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Original Article

Relationship between 11β-HSD2 mRNA and insulin sensitivity in term small-for-gestational age neonates after birth

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Abstract: The aim of this study was to investigate the relationship between serum 11β-HSD2 mRNA level and insulin sensitivity in term small-for-gestational age (SGA) neonates after birth. The 38 infants were divided into two groups, the SGA group and the appropriate-for-gestational age (AGA) group. The placental 11β-HSD2 mRNA abundance and concentration of cortisol, fasting glucose, fasting insulin, adiponectin, visfatin and insulin-like growth factor-I (IGF-1) in the umbilical vein plasma were measured. The results showed that in the SGA group, neonates had lower levels of placental 11β-HSD2 mRNA and serum cortisol, and higher fasting insulin and HOMA-IR compared to AGA group. For some insulin sensitivity relative factor, levels of serum adiponectin and IGF-1 were lower while visfatin was higher in the SGA group than AGA group. Correlation analyses revealed that 11β-HSD2 mRNA level had a negative correlation with fasting insulin, HOMA-IR and visfatin.

Keywords: 11β-HSD2, insulin sensitivity, SGA, term, neonates

Introduction

Epidemiological evidence suggests that an adverse prenatal environment permanently 'programs' physiology and increases the risk of cardiovascular, metabolic, neuroendocrine and psychiatric disorders in adulthood [1, 2]. Prenatal exposure to excess glucocorticoids might provide the link between fetal maturation and adult pathophysiology [3, 4]. In mammals, glucocorticoids are important for fetal growth, tissue development and maturation of various organs to prepare the fetus for extrauterine existence. However, supraphysiological levels of glucocorticoids have been shown to cause fetal growth retardation [1]. Glucocorticoids are lipophilic and readily cross placenta barriers. In fact, fetoplacental 11β-hydroxy steroid dehydrogenase type 2 (11β-HSD2), which catalyses the rapid metabolism of active cortisol and corticosterone to physiologically inert 11-keto forms (cortisone, 11-dehydro corticosterone), forms a barrier to transport of maternal glucocorticoids [5]. However, this barrier is not complete, as a minor proportion of maternal glucocorticoid crosses intact to the fetus; thus, maternal stress elevates fetal glucocorticoid levels. Therefore, deficiency in 11β-HSD2 would be expected to expose the fetus to increased glucocorticoid levels from the maternal circulation with subsequent effects on fetal development [6]. In support of this notion, the lowest placental 11β-HSD2 activity, and presumably the highest fetal exposure to maternal glucocorticoids, was seen in babies with the lowest birth weights [6].

An association between the low birth weight and the impairment of glucose homeostasis was first proposed by Hales and Barker in 1991 [7]. 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]. As mentioned above, high glucocorticoid caused by deficiency in placental 11β-HSD2 activity is kind of adverse prenatal environment. Therefore, the main aim of this study was to investigate the direct relationship between 11β-HSD2 and

Table 1. Primer sequences

		Primer sequence (5'-3')	Melting temperature
11β-HSD2	Fwd	GTAGCTGCATGGAGGTGAATT	56.9
	Rev	ACAGCTGAATGTGTCCATGAGT	
β-actin	Fwd	GCTACGAGCTGCCTGACG	57.3
	Rev	TCGTGGATGCCACAGGAC	

Table 2. Clinical and laboratory data of infants

	SGA	AGA
Number	18	20
Gestational week	39.4 ± 1.0	39.3 ± 1.1
Weight (g)	2264 ± 145**	3530 ± 306
Length (cm)	47.43 ± 1.22*	49.89 ± 0.96
Sex (male/female)	10/8	11/9
delivery mode (caesarean/eutocia)	7/11	9/11
11β-HSD2 mRNA	0.65 ± 0.06*	0.83 ± 0.07
Cortisol	205.5 ± 8.05*	283.4 ± 11.58
Fasting glucose (mg/dl)	64.7 ± 7.18	60.6 ± 6.02
Fasting insulin (μIU/ml)	13.82 ± 2.47**	2.08 ± 0.66
HOMA-IR	2.24 ± 0.61*	0.32 ± 0.31
Visfatin (ng/ml)	5.03 ± 0.62*	2.45 ± 0.58
Adiponectin (mg/ml)	2.76±0.61**	8.24±0.40
IGF-1 (U/ml)	0.19±0.03*	0.42±0.05

Data was presented as mean + SD; *Represents $P < 0.05$ and **represents $P < 0.01$ when SGA vs. AGA.

insulin sensitivity in term SGA neonates after birth.

Some insulin resistance relative factors were also find difference in low birth weight babies. The data demonstrated that the extremely low birth weight neonates had high serum visfatin levels and insulin resistance [9], it has been reported that adiponectin and visfatin appears to play a crucial role in the retardation of the fetal growth [10]. In addition, several studies have shown a positive association between birthweight and insulin-like growth factor-I (IGF-1) concentrations in umbilical venous blood at delivery [11, 12]. So, in the present study, we also explored relationships between 11β-HSD2 and adiponectin, visfatin, and IGF-1.

Materials and methods

Subjects

All subjects gave informed consent to participate in the study, which was approved by the University of Nanjing Medical Human Research Ethics Committee. Newborn infants delivered in the Nanjing Maternal and Child Health Hospital Affiliated to Nanjing Medical University between May 2011 and July 2014 were enrolled in this study. All of the newborns were healthy, and the infants who with maternal clinical conditions such as diabetes mellitus, or parathyroid, bone, renal, and gastrointestinal disorders were excluded from the study. None of the infants had any congenital malformations, chromosomal abnormalities, or intrauterine infections. Their mothers were in good health and had no remarkable complications during pregnancy.

The 38 babies were divided into two groups using the Lubchenco intrauterine growth curves. The SGA group included 18 low birth weight infants with birthweights lower than the 10th percentile or 2500 g. The AGA group included 20 normal birth weight infants with birthweights between the 10th and 90th percentiles, or those infants weighing between 2500 and 4000 g. The birthweight and length were obtained from each neonate immediately after birth. Immediately after delivery and before expulsion of the placenta, the umbilical cord was clamped in two places. Three millilitres of umbilical venous blood were collected into a plain tube and centrifuged within five minutes of collection at 3500 rpm for 10 minutes. Aliquots of sera were stored at -80°C until further analysis. The placenta was collected within 45 min of delivery and pieces were snap frozen in liquid nitrogen and stored at -80°C until use.

mRNA and biochemical assays

11β-HSD2 mRNA abundance was measured by quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) (ABI Prism 7700 sequence detector, Perkin Elmer Applied Biosystems), comparing abundance to that of the constitutively expressed gene, β-actin. Primers were designed using Primer Express version 1.0 (Perkin Elmer Applied Biosystems). Primers and the melting temperatures of each amplicon were shown in **Table 1**. Briefly, total

Table 3. Correlation of 11β-HSD2 mRNA abundance of neonates with the other parameters in SGA infants

	SGA	
	r	p
Weight (g)	0.23	0.21
Length (cm)	0.32	0.15
Cortisol	0.23	0.54
Fasting glucose (mg/dl)	0.10	0.67
Fasting insulin (μIU/ml)	-0.51	0.04
HOMA-IR	-0.42	0.05
Visfatin (ng/ml)	-0.43	0.05
Adiponectin (mg/ml)	0.33	0.08
IGF-1 (U/ml)	0.37	0.23

placental RNA was extracted using the trizol method (Life Technologies, Frederick, MD, USA). RNA was column purified (Qiagen Australia, Clifton Hill, Victoria, Australia) and reverse transcribed with the Taqman RT kit (Perkin Elmer, Branchburg, NJ, USA). Primers were made up to a concentration of 100 pmol/μl with RNase free water. 40 ng of cDNA was used in the PCR reaction, with SybrGreen (Applied Biosystems, Warrington, UK) as the fluorescent marker. PCR analysis was performed on triplicate samples with the following reaction conditions: 2 min at 50°C, 10 min at 95°C and 40 cycles of 15 sec at 95°C and 1 min at 60°C. A duplicate sample that had not been reverse transcribed was included as a control for each placental sample, along with duplicate no-template controls for each primer. The Comparative CT method (where CT is the threshold cycle) was used to derive a relative quantitative measure of 11β-HSD2 gene expression relative to β-actin expression.

The concentration of cortisol in the umbilical vein plasma was directly measured by commercial radioimmunoassay (Orion Diagnostica, Finland) according to the manufacturer's instructions. The sensitivity of the assay was 5 nmol/l and cross-reactivity of the cortisol antiserum with most other steroids was < 0.1 per cent.

Visfatin levels were determined by enzyme immunoassay studies (visfatin C-terminal [human] EIA; Phoenix Pharmaceuticals, Belmont, CA). The 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 the ELISA method using the Phoenix kit (Pharmaceuticals, Belmont, CA). The sensitivity of the adiponectin assay was 0.40 g/ml. The intraassay and interassay coefficients of variation were < 10% and < 15%, respectively. Serum IGF-1 was measured, after acid-ethanol extraction of its binding proteins, by radioimmunoassay (RIA) using apolyclonal rabbit antiserum (R557A) raised against purified human IGF-1. The sensitivity of this assay was 0.07 U/ml. Intra-assay coefficient of variation (CV) values were 11.3% at 0.23 U/ml and 6.5% at 1.23 U/ml. Interassay CV values were 10.5% at 0.38 U/ml and 12.1% at 0.99 U/ml. Fasting serum glucose was 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 (mU/l) × fasting glucose (mg/dl)/405) were chosen as measures of insulin sensitivity.

Statistics

All results were presented as means ± standard error of the mean. Statistical analysis was performed by SPSS10.0 software. Differences in the means of variables were tested using both parametric and nonparametric tests depending on the distribution of the variables. Correlation analyses were conducted using the Spearman or Pearson correlation coefficients depending once again on the distribution of the variables. A P value of < 0.05 was considered significant.

Results

The clinical and laboratory data of the newborns was shown at **Table 2**. The weight and length of SGA infants were significantly smaller than AGA babies ($P < 0.01$; $P < 0.05$; respectively). Placental 11β-HSD2 mRNA abundance was significantly lower in SGA group compared with AGA group ($P < 0.05$). The SGA group had higher fasting insulin and HOMA-IR ($P < 0.01$; P

< 0.05; respectively), but no difference in fasting glucose compared to the AGA group. Concentrations of cortisol, adiponectin and IGF-1 of the SGA neonates were significantly lower ($P < 0.05$; $P < 0.01$; $P < 0.05$; respectively), while visfatin was higher ($P < 0.05$) by contrast with the AGA infants.

The relation between placental 11β-HSD2 mRNA abundance and other parameters in the SGA infants was showed in **Table 3**. 11β-HSD2 mRNA showed a negative correlation with fasting insulin, HOMA-IR and visfatin (r = -0.51, P = 0.04; r = -0. 42, P = 0.05; r = -0.43, P = 0.05; respectively). There was a trend that 11β-HSD2 mRNA had a positive correlation with length, adiponectin and IGF-1 (r = 0.32, P = 0.15; r = 0.33, P = 0.08; r = 0.37, P = 0.23; respectively), however there was no significant difference.

Discussion

In humans, Deficiency in 11β-HSD2 was associated with low birth weight [13]. In the present study, we found the same result that the SGA infants had lower placental 11β-HSD2 mRNA abundance compared with the AGA group. The low placental 11β-HSD2 activity presumably made fetal exposure to high maternal glucocorticoids [6]. However, concentration of cortisol in SGA neonates was lower than AGA babies in the present study, which seems unreasonable. In fact, fetal hypothalamic-pituitary-adrenal axis (HPA axis) has matured in normal late pregnancy, which secretes large amounts of cortisol and becomes the main source of circulating cortisol of fetus [14, 15]. Therefore, the reason for lower cortisol levels in SGA newborns may be dysfunction in fetal HPA axis, rather than deficiency in 11β-HSD2.

Serum insulin levels and HOMA-IR were significantly increased in the SGA babies compared to the SGA infants. Insulin resistance is 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, insulin secretion in the absolute sense is normal or increased to compensate for insulin resistance [16, 17]. Adiponectin and visfatin, which are secreted by adipose tissue, play an important role in the metabolism and energy homeostasis. Plasma adiponectin levels decreased in subjects with insulin resistance or type 2 diabetes mellitus [18]. Visfatin has insulin-mimetic effects which lower plasma glucose levels [19]. In the present study, the SGA group also showed lower adiponectin and higher visfatin levels, which was consistent with previous studies. Several studies have shown a positive association between birthweight and IGF-1 concentrations in umbilical venous blood at delivery [12]. So we also checked the IGF-1 level, and the result showed that the SGA infants had a lower IGF-1concentration than the AGA babies. Furthermore, visfatin, adiponectin and IGF-1 can also be used as an early insulin resistance marker in SGA infants.

In the present study, we concentrated on the relationship between 11β-HSD2 mRNA and insulin sensitivity in SGA neonates. 11β-HSD2 mRNA level showed a negative correlation with fasting insulin, HOMA-IR and visfatin, and a trend of a positive correlation with adiponectin and IGF-1 in SGA babies. The reduced insulin sensitivity may result from an adaptation to an adverse in utero environment during a critical period of development [8]. Over exposure of the fetus to glucocorticoids, which induced by 11β-HSD2 deficiency, was a kind of adverse in utero environment. Since significant difference in 11β-HSD2 mRNA abundance between intrauterine growth restriction and AGA fetus was observed in the early third trimester and the term period [20]. In addition, the cortisol of full term SGA babies was lower in the present study. Therefore, we concluded that lower 11β-HSD2 may induce higher cortisol in the early third trimester with subsequent effects on insulin sensitivity. However, it still needs far more study.

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Disclosure of conflict of interest

None.

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