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The Positive Correlation between Cord Serum Retinol-Binding Protein 4 Concentrations and Fetal Growth

Te-Fu Chan[a–c] Yung-Chieh Tsai[f, g] Chen-Hsuan Wu[d] Chien-Hung Lee[e]
Shih-Han Wang[a, b] Juin-Huang Su[b, c]

[a]Department of Obstetrics and Gynecology, Kaohsiung Municipal Ta-Tung Hospital, [b]Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, [c]Department of Obstetrics and Gynecology, College of Medicine, Kaohsiung Medical University, [d]Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital – Kaohsiung Medical Center, Chang Gung University, College of Medicine, and [e]Graduate Institute of Public Health, School of Health Science, Kaohsiung Medical University, Kaohsiung, and [f]Department of Obstetrics and Gynecology, Center for Reproductive Medicine, Chi-Mei Medical Center, and [g]Department of Biotechnology, Southern Taiwan University of Technology, Tainan, Taiwan, ROC

Key Words
Retinol-binding protein 4 · Fetal growth · Cord serum

Abstract
Background/Aims: Retinol-binding protein 4 (RBP4) has been shown to be associated with insulin resistance and fatty acid metabolism. We hypothesize that RBP4 might play a role in fetal growth and that cord serum RBP4 may act as a marker of fetal growth, independent of fetal insulin levels. ***Methods:*** Twenty-one women having fetuses in the top quartile (>75th percentile) of birth weights for gestational age were enrolled into the trial, along with 21 women having fetuses in the bottom quartile (<25th percentile) of birth weights for gestational age. Serum RBP4 and insulin levels were analyzed. ***Results:*** Cord serum RBP4 and insulin concentrations were significantly higher in the top quartile group (14.3 ± 3.7 ng/ml, 3.8 ± 5.2 μIU/ml) than in the bottom quartile group (11.3 ± 2.6 ng/ml, 0.9 ± 1.4 μIU/ml; $p = 0.004$, $p = 0.017$). Cord serum RBP4 and insulin as well as gestational age ($r = 0.744$, $r^2 = 0.553$, $p < 0.001$) were significantly correlated with fetal birth weights. ***Conclusion:*** Cord serum RBP4 concentrations were higher in subjects with top quartile fetuses than in those with bottom quartile fetuses. Cord serum RBP4 concentrations were significantly correlated with fetal birth weight. These findings may indicate that cord serum RBP4 plays a regulatory role in fetal growth.

Introduction

Retinol-binding protein 4 (RBP4) belongs to the lipocalin family and functions as a transporter of retinol (vitamin A) [1, 2]. The main site of RBP4 synthesis is the liver, and when bound to transthyretin it prevents RBP clearance from the circulation by filtration in the renal glomeruli [2]. It has recently been suggested that RBP4 is secreted from adipose tissue and can be regarded as an adipokine [3]. It has also been shown to contribute to insulin resistance in mouse models [3, 4]. Since RBP4 inhibits glucose uptake by muscles, and interferes with insulin-mediated suppression of glucose production in the liver,

T.-F.C. and Y.-C.T. contributed equally to this work.

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Juin-Huang Su, MD, PhD
Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital
17 Floor, No.37, Mingjhe Road, Sanmin District
Kaohsiung City 807, Taiwan (ROC)
Tel. +886 7 312 1101, ext. 6428, E-Mail tefu.chan@msa.hinet.net

it may contribute to insulin resistance [4, 5]. Increased levels of RBP4 have been found to be connected with insulin resistance in obese subjects, impaired glucose tolerance, type 2 diabetes mellitus (DM), gestational DM and metabolic syndrome [6, 7].

Fetal growth is affected by maternal genetic, demographic and metabolic factors, fetal potential and the intrauterine environment [8]. Maternal hyperglycemia, which contributes to fetal hyperinsulinemia, is a known factor in fetal overgrowth [9, 10]. Recent studies have suggested that nutritional/metabolic factors other than glucose, such as triglyceride, may contribute to excessive fetal growth in the obese mother [8, 11, 12].

RBP4 has been shown to be associated with insulin resistance and fatty acid metabolism [13–16]. We therefore hypothesize that RBP4 might play a role in fetal growth and that cord serum RBP4 may act as a marker of fetal growth, independent of fetal insulin levels. To test this hypothesis we explored whether there was a difference in cord serum RBP4 levels between subjects in the top quartile (>75th percentile) of fetal birth weights for gestational age and those in the bottom quartile (<25th percentile). We also investigated the relationship between levels of RBP4 and insulin in cord serum and maternal serum as well as fetal birth weight to determine whether cord RBP4 is associated with fetal birth weight independently of insulin.

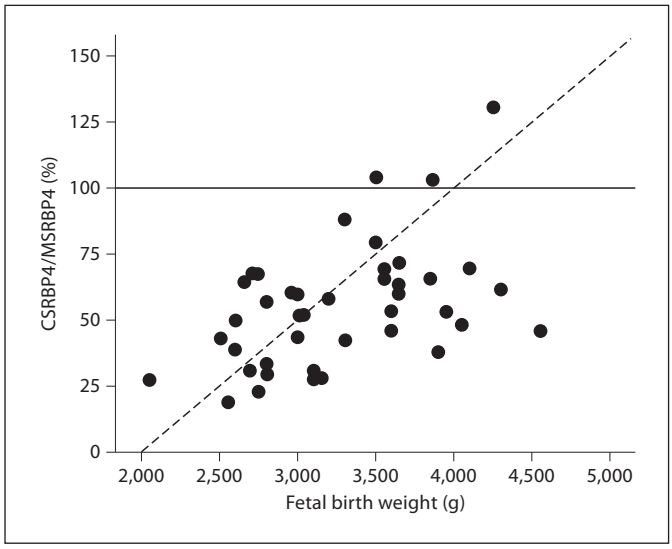

Fig. 1. CSRBP4/MSRBP4 ratios plotted against fetal birth weights. The dashed line indicates the regression line ($r = 0.480$, $r^2 = 0.231$, $p = 0.001$). The solid one indicates the reference line for the CSRBP4/MSRBP4 ratio at 100%. CSRBP4 = Cord serum RBP4; MSRBP4 = maternal serum RBP4.

Subjects and Methods

We invited all women visiting our outpatient clinic for routine prenatal examination to participate in the trial. Of those who agreed, 21 were found to have fetuses in the top quartile (>75th percentile) of birth weights for gestational age (women in top quartile). These were enrolled along with 21 others whose fetuses were in the bottom quartile (<25th percentile; women in bottom quartile). All of these women were at term (gestational age between 37 and 41 weeks). The mode of delivery was 19 vaginal deliveries and 2 cesarean sections for each group. A birth weight >75th percentile and <25th percentile of birth weights for gestational age referred to a national standard curve for Taiwanese singleton births after adjusting for gestational age and sex [17]. All women were of Han Chinese origin. Those with multiple pregnancies, fetal anomalies, pre-existing hypertension, DM, gestational DM, chronic diseases, maternal preeclampsia, low newborn APGAR score levels, maternal smoking and maternal chronic alcohol consumption were excluded. A precise medical and obstetric history that included BMI at admission was obtained and recorded. The approval of the institute review board was obtained.

For the purposes of making biochemical and hormonal determinations, blood samples were obtained from each subject directly from a cannulated vein following admission. Umbilical cord serum was also collected immediately after delivery. The serum was separated by centrifugation and stored at –80°C until further analysis. Estradiol, thyroxin-stimulating hormone and insulin were measured with a Coat-A-Count radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, Calif., USA). Serum RBP4 levels were analyzed by enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (Immundiagnostik AG, Bensheim, Germany). The intra- and interassay coefficients of variation were 6.3 and 11.2%, respectively.

Data were evaluated with SPSS software for Windows (version 12.0; SPSS Inc., Chicago, Ill., USA) and presented as means ± SD. Differences between groups were evaluated with nonparametric statistics. Pearson correlation, partial correlation, multiple and linear regression analysis were carried out to determine the relationships between the variables. All tests were two-tailed, and the significance level was defined as $p < 0.05$.

Results

Table 1 shows the clinical characteristics of our subjects. Cord serum RBP4 and insulin concentrations were significantly higher in the top quartile group (14.3 ± 3.7 ng/ml, 3.8 ± 5.2 μIU/ml) than in the bottom quartile group (11.3 ± 2.6 ng/ml, 0.9 ± 1.4 μIU/ml; $p = 0.005$, $p = 0.010$). Maternal serum RBP4 levels were significantly lower in the top quartile group than in the bottom quartile group (22.2 ± 5.7 vs. 28.7 ± 8.7 ng/ml, $p = 0.007$).

Table 1. Clinical data

	Bottom quartile (n = 21)	Top quartile (n = 21)	p value
Gestational age, weeks	38.9 ± 1.2	39.1 ± 1.0	0.520
Fetal birth weight, g	2,790.5 ± 259.6	3,755.2 ± 351.9	0.000*
Fetal gender (male/female)	12/9	8/13	0.222
Mode of delivery (VD/CS)	9/2	19/2	1.000
Maternal age, years	30.8 ± 3.8	31.2 ± 3.6	0.800
Maternal body weight, kg	65.9 ± 4.4	71.3 ± 8.5	0.04
Maternal body height, cm	159.3 ± 5.6	161.0 ± 4.7	0.306
Maternal BMI	26.0 ± 1.3	27.5 ± 3.5	0.178
Systolic blood pressure, mm Hg	116.7 ± 13.4	118.5 ± 12.2	0.493
Diastolic blood pressure, mm Hg	72.2 ± 7.4	74.2 ± 8.3	0.541
Maternal serum RBP4, μg/ml	28.7 ± 8.7	22.2 ± 5.7	0.007*
Maternal serum insulin, μIU/ml	10.2 ± 12.9	11.3 ± 19.1	0.428
Cord serum RBP4, μg/ml	11.3 ± 2.6	14.3 ± 3.7	0.005*
Cord serum insulin, μIU/ml	0.9 ± 1.4	3.8 ± 5.2	0.010*

Values are expressed as means ± SD. Nonparametric statistics were used. Bottom quartile: women having neonates in the bottom quartile (<25th percentile) of birth weights for gestational age. Top quartile: women having neonates in the top quartile (>75th percentile) of birth weights for gestational age.

VD = Vaginal delivery; CS = cesarean section. * $p < 0.05$.

The ratios of cord serum RBP4 to maternal serum RBP4 were significantly correlated with fetal birth weights ($r = 0.480$, $r^2 = 0.231$, $p = 0.001$). It is noteworthy that in some cases the ratio was higher than 100% (fig. 1). No correlation was found between maternal serum RBP4 levels and cord serum RBP4 levels ($r = 0.134$, $r^2 = 0.018$, $p = 0.397$).

Multiple linear regression analysis was performed to study the relationship between fetal birth weights and various demographic characteristics as well as maternal or fetal biochemical markers. Fetal birth weight was the dependent variable, and gestational age, fetal gender, cord serum RBP4 and insulin, maternal age, maternal BMI, and maternal RBP4 and insulin were independent variables. Cord serum RBP4 and insulin as well as gestational age ($r = 0.744$, $r^2 = 0.553$, $p < 0.001$) were significantly correlated with fetal birth weights (table 2).

Discussion

Although it has been suggested that insulin plays an important role in fetal growth, other factors, such as triglyceride, also appear to be involved [18, 19]. Our study found higher cord serum RBP4 and insulin levels as well as lower maternal RBP4 levels in subjects with fetuses in the top quartile than in those with fetuses in the bottom quartile (table 1). This study also showed cord serum RBP4 level to be an independent factor correlating with fetal birth weight, after adjustment for cord insulin level (table 2). While this finding is consistent with certain previous studies [7, 20], it is different from the finding of Laudes et al. [21], who observed no such correlation. This difference may be attributed to different sampling. While our study selected the top and bottom quartile of birth weights fetuses to enhance the effects of analysis, the other researchers selected neonates without referring to birth weight percentile charts.

This report demonstrated that RBP4 concentrations in maternal serum were not necessarily significantly higher than those in cord serum at delivery (fig. 1). Furthermore, there was no correlation between the two. Since the molecular weight of RBP4 is 21 kDa [22], maternal serum RBP4 is unable to contribute to RBP4 in fetal circulation. These observations suggested that fetal origin might be the main source of RBP4 production.

Although the role of RBP4 in fetal circulation is still unclear, it may have at least three possible effects on fetal growth. First, it has been suggested that RBP4 inhibits the effects of insulin on the muscles and in the liver. This may contribute to increased serum glucose levels and help the fetus meet the demands of high fetal glucose utilization [4, 5, 23]. Second, retinol is an important nutrient for fetal growth which recycles extensively between plasma,

Table 2. Multiple linear regression analysis of the possible fetal determinants for fetal birth weights

	Coefficients				p
	nonstandardized coefficients		standardized coefficients		
	β	SE	β	*t*	
Independent variables (constant)	–4,998.050	2,654.314		–1.883	0.069
Gestational age	163.945	65.884	0.314	2.488	0.018*
Fetal gender (male/female)	–111.016	144.658	–0.097	–0.767	0.448
Cord serum RBP4	50.175	21.175	0.307	2.370	0.024*
Cord serum insulin	46.068	18.164	0.323	2.536	0.016*
Maternal age	14.936	21.949	0.095	0.681	0.501
Maternal BMI	47.272	28.168	0.222	1.678	0.103
Maternal RBP4	–16.064	8.668	–0.223	–1.853	0.073
Maternal insulin	–1.974	4.595	–0.055	–0.430	0.670

The dependent variable is fetal birth weight. $r = 0.744$, $r^2 = 0.553$, $p < 0.001$.
Fetal gender expressed as dichotomy variable (male = 1/female = 2). * $p < 0.05$.

liver and extrahepatic tissues [24, 25]. RBP4 plays a role as a retinol transporter. When fetal body weight increases, more retinol is needed and thus an increased level of RBP4 was observed. Third, it has been suggested that fetal growth is influenced by certain metabolic factors other than glucose [26]. In addition, maternal hypertriglyceridemia is regarded as a significant predictor for fetal overgrowth [11]. Triglyceride levels are strongly associated with RBP4 levels [6]. Hypertriglyceridemia accompanied by hyperinsulinemia may trigger RBP4 synthesis and secretion in the liver or ectopic fat [15]. It is therefore possible that increased production of RBP4 by fetal liver or fat tissue is associated with triglyceride metabolism and accelerated fetal growth.

This study showed that maternal RBP4 levels in women with fetuses in the top quartile fetus were lower than in those with fetuses in the bottom quartile. The simple correlation model showed a negative relationship between maternal RBP4 levels and birth weights, but this correlation disappeared after adjustment for maternal age, maternal insulin levels, maternal BMI, and cord serum RBP4 and insulin (table 2). This finding is consistent with a recent study by Vaisbuch et al. [27] who argued that there is no significant difference in maternal plasma RBP4 levels between women with fetuses small for their gestational age and those with fetuses appropriate for their gestational age. However, a recent study conducted by Mazaki-Tovi et al. [28] reported that mothers without gestational DM but with large for their gestational age neonates had a higher maternal plasma concentration of RBP4 than women with appropriate for their gestational age newborns. It is contradictory to our findings. This study did not take maternal BMI into consideration and this may be the reason for these two contradictory findings. In fact, obesity, accompanied by metabolic changes, has also been suggested as the strongest independent predictor for fetal overgrowth [19, 26].

The significance of this study is that it provides evidence for another possible factor involved in fetal growth. This will help us to understand more about fetal growth and allow us to explore further ways of preventing overgrowth. However, the limitations of this study also need to be addressed. The concentration of many substances changed dramatically in maternal blood after delivery and especially after removal of the placenta [7]. We have no evidence that this is not the case for RBP4 and/or insulin. The cross-sectional design of the study limited our ability to infer a causal relationship between serum RBP4 concentrations and fetal growth. Further experiments, designed specifically to investigate the origin of RBP4 in the fetus, are therefore needed. This would help clarify the role of RBP4 in fetal growth. The small sample of participants in this study is another limitation. Small for their gestational age newborns are usually the subject of intrauterine growth restriction, which may imply an intrauterine insult, possibly caused by infection or chronic hypoxia, and this may cause a bias.

To sum up, our study found that cord serum RBP4 concentrations were higher in subjects with top quartile fetuses than in those with bottom quartile fetuses. Cord serum RBP4 concentrations were significantly correlated

with fetal birth weight after adjustment for the effect of cord serum insulin levels. These findings may indicate that cord serum RBP4 plays a regulatory role in fetal growth. However, further experiments are still required to elaborate this role.

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