

# Leptin and metabolic hormones in infants of diabetic mothers

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## Abstract

**Aims**—To investigate the effect of maternal diabetes on leptin in term newborns and to determine whether leptin correlates with insulin and its associated biochemical parameters in support of the hypothesis that a functional “adipoinular axis” might exist at this stage of development.

**Methods**—A total of 116 term newborns were prospectively enrolled and categorised into three groups: 44 were infants of non-diabetic mothers (control group C); 41 were infants born to mothers with gestational diabetes on dietary treatment (group D); and 31 were infants born to mothers with gestational or pregestational diabetes on insulin treatment (group I).

**Results**—No significant difference in serum leptin was observed between the three groups; the results of the study population were therefore pooled and analysed. Serum leptin correlated significantly with serum insulin, insulin:glucose ratio, birth weight, body length, body mass index, placenta weight, and maternal HbA<sub>1c</sub>. Female infants had significantly higher serum leptin than male infants. All parameters except placenta weight and body length remained significantly associated with serum leptin when multivariate stepwise regression analysis was applied. Subgroup analysis revealed a significant correlation between serum leptin and cortisol in group D.

**Conclusions**—There was no significant difference in serum leptin between infants born to diabetic and non-diabetic mothers, though infants born to mothers requiring insulin treatment had the highest median serum leptin concentrations. The significant association between serum leptin and insulin or insulin:glucose ratio supports the hypothesis that a functional adipoinular axis might exist in term newborns. Furthermore, the significant correlation between maternal HbA<sub>1c</sub> and circulating leptin of the studied infants suggests that the clinical control of maternal diabetes could affect the regulation of serum leptin in these infants.

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Keywords: leptin; insulin; diabetes; adipoinular axis

The recently discovered hormone leptin, a 16 kDa adipocyte derived protein encoded by the *ob* gene, has provided important insight into the physiology of neuroendocrine regulation of

body fat content, feeding behaviour, and energy homeostasis in rodents and humans.<sup>1–4</sup> Leptin functions as an afferent satiety signal from the peripheral fat mass to the hypothalamus, and controls the basal metabolic rate and energy expenditure of the body.<sup>5–6</sup> Accumulating evidence suggests that leptin exerts its metabolic effects by interacting with other hormonal systems. Leptin has been reported to suppress the secretion of insulin from the pancreatic  $\beta$  cells,<sup>7</sup> and modulate the action of insulin on hepatocytes.<sup>8</sup> A mutation in the *ob* gene in the rodent prevents normal leptin production and causes both obesity and diabetes,<sup>1</sup> whereas treatment of the *ob/ob* mice with leptin results in decrease of body weight and normalisation of blood glucose.<sup>9</sup> Leptin also modifies the functioning of both the hypothalamic–pituitary–adrenal (HPA) and the sympathetic–adrenomedullary axis in controlling fat cell proliferation and body metabolic activity.<sup>10–12</sup> In both rodents and humans, there is a strict reciprocal diurnal association between serum leptin and cortisol concentrations.<sup>13–14</sup> In addition, ACTH induced adrenal cortisol, aldosterone, and dehydroepiandrosterone secretion has been shown to be inhibited by leptin in a concentration dependent manner.<sup>15</sup> Circulating leptin also influences haematopoietic stem cell function<sup>16–17</sup> and oestradiol activity.<sup>18</sup>

As pregnancy is associated with profound changes in adipose tissue/lipid and hormonal metabolism such as increase in insulin, corticotrophin releasing hormone (CRH), cortisol, oestrogens, and progesterone concentrations, it is important to understand the physiological role of leptin in newborns with regard to normal and disease pregnancy states. To date, most newborn studies have concentrated on the relation of leptin on fetal growth and anthropometric measurements at birth.<sup>19–24</sup> Significant associations between umbilical cord serum leptin and birth weight, body mass index (BMI), and arm fat have been well documented.<sup>20–24</sup> However, the effects of pregnancy induced hyperglycaemia (gestational diabetes, one of the most frequent complications of pregnancy) and maternal insulin dependent diabetes on leptin and other metabolic hormones in newborns, have not been systematically evaluated. This study was, therefore, undertaken to investigate the effect of maternal diabetes on serum leptin in term newborns. The inter-relations between leptin and other metabolic hormones including insulin, insulin:glucose ratio (an index of glucose metabolism), corticotrophin (ACTH), cortisol, thyroid stimulating hormone (TSH), and free

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Table 1 Clinical characteristics of the study populations

	Group C (n = 44)	Group D (n = 41)	Group I (n = 31)
Birth weight (g)	3458 (684)	3250 (538)	3399 (572)
Length (cm)	49.6 (2.9)	48.7 (2.0)	48.7 (2.5)
BMI (kg/m <sup>2</sup> )	13.9 (1.7)	13.6 (1.6)	14.3 (1.6)
Placental weight (g)	570 (94)	556 (99)	563 (126)
Male:female (n)	23 : 21	28 : 13	15 : 16
Mode of delivery (n) (normal: caesarean section: forcep or ventouse)	22 : 13 : 9	18 : 14 : 9	16 : 12 : 3
Maternal smoker (n)	1	3	0
Apgar scores			
at 1 min	9 (8–9)	9 (8.75–9)	9 (8–10)
at 5 min	10 (9–10)	10 (10–10)	10 (9–10)
Arterial cord blood			
pH	7.27 (7.22–7.30)	7.27 (7.18–7.31)	7.25 (7.19–7.27)
Base excess	–6.6 (–3.8 to –7.9)	–6.7 (–5.4 to –8.5)	–6.3 (–4.6 to –8.2)
Maternal HbA <sub>1c</sub> *	—	4.84 (0.44)	6.05 (1.12)

Results are expressed as mean (SD) or median (interquartile range).

\*p < 0.05.

thyroxine (FT4) in these infants were also assessed.

### Patients and methods

#### STUDY POPULATION

A total of 116 term (37–42 weeks gestation) newborn infants admitted to the neonatal unit were prospectively enrolled between March 1998 and May 1999. The neonatal unit at Prince of Wales Hospital, Hong Kong admits all newborns with increased risk of infection such as those born to mothers who were carriers of group B streptococcus, and mothers who had peripartum fever or rupture of membranes longer than 24 hours. All infants of gestational and pregestational diabetic mothers are also monitored. Maternal gestational diabetes was defined as a venous plasma glucose concentration after fasting of >5.5 mol/l and/or of >8.0 mmol/l two hours after a 75 g oral glucose load.<sup>25</sup> Gestational age assessment was by the mother's last menstrual period, early ultrasound dating, and new Ballard Score examination.<sup>26</sup> Infants with major congenital abnormalities, chromosomal disorders, proven perinatal infection, and those whose mothers received corticosteroid or other hormonal therapy during pregnancy were excluded.

Enrolled infants were subdivided into three groups. The control group (group C) consisted of 44 term infants who have increased risk of perinatal infection but were subsequently proven to be non-infected with sterile blood and cerebrospinal fluid cultures and/or normal serial serum C reactive protein. The second group (group D) consisted of 41 term infants born to mothers with gestational diabetes requiring only a low energy diet (7.5 MJ/day) for blood glucose control. The third group (group I) consisted of 31 term infants born to mothers with gestational (n = 21) or pregestational diabetes (n = 10) requiring insulin treatment during pregnancy.

#### BLOOD SAMPLES

Blood samples were collected in the morning between 0800 and 1100 when the infants were approximately 24 hours of age (day 1). All collections were scheduled to coincide with the morning blood sampling procedure in order to minimise any unnecessary disturbances to the

patient. Blood was collected: (1) in a prechilled plain tube for serum leptin, insulin, cortisol, and FT4; (2) in a prechilled EDTA bottle for plasma ACTH; and (3) in a fluoride tube for plasma glucose measurement. These blood samples were immediately immersed in ice and transported to the laboratory for processing. All samples were centrifuged at 3500 rpm for 15 minutes at 4°C, and the resulting plasma/serum stored at –70°C until analysis. Umbilical cord blood TSH was routinely obtained immediately after birth for neonatal screening of congenital hypothyroidism.

#### HORMONE ASSAYS

Serum leptin and FT4 were measured by enzyme linked immunosorbent assay (ELISA, Diagnostic Systems Laboratories Inc., Webster, Texas, USA) and chemiluminescence immunoassay (ACS:180 analyser, CIBA-Corning Diagnostic Corp., Medfield, Massachusetts, USA), respectively. Serum insulin was measured by microparticle immunoassay (IMx analyser, Abbott Laboratories, Abbott Park, Illinois, USA) and plasma glucose was determined by the glucose oxidase method (Hitachi 911 analyser, Boehringer Mannheim GmbH, Mannheim, Germany). The IMx insulin assay does not show any cross reactivity with proinsulin (<0.005%). Plasma ACTH and serum cortisol were measured by double antibody radioimmunoassay (RIA, Nichols Institute Diagnostics, San Juan Capistrano, California, USA) and solid phase RIA (Diagnostic Products Corp., Los Angeles, California, USA), respectively as previously described.<sup>27</sup> The sensitivity and interassay coefficients of variation of leptin, ACTH, cortisol, FT4, insulin, and glucose were: 0.05 ng/ml, 3.5% at 2.2 ng/ml; 0.22 pmol/l, 5.2% at 7.8 pmol/l; 5.5 nmol/l, 4.2% at 407 nmol/l; 1.3 pmol/l, 2.9% at 13 pmol/l; 7.2 pmol/l, 5.6% at 38 pmol/l; and 0.2 mmol/l, 1.7% at 5.0 mmol/l, respectively. The plasma/serum volume required for hormonal analysis was: 50 µl, 200 µl, 200 µl, 200 µl, 180 µl, and 20 µl, respectively. The umbilical cord blood TSH was routinely measured by the Hong Kong Government Laboratory as part of the territory wide neonatal screening for congenital hypothyroidism. Leptin and metabolic hormones were analysed by the laboratory in a blinded fashion.

#### DATA COLLECTION

Anthropometric measurements including body weight and length were recorded at birth and BMI was calculated as body weight (kg)/square of length (m<sup>2</sup>).<sup>28</sup> Body length was measured by the Harpenden infantometer. Table 1 presents demographic and clinical data of the study population.

#### ETHICAL APPROVAL

Ethical approval of the study was obtained from the Clinical Research Ethical Committee of the Chinese University of Hong Kong. Informed parental consent was obtained for each patient before commencement of the test.

Table 2 Blood hormone and plasma glucose concentrations on the first day of life

	Group C (n = 43)	Group D (n = 40)	Group I (n = 31)
Age of first blood sample (h)	26.8 (6.4)	26.8 (7.0)	27.3 (8.9)
Leptin (ng/ml)	1.58 (0.47–6.33)	1.17 (0.50–2.05)	2.13 (0.58–9.04)
ACTH (pmol/l)	8.3 (4.9–18.6)	8.1 (4.8–15.2)	10.1 (6.1–16.5)
Cortisol (nmol/l)	308 (206–406)	264 (145–387)	340 (217–564)
Insulin (pmol/ml)†	24.4 (7.2–53.1)	32.7 (20.3–54.9)	56.0 (36.6–86.8)
Glucose (mmol/l)	4.1 (3.7–4.7)	4.4 (3.7–5.0)	4.2 (3.6–5.3)
Insulin:glucose ratio*	6.4 (2.2–10.8)	8.8 (4.4–13.4)	13.0 (8.2–23.0)
FT4 (pmol/l)	28.2 (22.6–33.3)	29.4 (24.8–34.1)	29.0 (25.2–33.2)
Cord TSH (mIU/l)	4.9 (3.9–7.3)	5.5 (4.2–7.2)	5.3 (3.6–9.1)

Results are expressed as mean (SD) or median (interquartile range).

\* $p < 0.002$ ; † $p < 0.001$ .

Table 3 Significant correlations between different metabolic hormones and clinical parameters

	Correlation (r)	p value
Leptin		
Insulin	0.26	< 0.01
Insulin:glucose ratio	0.28	< 0.005
Maternal HbA <sub>1c</sub> (groups D and I)	0.39	< 0.001
Placenta weight	0.30	< 0.001
Birth weight	0.54	< 0.001
Body length	0.34	< 0.001
BMI	0.53	< 0.001
Insulin		
Leptin	0.26	< 0.01
Glucose	0.22	< 0.05
Placental weight	0.25	< 0.01
Exogenous insulin dose (group I)	0.58	< 0.001
Maternal HbA <sub>1c</sub> (groups D and I)		
Leptin	0.39	< 0.001
Insulin:glucose ratio		
Leptin	0.28	< 0.005
Placental weight	0.24	< 0.05
Exogenous insulin dose (group I)	0.48	< 0.001
ACTH		
Cortisol	0.44	< 0.001

#### STATISTICAL ANALYSIS

The clinical and anthropometric parameters of the study populations were expressed as mean (SD). Apgar scores, umbilical arterial cord blood parameters, serum leptin, and other metabolic hormone concentrations followed a non-Gaussian distribution and were expressed as median (interquartile range). One way ANOVA and Kruskal–Wallis tests with post hoc pairwise multiple comparisons were used to compare the parametric and non-parametric parameters, respectively between the three groups. Spearman's correlation coefficient was used to evaluate the inter-relations between different metabolic hormones, and the relations of the metabolic hormones to birth weight, BMI, and other demographic or clinical parameters. The Mann–Whitney U test was also used to assess possible differences between various metabolic hormones and sex. Statistically significant parameters associated with leptin were further subjected to multivariate stepwise regression analysis. Logarithmic transformation of metabolic hormone concentrations was necessary as these concentrations were not normally distributed. Statistical tests were performed by SPSS for Windows (Release 9.0, SPSS Inc., Chicago, Illinois) and BMDP/Dynamic (Release 7, BMDP, Statistical Software Inc., Los Angeles, California). The level of significance was set at 5% in all comparisons.

#### Results

Table 1 summarises the clinical characteristics of the study population. Maternal HbA<sub>1c</sub> was

significantly higher in group I than in group D ( $p < 0.05$ ), but none of the other parameters listed in the table differed significantly between the three groups.

Table 2 summarises the results of the metabolic hormones and plasma glucose. No significant difference in serum leptin was observed between the three groups. Serum insulin was significantly higher in group I than in group C; and insulin:glucose ratio was significantly higher in group I than in groups C and D. In addition, there were significant correlations between serum leptin and cortisol in group D ( $p < 0.05$ ,  $r = 0.36$ ).

The results of the study population were then pooled and analysed. Table 3 summarises significant associations between different metabolic hormones and clinical parameters. Serum leptin was found to correlate significantly with serum insulin, insulin:glucose ratio, birth weight, body length, BMI, placenta weight, and maternal HbA<sub>1c</sub> (groups D and I). Female infants had significantly higher serum leptin concentration than male infants ( $p < 0.002$ ). When these parameters (maternal HbA<sub>1c</sub> excluded) were subjected to multivariate stepwise regression analysis, all except placenta weight and body length remained significantly correlated with serum leptin.

#### Discussion

This study investigated the inter-relations between serum leptin and other major metabolic hormones in newborns of diabetic and non-diabetic mothers. Although it has been shown in adults that leptin is closely associated with insulin,<sup>29–34</sup> a similar relation has not been consistently shown in newborn infants. Most newborn studies have concentrated on the association between serum leptin and anthropometric parameters at birth.<sup>19–23</sup> Very few studies have investigated the relation between serum leptin and insulin,<sup>22 24 35 36</sup> and to our knowledge, only one has shown a significant connection between serum leptin and insulin in newborn infants.<sup>24</sup> Moreover, the influence of maternal diabetes on leptin, and the interaction of leptin with other major metabolic pathways such as the pituitary–thyroid and HPA axis in newborns are not yet fully elucidated. Hence, we sought to determine whether an “adiposular” or other leptin related hormonal axis exists in term newborns and whether such relations are affected by maternal diabetes.

Our results did not show a significant difference in serum leptin between term infants born to diabetic and non-diabetic mothers. However, it is worth noting that infants born to mothers requiring insulin treatment (group I) had the highest median serum leptin concentrations (table 2). The wide variation in serum leptin and the relatively small sample size in individual groups might have contributed to these insignificant differences. More importantly, significant associations between serum leptin and insulin, insulin:glucose ratio and maternal HbA<sub>1c</sub> support the hypothesis that a functional “adiposular axis” might exist in term newborns. It is most likely that excess

maternal glucose crosses the placenta and stimulates an increase in insulin production of the fetus. Fetal insulin up regulates the *ob* gene expression and induces leptin production by the adipocytes. Leptin functions as a counter regulatory hormone and inhibits insulin production by activating the ATP sensitive potassium channels in the pancreatic  $\beta$  cells.<sup>31</sup> This negative feedback mechanism has been extensively studied in rodents both *in vivo* and *in vitro*,<sup>37,38</sup> and has also been observed in human adults after prolonged administration of insulin.<sup>34</sup> Three studies have investigated the effect of maternal diabetes on newborn serum leptin and showed that infants of diabetic mothers, especially those on insulin treatment, have higher umbilical cord serum leptin concentration.<sup>36,39,40</sup> None of these studies, however, showed a significant correlation between serum leptin and insulin or insulin:glucose ratio.<sup>39,40</sup> Only the subgroup analysis by Maffei *et al* revealed a significant association between serum leptin and insulin, C peptide, and maternal HbA<sub>1c</sub> in eight neonates born to insulin dependent diabetic mothers.<sup>36</sup> In addition, a significant association between maternal HbA<sub>1c</sub> and circulating leptin of our studied infants suggests that the regulation of serum leptin concentrations in term newborns may be influenced by the clinical control of maternal diabetes.

Our subgroup analysis also showed a significant association between serum leptin and cortisol in group D infants. As pharmacological doses of dexamethasone have been shown to induce leptin production<sup>29,40-42</sup> and insulin could block the dexamethasone stimulated increase in leptin release,<sup>43</sup> our observation suggests the possibility that serum leptin in term newborns may be influenced by circulating cortisol. However, it would be unlikely that cortisol could act directly by stimulating *ob* gene transcription,<sup>29</sup> but cortisol might interact indirectly with other hormonal systems such as the insulin/glucose pathway in affecting serum leptin.

Our study also confirms the consistent relation between serum leptin and birth weight or BMI.<sup>19-21,24,39,40</sup> Again, a sex difference in serum leptin concentration was observed. Female infants had significantly higher serum leptin concentrations than male infants suggesting that sexual dimorphism exists in utero.<sup>19,24</sup>

In summary, our results did not show a significant difference in serum leptin between infants born to diabetic and non-diabetic mothers, though infants born to mothers requiring insulin treatment had the highest median serum leptin concentrations. There were significant associations between serum leptin and insulin or insulin:glucose ratio, supporting the hypothesis that a functional adiposular axis might be present in the term newborns. In addition, a significant correlation between maternal HbA<sub>1c</sub> and serum leptin in the studied infants suggests that the clinical control of maternal diabetes could influence the concentration of circulating leptin in these infants. Accumulating evidence indicates that maternal diabetes could affect fetal leptin

### Key message

An active "adiposular axis" is likely to exist in term newborns. The clinical control of maternal diabetes may affect the regulation of serum leptin and possibly may also influence the growth of the fetus in utero

metabolism.<sup>39,40</sup> Although a definite relation could not be established between leptin and cortisol at this stage, the present data suggest the possibility that serum leptin in newborns may be influenced by circulating cortisol. Despite the associations between leptin and various metabolic hormones, further experimental and clinical investigations are required to determine their causal relations and their physiological role in controlling fetal and neonatal growth at this crucial stage of human development.

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