European Review for Medical and Pharmacological Sciences 2013; 17: 501-506

Adiponectin and visfatin levels in extremely low birth weight infants; they are also at risk for insulin resistance

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Abstract. – AIM: The aim of this study was to assess adiponectin, visfatin, HOMA-IR, glucose and triglyceride levels in term, preterm and extremely low birth weight (ELBW) babies. Each of these three groups was subdivided into two groups as small-for-gestational age (SGA), and appropriate-for-gestational age (AGA). 30 term, 30 preterm and 30 extremely low birth weight infants were included into the study.

RESULTS: There was no significant difference in term and preterm infants for serum adiponectin, visfatin, and HOMA-IR levels. There were also no significant differences between term and preterm infants for glucose and triglycerides. The serum visfatin, insulin and HOMA-IR levels ($p = 0.001$, $p = 0.001$ and $p < 0.05$, respectively) were higher in ELBW group than preterm group. Comparing the subgroups as SGA and AGA in all main groups, only in ELBW group there were no significant differences in serum adiponectin, visfatin, HOMA-IR and insulin levels.

CONCLUSIONS: We suggest that visfatin can be used as an early indicator of insulin resistance. Independent of being SGA, ELBW itself may be a risk factor for insulin resistance. In the follow-up of these babies the risk of obesity, metabolic syndrome and cardiovascular diseases may be increased as in SGA babies.

Key Words:
Visfatin, Adiponectin, Insulin resistance, Neonate.

Introduction

In the term baby there is a hormonal and metabolic adaptation in the perinatal period to ensure an adequate fuel supply to the brain and other vital organs after the delivery. However, in the preterm infant, abnormalities of glucose homeostasis are customary[1,2]. In fact in preterm infants hypoglycemia is common, but after this initial hypoglycemia, due to limited glycogen and fat stores, preterm babies often become hyperglycemic because of a combination of insulin resistance and relative insulin deficiency[3-7]. These infants are unable to suppress glucose production within a large range of glucose and insulin concentrations; insulin secretory response is inappropriate and insulin processing is immature. There is also an increased ratio of the glucose transporters Glut-1/Glut-2 in fetal tissues, which limits the sensitivity and the hepatocyte reaction to increments in glucose/insulin concentration during the hyperglycemia[8,9]. It has been proposed that the reduced insulin sensitivity may result from an adaptation to an adverse *in utero* environment during a critical period of development[8,9]. An association between the low birth weight and the impairment of glucose homeostasis was first proposed by Hales and Barker in 1991[10]. Several studies have confirmed these findings, thereby underscoring the importance of the intrauterine environment in the development of diseases in adulthood, including hypertension, cardiovascular diseases and impaired glucose tolerance.

Adipose tissue secretes a number of hormones called adipocytokines, two of which, visfatin and adiponectin, appear to play an important role in the metabolism and energy homeostasis[11,12]. Adiponectin is involved in glucose and lipid metabolism and is exclusively expressed and secreted by the adipose tissue. Hypoadiponectinaemia has been shown to be associated with insulin resistance in animal and human investigations[13]. Plasma adiponectin levels are decreased in subjects with obesity, insulin resistance or type 2 diabetes mellitus, and are inversely correlated with

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visfatin and fasting insulin levels[14,15]. Visfatin has insulin-mimetic effects which lower plasma glucose levels[16,17]. Moreover, circulating visfatin concentrations have been shown to increase in parallel with hyperglycemia[18]. Low birth weight has been related with an increased risk for developing metabolic syndrome in adulthood as well. Recently, we reported that the excessive or the retardation of the fetal growth is linked with the glucose metabolism and adiponectin; Visfatin appears to play a crucial role[19].

The aim of this study was to investigate the relation between adipokines and insulin sensitivity markers in term, preterm and extremely low birth weight (ELBW) infants and the relationship between other metabolic features with adipocytokines.

Materials and Methods

Subjects

Newborn infants born in GATA Medical Faculty between December, 2007 and November, 2008 by delivered only cesarean section were enrolled in the present study. The gestational age was assessed by maternal menstrual dating, ultrasound and confirmed by the Dubowitz scoring[20]. Maternal data including age, body mass index (BMI), history of smoking, obesity, chorioamnionitis and glucose intolerance were recorded. Women admitted before 34 weeks of gestation within the 24 hours of preterm delivery were routinely given betametasone. A total of 44 mothers were given antenatal steroids. None of the participating mothers smoked during pregnancy. Women with singleton term pregnancies known to be in good health and without any underlying medical disorders were recruited randomly from the delivery suite. All mothers were screened for gestational diabetes at 22 weeks of gestation by glucose tolerance test. During the study period, a total of 948 infants (220 preterm and 728 term) were born and infants with maternal clinical conditions such as diabetes mellitus, chorioamnionitis, preterm premature rupture of membranes or parathyroid, bone, renal, and gastrointestinal disorders were excluded from the study. All mothers at the time of delivery received same amount and composition of intravenous fluids. As a result, a total of 90 infants were included randomly to this investigation.

The subjects were divided into three groups as term, preterm and ELBW. Each of these three groups were then subdivided into two groups as SGA or AGA by using Fenton intrauterine growth curves[21]. Gestational age was defined according to first day of last menstrual period and dating ultrasonography performed at 16 weeks of gestation.

Small for gestational age (SGA) Group: Low birth weight infant, included 49 babies of birthweight lower than 10st percentile according to Fenton curves[21];

Appropriate for gestational age (AGA) Group: Normal birth weight, included 41 babies between 10st and 90st percentiles of Fenton curves.

None of the infants had any congenital malformations, chromosomal abnormalities, or intrauterine infections. The 5st minute Apgar scores were ≥ 8 and their physical examinations were normal. Those with chronic conditions, systemic infections and nutrition defects were excluded from the investigation. All infants were breastfed and were studied at birth. Anthropometric measurements (height and weight) were recorded and blood samples were taken. The birthweight and length were obtained from each neonate immediately after the birth. The babies were weighed naked with an electronic weighing machine. The approval of the GATA Medical Faculty Ethics Committee and the informed consents of the relatives of the patients were obtained before the blood samples were taken.

Hormone and Biochemical Assays

Neonatal blood was collected from the umbilical vein. The blood samples were immediately centrifuged after clotting and the supernatant serum was kept frozen at –80°C until the assay.

Visfatin levels were determined by enzyme immunoassay studies (visfatin C-terminal [human] EIA; Phoenix Pharmaceuticals, Belmont, CA, USA). Minimum detectable concentration and intraassay and interassay coefficients of variation were 0.1 ng/mL and 5% and 12%, respectively. Serum adiponectin levels were measured by ELISA method using the kit of Phoenix Pharmaceuticals Inc. (Belmont, CA, USA). The sensitivity of the adiponectin assay was 0.40 g/ml. The intraassay and interassay coefficients of variation were <10% and <15%, respectively. Fasting serum glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured using standard enzymatic methods. Fasting plasma insulin concentrations were determined by a solid-phase, two-site chemiluminescent immunometric assay (Immulite, 2000;

DPC DIPESA S.A., Madrid, Spain). The sensitivity of the assay was 2·0 mU/l and the intra- and interassay coefficients of variation (CVs) were less than 8%. Homeostasis assessment model for insulin resistance [HOMA-IR = fasting insulin (μU/l) × fasting glucose (mg/dl)/22.5) were chosen as measures of insulin sensitivity[22].

Statistics

Data was expressed as mean ± SD. Differences in the means of variables were tested using both parametric and non-parametric tests. We used Student *t*-test for parametric variables and Mann Whitney-U for non-parametric data. A probability value of less than 0.05 was considered significant. SPSS version 17 for Windows (SPSS Inc., Chicago, IL, USA) was used for analysis.

Results

The clinical and laboratory data of term, preterm and ELBW infants are shown in Table I and II. There was no significant difference in term and preterm infants for serum visfatin, adiponectin and insulin levels. Serum Visfatin levels were slightly higher in term babies (3.5 ± 2.5 ng/ml) than in preterm babies (2.5 ± 1.9 ng/ml). There were also no significant differences between term and preterm infants for glucose, HOMA-IR and triglycerides. There was a significant difference in cholesterol levels between these two groups.

However; there were significant differences between preterm and ELBW infants in HOMA-IR, visfatin and insulin levels. Serum insulin levels were different for preterm and ELBW infants. All other parameters and *p* values for these groups are shown also in Table I.

In term infants between SGA and AGA neonates there was statistically difference for serum adiponectin and insulin ($p = 0.001$). Preterm SGA and AGA infants also were found high visfatin levels ($p = 0.001$), insulin ($p = 0.001$) and adiponectin ($p = 0.002$). HOMA-IR were found to differ between SGA and AGA infants ($p = 0.001$).

There was no statistically significant difference between SGA and AGA in ELBW infants for serum visfatin ($p = 0.174$), serum adiponectin ($p = 0.49$), serum insulin ($p = 0.08$) and HOMA-IR ($p = 0.11$). All other parameters for SGA and AGA infants are summarized in Table II. There were no significant differences between study groups with respect to maternal characteristics including maternal age and BMI.

Discussion

To the best of our knowledge, this study is the first to focus on the insulin resistance which may occur in ELBW neonates independently from being SGA. Our data demonstrated that ELBW neonates have high serum visfatin levels, high insulin resistance as like as seen in SGA infants. Furthermore, visfatin can be used

Table I. The clinical and laboratory data of term, preterm and ELBW infants.

	Term	Preterm	ELBW
N	30	30	30
Gestational week	39 ± 0.8	34 ± 1.1*	28.5 ± 1†‡
Antenatal steroids	0 (0%)	16 (53%)	28 (93%)
Weight (g)	2830 ± 656	1963 ± 381*	909 ± 79†‡
Length (cm)	49 ± 2.2	45 ± 2.1*	37 ± 1.4†‡
Fasting glucose (mg/dl)	59 ± 10	60 ± 10	63 ± 11
Fasting insulin (μIU/ml)	2.7 ± 1.4	3.2 ± 1.1	15 ± 11†‡
HDL-cholesterol (mg/dl)	43 ± 10	42 ± 15	43 ± 16
Triglycerides (mg/dl)	127 ± 37	107 ± 41	94 ± 41
Total cholesterol (mg/dl)	141 ± 28	172 ± 40*	187 ± 43†
Visfatin (ng/ml)	3.2 ± 0.6	3.2 ± 1.1	23 ± 13†‡
Adiponectin (μg/ml)	3.5 ± 2.5	2.5 ± 1.9	1.9 ± 0.5
HOMA-IR	0.49 ± 0.57	0.48 ± 0.2	2.3 ± 1.7†‡

Values are presented as mean ± SD. Data are given as means ± SD. difference at $p < 0.05$ level. HOMA-IR: homeostasis model assessment for insulin resistance (fasting insulin (μU/mL) × fasting glucose (mg/dL) /405. *$p < 0.05$ term vs. preterm. †$p < 0.05$ term vs. ELBW. ‡$p < 0.05$ preterm vs. ELBW.

Table II. Comparison of clinical and laboratory data of term, preterm and ELBW infants.

	Term		p	Preterm		p	ELBW		p
	SGA n:17	AGA n:13		SGA n:18	AGA n:12		SGA n:14	AGA n:16	
Weight(g)	2236 ± 107	3424 ± 353	0.001	1636 ± 223	2289 ± 159	0.001	883 ± 74	915 ± 29	0.02
Length (cm)	47 ± 1.2	50 ± 1.8	0.001	44 ± 1.6	47 ± 1.7	0.001	36 ± 1.5	37 ± 0.9	ns
Head circumference (cm)	32.6 ± 1.2	34.5 ± 1.3	0.02	30 ± 1	32.5 ± 0.8	0.01	27.5 ± 1.2	27.2 ± 1	ns
Gestional week	39 ± 0.6	39 ± 0.8	ns	34 ± 1.2	34 ± 1	ns	29 ± 1	28 ± 0.5	0.014
Visfatin(ng/ml)	3.4 ± 0.6	2.9 ± 0.63	0.04	4.1 ± 0.7	2.2 ± 0.6	0.001	20 ± 14	27 ± 10	0.174
Adiponectin(µg/ml)	1.4 ± 0.3	5.7 ± 2.5	0.001	1.5 ± 0.5	3.6 ± 2.2	0.002	1.8 ± 0.43	1.9 ± 0.6	ns
Insulin (µIU/ml)	3.8 ± 0.7	1.6 ± 1	0.001	4.3 ± 0.5	2.2 ± 0.5	0.001	11.7 ± 8.9	18 ± 11	ns
Glucose (mg/dl)	61 ± 5	58 ± 14	ns	59 ± 11	60 ± 9.5	ns	63 ± 11	64 ± 12	ns
Homa-IR	0.54 ± 0.2	0.43 ± 0.8	ns	0.63 ± 0.17	0.34 ± 0.1	0.001	1.8 ± 1.6	2.8 ± 1.7	ns
HDL (mg/dl)	47 ± 1.9	40 ± 13	0.65	40 ± 20	43 ± 8.3	0.8	31 ± 6.6	56 ± 13	0.001
Triglycerides (mg/dl)	122 ± 9	131 ± 52	0.52	105 ± 41	109 ± 43	0.77	77 ± 46	112 ± 25	0.018
Total cholesterol (mg/dl)	134 ± 13	148 ± 37	0.17	194 ± 41	151 ± 24	0.002	199 ± 43	174 ± 41	0.12

Values are presented as mean ± SD. Data are given as means ± SD. difference at $p < 0.05$ level.

as an early insulin resistance marker also in ELBW infants, as we mentioned before in term neonates[19].

Insulin resistance in many tissues, including the liver, adipose tissue and muscle, is a central defect of metabolism in the early pre-diabetic as well as the overt type 2 diabetic states. In fact, insulin resistance is also considered a major programmed defect of metabolism linking the adverse intrauterine environment and a subsequent SGA birth with an increased risk of type 2 diabetes in later life. Thus, although insulin secretion in individuals with low birth weight is disproportionately reduced when corrected for *in-vivo* insulin action very early in life[23], insulin secretion in the absolute sense is normal or increased to compensate for insulin resistance[24]. In our study, serum visfatin and insulin levels and HOMA-IR were found to be significantly increased in the ELBW group compared to the term and preterm groups. When the subgroups of AGA and SGA were examined within each group, the insulin level, HOMA-IR, serum visfatin levels were found to be higher and the serum adiponectin levels to be lower in SGA babies compared to AGA babies in the preterm and term groups. However, no significant difference in the insulin levels, HOMA-IR and visfatin levels could be detected among the SGA and AGA babies in the ELBW group. When the SGA and AGA babies were compared among the main groups, the insulin resistance was found to increase with increasing levels of prematurity and the state of SGA. Similarly, the findings of significantly increased levels of serum insulin, HOMA-IR and serum visfatin and significantly decreased levels of serum adiponectin in AGA babies in the ELBW group, has shown that the degree of prematurity, and especially being ELBW, is an important risk factor for insulin resistance independent from being SGA. There are only a few studies of glucose/insulin abnormalities within the perinatal period that compare infants who were born SGA with those born AGA. To investigate whether intrauterine growth restriction (IUGR) is associated with a decreased insulin sensitivity, Leipala et al[25] used an abbreviated minimal model to study insulin sensitivity in preterm newborn infants at a mean of 7 ± 3 days with a birth weight of less than 1.500 g. Basal insulin sensitivity and insulin sensitivity index did not differ between infants born AGA and those born SGA, but steroids decreased insulin sensitivity only in the SGA group. A simi-

lar observation was performed in a larger group of preterm newborns in South Africa[26]. One-hundred premature infants were recruited, and fasting and postprandial (standardized milk feed) glucose/insulin levels were measured. Assessment occurred within 1-65 days after birth. Infants born SGA had higher 60-min serum insulin levels than neonates born AGA, despite similar glucose levels and similarly in another study, Soto et al[27] found that postnatal growth velocity correlated negatively and independently with birth weight and insulin resistance.

Plasma visfatin is known to increase in parallel with obesity, have insulin-mimetic effects and lower plasma glucose levels[16]. Furthermore, plasma adiponectin is inversely correlated with visfatin and fasting insulin levels[17,28]. Malamitsi-Puchner et al[29] and our another study showed higher visfatin levels in the SGA group than the AGA group and hypothesized that visfatin was an early marker for the insulin resistance[19,29]. We suggest that visfatin can be used as an early indicator of the insulin resistance. Independent of being SGA, ELBW itself may be a risk factor for insulin resistance and in the follow-up of these babies the risk of obesity, metabolic syndrome and cardiovascular diseases may have increased as in SGA babies. However, further studies are needed on this topic.

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