secreted by adipocytes and liver, could be an early marker of insulin resistance (IR). We determined whether serum RBP4 was increased in low birth weight (LBW)-young women as a model of early-onset IR, through a historical prospective study. The study-population included 35 LBW and 35 born at term appropriate for gestational age (term AGA) young women. Metabolic evaluations included the composite-insulin sensitivity index (composite ISI). Serum RBP4 was measured with a competitive enzyme-linked immunosorbent assay (ELISA). RBP4 levels were similar in LBW and term AGA women, while composite ISI was significantly lower in the former group. With multivariate logistic regression analysis hormonal contraception (HC) use but not birth weight, diabetes in either parents and body mass index was significantly associated with higher RBP4 levels: odds ratio = 10.6; 95% confidence interval (CI) = 2.4–76.6. In spite of higher RBP4 levels in women under HC, composite ISI was similar in women with or without HC. Women under HC also exhibited significantly higher levels of sex hormone binding globulin (SHBG), triglycerides, cholesterol, and C-reactive protein (CRP), and all of them, but not composite ISI, were significantly correlated with RBP4 levels. In conclusion, RBP4 serum level was not a marker of IR but, for the first time, it is documented a sustained increase of serum RBP4 under HC. Pathophysiological and clinical significance of this novel finding requires further investigations

Keywords Retinol binding protein 4 · Low birth weight · Insulin resistance · Hormonal contraception

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Introduction

Recent studies in mice suggest that adipose tissue behaves as a glucose sensor and regulates whole-body insulin sensitivity through release of a circulating factor in response to decreased intracellular glucose concentrations [1]. Retinol binding protein 4 (RBP4), a molecule secreted by liver and adipocytes, may represent this adipose-derived circulating factor [2]. In mice, selective knockout of adipocytes glucose-transporter 4 (GLUT4) resulted in increased serum levels of RBP4, and injection of purified RBP4 or transgenic over-expression of RBP4 impaired insulin signaling in muscle and increased hepatic gluconeogenesis by stimulating the expression of the phosphoenolpyruvate carboxykinase [2]. Therefore, according to this model, the reduced expression of adipocytes GLUT4, early occurrence in insulin-resistant states, would promote systemic insulin resistance (IR).

In humans, elevated serum RBP4 levels have been reported in insulin-resistant states including obesity, type 2 diabetes, and impaired glucose tolerance [2–6]. Recently, serum levels of RBP4 correlated with IR in non-obese, non-diabetic subjects, suggesting that increased serum levels of RBP4 would be an early marker of IR [5, 7]. However, some recent studies questioned the role for RBP4 as a marker of IR [8–10].

As low birth weight (LBW) predisposes to early development of IR [11–14], we measured serum RBP4 in young women with and without a LBW. The aim was to determine whether serum RBP4 was increased in LBW-young women as a model of early-onset IR.

Results

Table 1 shows demographic, clinical and metabolic parameters in LBW women and term AGA controls. Only birth weight and the length of gestation were significantly different. The proportion of women with type 2 diabetes in either parents, and the proportion of those under HC were not significantly different. Third generation pill with either 20 or 30 μg of ethinylestradiol was being used in all cases. No subject was under antiandrogenic therapy. Composite-insulin sensitivity index (cISI) was significantly lower in the LBW group, whereas serum RBP4 was similar in the two groups.

With multivariate logistic regression analysis using dichotomized variables, including hormonal contraception (HC) use, birth weight (LBW or term AGA), diabetes in either parents and BMI (< or ≥median value), only HC use was significantly associated with higher (≥median value) RBP4 levels: odds ratio (OR) = 10.6; 95% confidence interval (CI) = 2.4–76.6. None of the other variables was significantly associated with high levels of RBP4, even when women under HC were excluded from the analysis. As shown in Table 2, RBP4 levels were strongly increased in women under HC compared to women without HR, but composite ISI was similar in women with or without HC. Excluding from the analysis women under HC, composite ISI remained significantly lower in the LBW group compared to term AGA controls (median [interquartile range]: 4.4 [2.6–5.2] vs. 5.7 [3.7–9.0]; P=0.011), whereas serum RBP4 was similar in the two groups (26.8 [22.7–30.7] vs. 26.3 [23.4–30.3]; P=0.99).

Women under HC also exhibited significantly higher levels of sex hormone binding globulin (SHBG), C-reactive protein (CRP), triglycerides, cholesterol, and low-density lipoprotein (LDL)-cholesterol (Table 2). Body mass index and WHR were similar in women with or without HC (22.07 ± 3.1 vs. 21.9 ± 3.2 and 0.73 ± 0.1 vs. 0.74 ± 0.1, respectively).

Serum RBP4 was significantly correlated with CRP ($r = 0.43$; $P = 0.0002$), triglycerides ($r = 0.39$; $P = 0.0009$), total cholesterol ($r = 0.32$; $P = 0.007$), and SHBG ($r = 0.30$; $P = 0.01$), but not with composite ISI ($r = 0.10$; $P = 0.4$). When women under HC were excluded from the analysis, all these correlations were lost, and no other correlation was revealed.

Discussion

Recent reports have suggested that increased serum levels of RBP4 would be an early marker of IR [5, 7]. As LBW predisposes to early development of IR, as revealed in childhood [11, 13] and early adulthood [12, 14], our study population represented a suitable model to confirm the role for elevated RBP4 levels as an early marker of IR. We were not able to confirm this role for RBP4 in our study population, as the reduced insulin sensitivity revealed in LBW-young women was not associated to increased serum levels of RBP4. Moreover, no relationship was found between insulin sensitivity and RBP4 levels. We assessed insulin sensitivity by the composite ISI instead of the euglycemic insulin clamp, used in the two previous reports [5, 7]; however, composite ISI is highly correlated with the rate of whole-body glucose disposal during the euglycemic insulin clamp [15]. Actually, in disagreement with previous

Table 1 Demographic, clinical and metabolic parameters in low birth weight (LBW) and controls (born at term, appropriate for gestational age—Term AGA) women

	LBW (35)	Term AGA (controls) (35)	P*
Age (years)	21.8 ± 1.8	22.3 ± 1.6	n.s.
Birth weight (gr)	2093 ± 414	3259 ± 311	<0.0001
Gestational age (weeks)	35.8 ± 3.0	39.5 ± 1.5	<0.0001
WHR	0.75 ± 0.08	0.73 ± 0.13	n.s.
BMI (Kg/m^2)	21.9 ± 3.5	21.9 ± 2.8	n.s.
Sistolic blood pressure (mmHg)	109.9 ± 10.3	111.4 ± 10.0	n.s.
Diastolic blood pressure (mmHg)	71.4 ± 6.9	73.8 ± 5.7	n.s.
Diabetes in either parents—no (%)	9 (14.3)	5 (11.4)	n.s.
Hormonal contraception—no (%)	6 (17)	9 (26)	n.s.
Composite ISI	4.8 ± 3.8	6.9 ± 4.5	0.028
Median (interquartile range)	4.42 (2.6–6.3)	5.5 (3.8–8.9)	
RBP4 (μg/ml)	29.8 ± 8.9	30 ± 8.5	n.s.
Median (interquartile range)	27.1 (23.2–34.4)	27.3 (24.2–34.6)	

* Wilcoxon rank sum test for continuous variables and chi-square test for proportions

Table 2 Metabolic parameters in women with or without hormonal contraception (HC)

	Women without HC (55)	Women with HC (15)	*P**
Composite ISI			
Mean ± SD	5.5 ± 3.6	7.2 ± 5.2	0.46
Median (interquartile range)	4.7 (3.1–6.5)	4.6 (3.8–11.5)	
RBP4 (μg/ml)			
Mean ± SD	27.1 ± 6.1	40.1 ± 9.0	<0.0001
Median (interquartile range)	26.7 (23.1–30.9)	41.7 (35.2–44.7)	
SHBG (nmol/L)			
Mean ± SD	62.3 ± 34.7	186.4 ± 88.3	<0.0001
Median (interquartile range)	54.6 (39.2–76.7)	169 (133–254)	
CRP (mg/dl)			
Mean ± SD	0.10 ± 0.16	0.29 ± 0.18	<0.0001
Median (interquartile range)	0.05 (0.03–0.08)	0.19 (0.15–0.4)	
Triglycerides (mg/dl)			
Mean ± SD	65.8 ± 45.5	102.1 ± 39.1	0.0003
Median (interquartile range)	52 (42–73)	91 (80–125)	
Total cholesterol (mg/dl)			
Mean ± SD	174.7 ± 26.6	213.4 ± 52.2	0.002
Median (interquartile range)	173 (157–192)	204 (182–237)	
Cholesterol LDL (mg/dl)			
Mean ± SD	105.2 ± 24.4	130.7 ± 44.6	0.032
Median (interquartile range)	104 (89.6–119.2)	122 (107–149)	

* Wilcoxon rank sum test

reports [2–6], recent studies did not find any relationship between RBP4 levels and indices of IR in various hyperinsulinemic states [8–10]. Differences in the design of the studies, in the assessment of IR and in the performance of commonly used RBP4 assays [16] may account for the divergent findings. Nevertheless, the actual relationship between RBP4 and IR needs to be further clarified.

The highly increased levels of RBP4 in HC-users, is a novel finding with possible pathophysiological and clinical implications. In HC-users, high levels of RBP4 were associated with increased levels of CRP, triglycerides, cholesterol, and SHBG, whose liver secretion was likely stimulated by HC. Furthermore, all of them were significantly correlated with RBP4 levels in the whole population, and all these correlations were lost excluding from the analysis women under HC. These data strongly suggest that HC stimulates RBP4 secretion by the liver. However, a possible additional effect on RBP4 production by adipocytes cannot be excluded, as, recently, 17β-estradiol significantly increased RBP4 mRNA expression, protein levels, and secretion into the colture media in human subcutaneous and omental adipose tissue explants [17].

Noteworthy, in the present study, in spite of the evidence that RBP4 levels were highly increased in women under HC, insulin sensitivity was not lower than in women without HC. This finding could suggest that RBP4 does not play a causal role in the pathogenesis of IR in humans, differently from the evidence produced in mice [2].

Repeatedly, serum RBP4 levels were reported to correlate with triglycerides [5, 18, 19], total cholesterol [9, 19], and LDL-cholesterol [9, 19]. The present data concerning the association of highly increased levels of RBP4 with increased levels of triglycerides and cholesterol under HC, reinforces the evidence of this relationship. But the nature of this relationship is not known, although a possible role for RBP4 in lipid metabolism has been suggested [9].

The magnitude of the increase of circulating RBP4 under HC was much higher than that generally reported when an association between high RBP4 levels and IR states was found. This indicates that also in humans the liver represents the major source of the circulating RBP4.

In conclusion, the role for increased RBP4 as an early marker of IR is not supported by the present study, which demonstrates for the first time a sustained increase of serum RBP4 levels under HC. Pathophysiological and clinical significance of this novel finding requires further investigations.

Materials and methods

Study population

All women were selected according to their birth weight. Subjects were randomly recruited from the neonatal-unit registry of the University of L'Aquila General Hospital, Italy. In total 85 female subjects, who were born with a birth weight

<2,500 g, were identified. Exclusion criteria were malformations and chromosomal abnormalities as well as major neonatal and pregnancy complications including gestational diabetes and pre-eclampsia. Control subjects were selected as the next full-term singleton female in the registry, with birth weight ≥3,000 g and appropriate for gestational age (between the 25th and 75th percentile; term AGA), using the same exclusion criteria. In total, 15 subjects with LBW and 10 controls were not traceable, and 53% of the LBW women ($n = 37$) and 47% of controls ($n = 35$) agreed to participate in the study. Exclusion criteria included pregnancy, diabetes, congenital adrenal hyperplasia, Cushing's syndrome or corticosteroids treatments, hyperprolactinemia, thyroid dysfunction, and abnormal kidney or liver function, on the basis of clinical and laboratory evaluation. One LBW woman was excluded for pregnancy and another for thyroid dysfunction. In the LBW group, 19 out of the 35 women were born SGA (below the 10th percentile) either at term (≥37 weeks) or preterm (<37 weeks). The other 16 LBW women were born preterm with a birth weight that was appropriate for gestational age (≥10th percentile; premature AGA). We included all women regardless of HC.

The study protocol was approved by the local Ethical Committee and informed written consent was obtained from all subjects.

Study protocol and biochemical assays

All women underwent clinical and metabolic evaluations, including the composite-insulin sensitivity index (Composite ISI) as surrogate measure of insulin sensitivity. It was derived from the measurements of glucose and insulin during the oral glucose tolerance test (OGTT), using the following formula: (10,000/square root of [fasting glucose x fasting insulin] x [mean glucose x mean insulin during OGTT]) [15]. Glucose was determined by the glucose oxidase method (Aeroset System, Abbott Laboratories, Abbott Park, IL, USA). Insulin was measured by electrochemi-luminescent assay (Elecsys System, Roche diagnostic, Basel, Switzerland). Serum RBP4 was measured in duplicate with a competitive enzyme-linked immunosorbent assay (ELISA) (AdipoGen, Soul, Korea), and intra- (five replicates of four samples) and interassay coefficients of variation were 6.4% and 9.4%, respectively. All blood samples were collected after an overnight fast, during the early follicular phase (day 3–7) of menstrual cycle.

Statistical analysis

Statistical analysis was performed by the SAS statistical software (version 9.1, 2003; SAS Institute, Inc., Cary, NC, USA). Differences in continuous variables were analyzed by the Wilcoxon rank sum test. Proportional differences were analyzed by the chi-square test. Multivariate logistic regression analysis using dichotomized variables was used to assess independent associations with higher (≥ median values) RBP4 levels. Correlations were evaluated using the Spearman correlation test.

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