

## Relationship between Leptin Concentration in Cord Blood and Foetal Growth and Maternal Anthropometry of GDM Mothers at Delivery

S JAHAN<sup>a</sup>, TR DAS<sup>b</sup>, KB BISWAS<sup>c</sup>

### Summary:

**Background and Aims:** Cord blood leptin may reflect the leptinemic status of a newborn at birth more accurately than the leptin values of blood collected from other sites. The present study was undertaken to determine the relationship of cord serum leptin concentration at birth with neonatal and maternal anthropometric parameters. **Materials and Methods:** Blood was taken from the umbilical cord of the babies at delivery. Maternal anthropometric measurements were recorded at admission for delivery. Neonatal anthropometric measurements were recorded within 48 hours after delivery. Linear regression analysis was used to explore the relationship between cord serum leptin concentration and anthropometric parameters of the baby and the mother. Both Serum leptin and serum C-peptide levels

were measured by chemiluminescence-based ELISA method. **Results:** The leptin concentration (ng/ml, mean $\pm$ SD) in cord blood was 39.13 $\pm$ 14.44. Cord leptin levels correlated with birth weight ( $r=0.673$ ,  $p<0.0001$ ), ponderal index ( $r=0.732$ ,  $p<0.0001$ ) but it did not correlate with maternal body mass index, gestational age ( $r=0.135$ ,  $p=0.349$ ) at delivery or cord serum C-peptide concentration ( $r=-0.049$ ,  $p=0.735$ ) or placental weight ( $r=0.203$ ,  $p=0.157$ ). **Conclusion:** There are associations between cord leptin concentration at delivery and birth weight, ponderal index (PI) of the babies but not body mass index (BMI) of the mothers. High leptin levels of the baby could represent an important feedback modulator of substrate supply and subsequently for adipose tissue status during late gestation.

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### Introduction:

The mechanisms by which foetal weight is regulated during pregnancy are poorly understood. Pregnancy is a hypermetabolic state associated with a physiological increase in maternal body fat and weight. Leptin is 16 kDa protein, the product of the "ob"- gene, secreted by adipocytes.<sup>1,2</sup> It regulates body weight through a negative feedback signal between the adipose tissue and the hypothalamic centers of satiety,<sup>3</sup> therefore causing a decrease in food intake<sup>1,4,5</sup> and an increase in body temperature and energy expenditure.<sup>1,4</sup> In

obese or normal weight children, as in adults, serum leptin concentrations closely correlate with body weight and percentage of body fat.<sup>6,7</sup> Several recent studies have demonstrated a positive correlation between leptin concentrations in cord blood and body weight at birth<sup>8-10</sup>. The last trimester of gestation is of considerable importance for the growth and development of adipose tissue, with an exponential accumulation of fat mass.<sup>11</sup> The mechanism regulating foetal growth is poorly understood. Chromosomal aberrations<sup>12</sup> and environmental factors or toxin exposure during pregnancy,<sup>13</sup> as well as hormonal factors<sup>14</sup> all have been implicated in addition to genetic predisposition, the primary determinant of foetal weight. In 1994, leptin - a 16 kDa hormone, was identified as the product of the obesity (ob) gene.<sup>15</sup> Subsequent work clearly demonstrated the role of leptin in the regulation of body weight and has lead to hypothesis explaining the mechanism(s) of how leptin may affect body weight. Mouse leptin is secreted into the circulations by large adipocytes,<sup>16</sup> crosses the blood - brain barrier<sup>17</sup> and binds to its hypothalamic receptors,<sup>18</sup> where the expression of neuropeptide Y (NPY) is down

- a. Dr. Samsad Jahan, MS, Assistant Professor, Department of Gynaecology and Obstetrics, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Shahbag, Dhaka 1000, Bangladesh.
- b. Dr. Tripti Rani Das, FCPS, Assistant Professor, Department of Gynaecology and Obstetrics, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka 1000, Bangladesh.
- c. Kazal Boron Biswas, MSc, Research Fellow, Biomedical Research Group, BIRDEM, Shahbag, Dhaka 1000, Bangladesh.

**Address of Correspondence:** Dr. Samsad Jahan, MS, Assistant Professor, Department of Gynaecology and Obstetrics, BIRDEM, Shahbag, Dhaka 1000, Bangladesh.

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regulated<sup>18</sup>. Consecutively, a loss of food intake and increase in energy expenditure leads to a reduction of body fat and body weight<sup>19,20</sup>. Human obesity in children and adults are associated with elevated serum leptin levels.<sup>6,21</sup> A genetic defect in the “ob” gene leads to extreme adiposity.<sup>22</sup> These data strongly indicate an important role for leptin in regulation of body weight in humans also. Insulin has been shown to induce expressions of the “ob” gene in rats and humans,<sup>23</sup> although in the later this effect appears to be indirect rather than direct.<sup>21,24,25</sup> Because, insulin is also an important growth factor for the fetus, the present study investigated whether cord blood insulin - leptin levels correlate. Particular focus was put on potential correlations between birth weight and leptin and insulin or C-peptide levels in the offspring of GDM mothers. Haemolysis to any degree can affect the accuracy of the insulin assay but not the C-peptide concentration.<sup>26</sup> Therefore C-peptide was measured in all the samples of cord blood. The influence of this hormone on the developing fetus is still unknown. Leptin is regularly present in foetal serum at term.<sup>8,10,27</sup> Its cord serum level positively correlate with foetal birth weight, whereas no such correlation has been found with maternal serum leptin. Available evidence indicates leptin’s importance for intrauterine growth and development.

#### **Materials and Methods:**

The mothers with GDM were admitted in the Gynaecology and Obstetric department of BIRDEM Hospital and underwent clinical examination. Laboratory investigations were done at research division, BIRDEM Hospital during the period of January, 2002 to December, 2002. Gestational age at delivery was calculated from the first day of last menstrual period (LMP) and confirmed by USG during the first trimester, to ensure the pregnancy was singleton. Age of all mothers was within 25-35 years. The inclusion criteria were women recorded at <12 weeks of gestation with a singleton pregnancy. All women were explained about the study. Written informed consent was obtained from all the participants. All the newborns were healthy and their mothers had no remarkable illnesses during their pregnancies and none was taking any medication except for vitamins and iron supplements. Neonates born to mother who experienced medical complications that could significantly affect foetal growth during or

before pregnancy were excluded. A sample of venous cord blood was collected from each new born consecutively, enrolled for vaginal and caesarean full term deliveries just after birth from placental side of the umbilical cord. The serum was immediately separated and frozen at -70°C until analysis. Birth weight of the babies was measured in the first 60 minutes of life using standard weighing balance. Each baby was weighed naked. Weight was recorded in kilogram to the nearest of 10 gram. Placentas were delivered within 10 minutes after delivery of the babies. Before weighing the placenta, it was drained off blood and freed from membranes and cord within 2 centimeters of its insertions. Placental weight was measured by weighing balance. Neonatal length was measured in the first 60 minutes using a stadiometer in centimeters (cm). Neonatal ponderal index (PI) was calculated from the formula (weight / height<sup>3</sup> x 100).<sup>28</sup> For each anthropometric measure, three separate measurements were taken and the mean value was recorded. The height of each woman was measured using a stadiometer in meters (m) to the nearest of 0.01 m. Weight was measured with no foot wear and only light clothing and recorded in kilograms (kg) to the nearest of 0.1 kg. Body mass index was calculated using the formula weight / height.<sup>29</sup> Laboratory techniques: Both serum leptin level and serum c-peptide level were measured by chemiluminescence based ELISA method (DPC, USA). GDM was diagnosed by two samples of fasting, and two hour after 75 gram of oral glucose intake. Women who had repeatedly elevated fasting (>7.0 mmol/L) or postprandial (9 mmol/L) blood glucose values and were treated with diet alone, received insulin therapy.

#### **Statistical Analysis:**

Results were expressed as mean±SD. Linear regression analysis was used to explore the relationship between cord serum leptin concentration and anthropometric parameters of the baby and the mother. Statistical analysis was done with SPSS, P<0.05 as significant.

#### **Results:**

All new born babies (n = 50) and their mothers with GDM did not require special medical attention after birth. Birth weight of the baby was (kg) 3.39±0.61, Birth length (cm) 51.45±3.13, Ponderal index (kg/m<sup>3</sup>) 2.54±0.69, Placental weight (gm) 649.52±107.74, Serum C-peptide (ng/ml) 2.05±0.69,

Serum leptin (ng/ml)  $39.13 \pm 14.44$  (Table I). Weight of the mother (kg)  $71.76 \pm 5.54$ , Height of the mother (m)  $1.61 \pm 3.49$ , Body mass index (BMI)  $27.82 \pm 2.33$ . Mean gestational age at delivery (weeks) was  $38.43 \pm 1.55$  (Table 2).

**Table-I**

*Anthropometric and Biochemical Parameters of the fetuses of GDM mothers (n= 50).*

Birth weight (kg)	$3.39 \pm 0.61$
Male birth weight (Kg)	$3.16 \pm 0.54$
Female birth weight (Kg)	$3.61 \pm 0.46$
Birth length (cm)	$51.45 \pm 3.13$
Ponderal index (kg/m <sup>3</sup> )	$2.54 \pm 0.69$
Placental weight (gm)	$649.52 \pm 107.74$
Serum leptin (ng/ml)	$39.13 \pm 14.44$
Leptin level in male (ng/ml)	$35.42 \pm 12.96$
Leptin level in female (ng/ml)	$42.84 \pm 15.13$
Serum C-peptide (ng/ml)	$2.05 \pm 0.69$

Results were expressed as mean  $\pm$  SD.

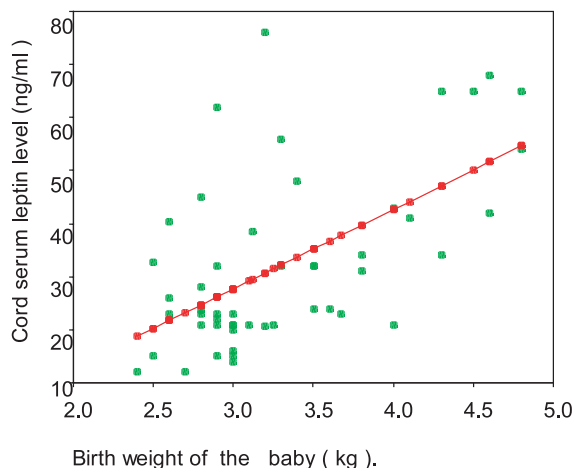
**Table-II**

*Anthropometric characteristics of the GDM mothers (n = 50)*

Age of the mother (years)	$26.60 \pm 3.90$
Gestational age (weeks)	$38.43 \pm 1.55$
Body weight (kg)	$71.76 \pm 5.54$
Height (m)	$1.61 \pm 3.49$
BMI (Kg/m <sup>2</sup> )	$27.82 \pm 2.33$

BMI=Body Mass Index; Results were expressed as mean  $\pm$  SD.

Leptin concentrations in cord blood was strongly correlated with birth weight of the babies ( $r=0.673$ ,  $p=0.0001$ ) (Figure I). Leptin levels also was correlated with ponderal index of the baby ( $r=0.732$ ,  $p=0.0001$ ), but there was no correlation with placental weight ( $r=0.203$ ,  $p=0.157$ ), serum C-peptide level ( $r= -0.049$ ,  $p=0.735$ ), gestational age at

**Fig.-1:** Correlation of serum leptin levels with birth weight of the baby of GDM mothers.

delivery ( $r=0.135$ ,  $p=0.349$ ) (Table-III). There were no significant differences in height, weight, BMI, mid arm circumference (MAC) of the mothers.

**Table-III**

*Cord serum leptin and its relationship with neonatal anthropometry and other factors*

Factors	r	p
Birth weight	0.673	0.0001
Ponderal index	0.732	0.0001
Placental weight	0.203	0.157
Gestational age at delivery	0.135	0.349
Serum c-peptide concentrations	-0.049	0.735

**Discussion:**

The role of leptin among the complex network of factors controlling foetal growth is incompletely understood. Leptin is positively associated with birth weight, but the mechanism underlying this association remains unknown. This association may reflect either a simple relationship with adipose tissue mass or an active role for leptin in foetal growth. Although the nature of the relationship of insulin and leptin remains unclear, some reports have suggested that insulin has a stimulatory effect on leptin secretion in vitro and in vivo.<sup>30</sup> In contrast, we found no statistical correlation between cord leptin level and c-peptide level. This is consistent with a previous report in which no

correlation was found between cord leptin and C-peptide level in newborn infants.<sup>31</sup> These findings suggested the possibility of an independent association between leptin and birth weight. We found a statistically significant relationship between cord leptin level and birth weight that is independent of C-peptide levels. The correlation of cord leptin level with birth weight is consistent with earlier reports.<sup>32,33,34,35</sup> The basis of this correlation may be related to the direct relationship between adipose tissue mass and circulating leptin concentration.<sup>36</sup> The increase in serum leptin concentration in cord blood is consistent with the known exponential increase in fat mass (115%), during the last trimester of gestation.<sup>37</sup> This hypothesis is supported by the strong association between leptin concentration and body weight at birth. Previous studies have demonstrated that the fat mass accumulated during the last trimester of gestation is dramatically reduced in fetuses or newborns with IUGR.<sup>38,39</sup> There was no association between cord leptin concentration and gestational age at delivery. Unlike Schrubing et al,<sup>10</sup> we did not find a significant correlation between cord leptin concentration and placental weight. This result suggests that foetal leptin synthesis and secretion could be dependent only on the fetus. Jaquet et al<sup>40</sup> showed that leptin was detectable in foetal cord blood in all subjects (n=79) as early as 18 weeks of gestation and that levels dramatically increased after 34 weeks of gestation, suggesting that development of adipose tissue and the accumulation of fat mass are the major determinants of foetal and neonatal serum leptin levels. The correlation between umbilical cord insulin level and birth weight was not statistically significant. Our data showed umbilical cord leptin concentration was an independent risk factor for foetal macrosomia. As leptin plays an essential role in determining satiety and it is an indicator of body fat mass, it seems reasonable to propose that this peptide may be involved in foetal growth in the third trimester, when foetal fat is laid down. Leptin may also provide a link between events in early life and the subsequent development of adult diseases. Further studies with larger numbers are warranted for conclusions: There are associations between cord leptin concentrations at delivery and birth weight, ponderal index of the babies but not body

mass index of the mother. High leptin levels of the baby could represent an important feedback modulator of substrate supply and subsequently for adipose tissue status during late gestation.

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