Retinol-binding protein 4 in neonates born small for gestational age

C. Giacomozzi[1], P. Ghirri[2], R. Lapolla[1], A. Bartoli[2], G. Scirè[1], L. Serino[2], D. Germani[1], A. Boldrini[2], and S. Cianfarani[1]

[1]Molecular Endocrinology Unit, D.P.U.O. 'Bambino Gesù' Children's Hospital, 'Rina Balducci' Center of Pediatric Endocrinology, Tor Vergata University, Rome; [2]Division of Neonatology, S. Chiara Hospital, University of Pisa, Pisa, Italy

ABSTRACT. *Background:* Retinol-binding protein 4 (RBP4) is an adipocyte-derived 'signal' that may contribute to the pathogenesis of insulin resistance and Type 2 diabetes. The relationship of RBP4 with insulin resistance and metabolic risk in human beings has been the subject of several studies. Subjects born small for gestational age (SGA) are at risk of insulin resistance and Type 2 diabetes. Though RBP4 could represent an early marker of insulin resistance, to date, none have determined RBP4 in SGA children. *Aim:* Our aim was to measure RBP4 concentrations in cord blood of SGA newborns compared with those in children born with a birth weight appropriate for gestational age (AGA) and to determine whether serum RBP4 levels at birth correlate with insulin sensitivity markers. *Subjects and methods:* Sixty-four newborns, 17 born SGA (mean gestational age: 36.4±2.1 weeks), and 47 born AGA (mean gestational age: 37.0±3.6 weeks) were studied. The main outcome measures included anthropometry, lipid profile, insulin, homeostasis model assessment, quantitative insulin-sensitivity check index, adiponectin, and RBP4. *Results:* RBP4 concentrations were significantly reduced in SGA newborns (*p*<0.002). No relationship was found between RBP4 and insulin sensitivity parameters. Stepwise regression analysis revealed that birth weight was the major predictor of RBP4 serum concentrations (*p*<0.001). *Conclusion:* RBP4 is reduced in SGA newborns, birth weight representing the major determinant of RBP4 concentrations, and is not related to insulin sensitivity. No significant difference in adiponectin levels and insulin sensitivity markers was found between SGA and AGA neonates.
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INTRODUCTION

Retinol-binding protein 4 (RBP4) is an adipocyte-derived 'signal' that may contribute to the pathogenesis of insulin resistance (IR) and Type 2 diabetes. RBP4 could play a role in obesity-induced IR, since chronic elevation of RBP4 in mice increases hepatic glucose production, down-regulates insulin signaling in muscle, and causes systemic IR (1). Furthermore, lowering elevated serum RBP4 levels in obese mice with a synthetic retinoid improves insulin sensitivity and normalizes glucose tolerance (1). In humans, the link between RBP4 and IR is less clear. In adulthood, serum RBP4 levels have been shown to correlate with the magnitude of IR in subjects with obesity, impaired glucose tolerance, and Type 2 diabetes (2). Moreover, elevated serum RBP4 was associated with components of the metabolic syndrome, including increased body mass index, waist-to-hip ratio, serum triglyceride levels, and systolic blood pressure and decreased HDL cholesterol levels (2). In childhood, a relationship between serum RBP4 and obesity and components of the metabolic syndrome has been described in pre-pubertal and early pubertal children (3).

Increased risk of developing IR and Type 2 diabetes in adulthood is associated with low birth weight (4). Although this association is clearly established in adults (5), results in children are still conflicting (6). Some authors have suggested that IR, as a consequence of intrauterine growth retardation would be detectable as early as birth (7, 8). On the other hand, there is evidence that increased insulin sensitivity induced by the fetal environment aims at ameliorating nutrient disposal and utilization, ultimately leading to fetal growth (9). In this respect, an increase in insulin sensitivity would be a physiological adaptation to fetal growth restriction because insulin is a major regulator of fetal growth. Consistent with this, we and others have reported that small-for-gestational age (SGA) newborns display increased insulin sensitivity (10-12).

Although RBP4 may play a pivotal role in the development of IR in humans, to date, however, no study has been carried out in low birth weight subjects. We set out to measure RBP4 concentrations in cord blood of SGA newborns compared with those in children born with a birth weight appropriate for gestational age (AGA) and to determine whether serum RBP4 levels correlate with insulin sensitivity parameters at birth.

SUBJECTS AND METHODS

Subjects

Sixty-four newborns, 17 born SGA (9 males and 8 females; mean gestational age: 36.4±2.1 weeks), and 47 born AGA (24 males and 23 females; mean gestational age: 37.0±3.6 weeks), matched for sex and type of de-

livery (vaginal or cesarean section) were enrolled. The newborns were defined as SGA if the deviation in birth weight was more than 2 SD below the gestational age-related mean of the population (13). The newborns were defined as AGA if birth weight was above the 10th and less than 90th percentile according to Italian standards (13). Neonates with birth weight between the 3rd and the 10th centile were excluded to avoid any overlapping. All pregnancies were dated by ultrasound at 17-18 gestational weeks. Newborns with congenital malformations, chromosomal anomalies, sepsis, endocrine disorders, maternal diabetes, maternal corticosteroid therapy during pregnancy, and more than one pre-natal course of dexamethasone or betamethasone, were excluded. Karyotype was carried out in all SGA newborns with weight and length at birth more than 2 SD scores (SDS) below the mean. The study was approved by the Committee for Research Ethics at Pisa University, and informed consent was obtained from the parents of all newborns.

Measurements

Supine length at birth was measured with a wooden box consisting of a fixed board for the infant's head and a movable board allowing feet to be placed perpendicular to the longitudinal axis of the infant. Weight was measured using a manual scale with a 10-g gradation (Seca, Hamburg, Germany). Weight and length at birth were converted into SDS to adjust for gestational age using local normative data (13). Ponderal index was calculated according to the following formula: [weight (kg)/length (m)^3] (14). As surrogate estimates of insulin sensitivity, we measured: insulin level (I_F), glucose (G_F), the homeostasis model assessment for IR (HOMA-IR) = [(fasting insulin in mU/l) × (fasting glucose in mM)/22.5] (15) and the quantitative insulin sensitivity check index (QUICKI) = $1/[\log(I_F) + \log(G_F)]$ (16).

Assays

At birth, serum samples were collected from the umbilical cord and stored at –80 C until required for assay. Serum insulin was measured using Human Insulin enzyme-linked immunososrbent assay (ELISA) kit (Linco Research Inc., St. Charles, Missouri, USA). The sensitivity of this assay is 2 µU/ml. Intra- and interassay coefficients of variation (CV) are 6.8 and 5.9%, respectively. Serum adiponectin was measured using human adiponectin ELISA Kit (Linco Research Inc., St. Charles, Missouri, USA). The sensitivity of this assay is 0.78 ng/ml. Intra- and interassay CV are 7.4 and 8.4%, respectively. Serum RBP4 was measured using quantikine human RBP4 immunoassay (R&D Systems Minneapolis, Minnesota, USA). The sensitivity of this assay is 0.224 ng/ml. Intra- and interassay CV are 5.7 and 5.8%, respectively. Blood glucose was measured immediately, by the glucose oxidase method, using a glucose analyzer (YSI, Inc., Yellow Springs, Ohio, USA).

Statistics

Results are reported as the mean±SD. Differences between means were assessed using an unpaired two-tailed t test. After ascertaining that variables were nor-

Table 1 - *Anthropometric and biochemical variables. Comparison between small for gestational age (SGA) and appropriate for gestational age (AGA) newborns.*

	SGA (no.=17) Mean±SD	AGA (no.=47) Mean±SD	p
Gestational age (weeks)	36.4±2.0	37.0±3.6	ns
Birth weight (g)	1968±411	2729±771	<0.001
Birth weight (SDS)	–2.38±0.49	–0.67±0.75	<0.001
Birth length (cm)	43.4±3.2	46.7±4.6	<0.01
Ponderal index [weight (kg)/length (m)^3]	23.8±1.6	25.8±2.9	<0.01
Glucose (mg/dl)	52.3±10.8	52.6±20.9	ns
Insulin (µU/ml)	4.3±2.5	7.7±4.7	ns
Total cholesterol (mg/dl)	61.2±13.5	67.1±18.2	ns
HDL cholesterol (mg/dl)	22.0±6.0	27.4±7.7	<0.05
Triglycerides (mg/dl)	58±112	30±16	ns
HOMA	0.5±0.3	0.9±0.7	ns
QUICKI	0.4±0.03	0.4±0.05	ns
Adiponectin (ng/ml)	19.6±8.3	23.3±11.5	ns
RBP4 (ng/ml)	10.5±5.8	16.3±6.1	<0.002

SDS: SD score; HOMA: homeostasis model assessment; QUICKI: quantitative insulin-sensitivity check index; RBP4: retinol-binding protein 4.

mally distributed, the relationships among parameters were evaluated by Pearson correlation. Significance was assigned for $p<0.05$. SPSS 17.0 computer program was used for all statistical calculations (SPSS Inc, Chicago, IL, USA).

RESULTS

The whole SGA and AGA populations were analyzed to investigate differences among anthropometric and biochemical variables (Table 1). No significant differences in gestational age, glucose, total cholesterol, triglycerides, insulin, HOMA, QUICKI, and adiponectin were found. SGA children showed significantly reduced ponderal index ($p<0.01$), HDL cholesterol ($p<0.05$), and RBP4 ($p<0.002$).

RBP4 was closely related to birth weight expressed both in grams (r=0.47, $p<0.001$) and SDS (r=0.38, $p=0.002$), birth length (r=0.35, $p=0.005$), gestational age (r=0.35, $p=0.005$), ponderal index (r=0.36, $p<0.005$), and adiponectin (r=0.40, $p=0.001$). No significant relationship between RBP4 and insulin sensitivity parameters was found. Multiple regression and stepwise regression analyses revealed that the major predictor of RBP4 serum concentrations was birth weight (β-coefficient =0.47, adjusted R^2= 0.21, $p<0.001$) (Fig. 1). The higher predictive value of birth weight was confirmed even considering the two populations (SGA and AGA) separately.

Adiponectin was closely related to birth weight expressed both in grams (r=0.63, $p<0.0001$) and SDS (r=0.27, $p=0.03$), birth length (r=0.57, $p<0.0001$), gestational age (r=0.62, $p<0.0001$), ponderal index (r=0.40, $p=0.001$) and RBP4 (r=0.40, $p=0.001$). No significant relationship between adiponectin and insulin sensitivity parameters were found. Multiple regression and step-
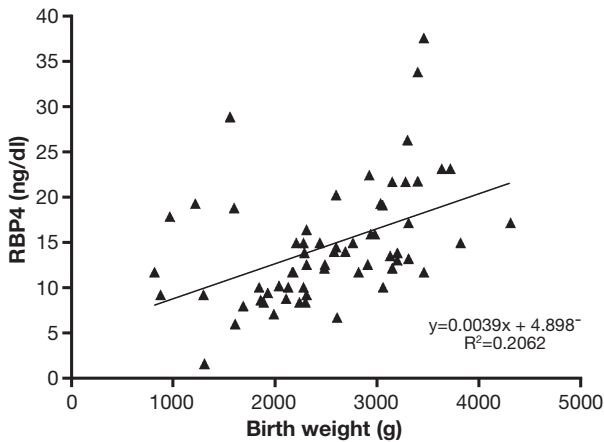
Fig. 1 - *Correlation between birth weight and serum retinol-binding protein 4 (RBP4) levels in cord blood of small for gestational age (no.=17) and appropriate for gestational age (no.=47) newborns (r=0.47, p<0.001).*

wise regression analyses revealed that the major predictor of adiponectin serum concentrations was gestational age (β-coefficient =0.62, adjusted R^2=0.37, *p*<0.0001).

DISCUSSION

Low birth weight has repeatedly been reported to be a risk factor for the development of IR, Type 2 diabetes, and cardiovascular disease in adulthood (4). However, the physiological mechanisms that link fetal growth and adverse outcome remain unknown. RBP4 was found to be secreted by adipocytes and to be up-regulated in adipose tissue and serum of insulin-resistant human beings (17). These findings suggest that RBP4 may play an important role in metabolic homeostasis in insulin-resistant states. To date, however, RBP4 has never been measured in SGA children. Therefore, we set out to test whether RBP4 could represent a helpful early marker of IR able to identify those low birth weight neonates at higher risk of developing metabolic disturbances. Our data show, for the first time, that RBP4 circulating levels are reduced in SGA newborns. There are conflicting data on the relationship between RBP4 and weight at birth (18, 19). Our findings are consistent with a previous report showing a relationship between birth weight and RBP4 in neonates with AGA birth weight (18). On the contrary, a recent study has found no correlation between RBP4 concentrations and birth weight (19). Interestingly, our data show that RBP4 concentrations do not correlate with insulin sensitivity parameters at birth. The fact that umbilical cord samples partly contain maternal blood may represent a potential confounding factor in interpreting our results. However, the close relationship between RBP4 and birth size measurements as well as the significant difference in RBP4 between SGA and AGA newborns strongly suggest that measured RBP4 was mainly from fetal origin.
RBP4 levels have been linked to visceral adiposity, vis-

ceral fat representing a major source of RBP4 in conditions associated with IR (17). Data from absorptiometry studies show that the amount of fat mass is strikingly reduced in neonates who suffered intrauterine growth retardation, indicating an altered development of adipose tissue (12). Early catch-up growth following fetal growth restriction promotes restoration of body size and fat stores by the 1st year of post-natal life (20). These findings lead us to speculate that low fat mass at birth may account for reduced RBP4 in SGA newborns. As visceral fat excess in SGA children has been reported to be already present at the age of 6 yr (21), further longitudinal studies aimed at determining RBP4 fluctuations in relation to body composition and insulin sensitivity parameters would establish whether RBP4 may represent an early marker of metabolic risk.
Finally, the results of our study indicate that SGA newborns do not show impaired insulin sensitivity, suggesting that post-natal growth trajectory and accumulation of visceral fat, play the major role in determining the long-term metabolic risk.

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