Metabolic hormones profile in 2 weeks old healthy infants of diabetic mothers

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ABSTRACT

Objectives: To determine the concentration of plasma leptin and other metabolic hormones in offspring of diabetic and none diabetic mothers after 2 weeks of age. The relationship between leptin and metabolic hormones was also investigated.

Methods: Included in the study were 79 newborns from the Neonatal Unit at King Abdul-Aziz University Hospital, Jeddah, Kingdom of Saudi Arabia from January 2004-January 2005. The newborns were categorized into 3 main groups: the control group, consisting of 32 infants of non-diabetic mothers; the gestational diabetes mothers (GDM) group, consisting of 26 infants; and the frank diabetic mothers (FDM), consisting of 21 infants. Infants of diabetic mothers were further subdivided into those of dietary (d) or insulin (i) dependent mothers. Plasma leptin, insulin, cortisol and free thyroxin levels were measured by enzyme link immunosorbent assay.

Results: No significant difference in plasma leptin was observed between the studied groups. The GDM-d and FDM-d showed lower glucose versus controls ($p<0.001$ and $p<0.05$). There was significant correlation between leptin and glucose in the GDM group (r=0.18, $p< 0.05$) and with insulin in GDM-d on diet control (r=0.37, $p< 0.01$).

Conclusion: After 2 weeks of life, no difference in plasma leptin between infants of diabetic and non-diabetic mothers was observed, which may be important for the stimulation of feeding behavior and acquisition of energy homeostasis. Significant association between plasma leptin and insulin in offspring of GDM supports the hypothesis that functional adipoinsular axis might exist in term newborns.

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In addition to its role as a storage depot for fat, adipose tissue also produces and secretes a number of hormones, collectively called adipocytokines. Those hormones are of importance in modulating metabolism and energy homeostasis. Leptin is a member of the adipocytokine family.[1] Leptin is detectable in cord blood as early as the second trimester, and its level in the cord blood increases from the middle of the third trimester towards term, in parallel with the development of fetal adipose tissue.[2] It had been found that cord blood leptin level positively correlated with fetal adiposity at birth.[3] However, the early appearance of this hormone during fetal development, and the recognition of the placenta as another source of leptin production, suggests that leptin is important in fetal growth, beyond its function as an indicator of fetal adipose tissue mass.[4]

Leptin functions as an afferent satiety signal from the peripheral fat mass toward the hypothalamus, and controls the basal metabolic rate and energy expenditure of the body.[5] Accumulating evidence suggests that leptin exerts its metabolic effects by

interacting with other hormonal systems.[6] Leptin has been reported to suppress the secretion of insulin from the pancreatic β cells,[7] and modulates the action of insulin on hepatocytes.[8] Meanwhile, insulin plays a crucial role in regulating fetal growth and may increase leptin levels. Increase fetal insulin and leptin levels have been associated with increased adiposity at birth.[9] Leptin also modifies the function of both the hypothalamic–pituitary–adrenal axis (HPA), and the sympathetic–adrenomedullary axis in controlling fat cell proliferation and body metabolic activity.[10] In both rodents and humans, there is a strict reciprocal diurnal association between serum leptin and cortisol concentrations.[11] In addition, adrenocorticotrophic hormone induced adrenal cortisol, aldosterone, and dehydroepiandrosterone secretion has been shown to be inhibited by leptin in a concentration dependent manner.[12]

As pregnancy is associated with profound changes in adipose tissue/lipid and hormonal metabolism such as increase in insulin, corticotrophin releasing hormone (CRH), cortisol, estrogens, and progesterone concentrations, it is important to understand the physiological role of leptin in newborns of diabetic and none diabetic mothers. In both healthy mothers and insulin dependent diabetic ones (type 1), the concentration of both maternal free and bind leptin increase during pregnancy. In addition, diabetic mothers have higher levels of soluble leptin receptor.[13] Together the above data suggest that insulin or altered glucose metabolism, or both may affect concentrations of free and bound leptin. Infants of diabetic mothers have higher cord blood leptin concentrations than infants of healthy mothers.[14] The infants of diabetic mothers (IODM) have a higher risk of complications in the neonatal period and at a later age.[15] The occurrence rate of postnatal disorders is strongly correlated to the level of diabetes control during pregnancy and genetic background.[16]

To date, most newborn studies have concentrated on the relation of leptin level on fetal growth and the anthropometric measurements at birth.[9] However, the effects of gestational diabetes (pregnancy induced hyperglycemia) and maternal insulin dependent diabetes on leptin and other metabolic hormones in newborns have not been systematically evaluated.[6]

This study was, therefore, undertaken to investigate the effect of maternal diabetes (gestational or frank diabetes mellitus) on plasma leptin in term newborns during the second week of life. The inter-relations between plasma leptin and glucose and other metabolic hormones including insulin, insulin: glucose ratio (an index of glucose metabolism), cortisol and free thyroxin (FT4) in those infants were also assessed.

Methods. A total number of 79 term newborns (37–42 weeks gestational age) who were admitted to the Neonatal Unit at King Abdul-Aziz University Hospital, Jeddah, Kingdom of Saudi Arabia, from January 2004 till January 2005 were included in the study. The total number of births during this year was 4598 and the percentage of diabetic mothers per 1000 birth was 17.2/1000. All infants of gestational diabetic mothers (GDM) and frank diabetic mothers (FDM) were enrolled. Maternal gestational diabetes was defined as a venous fasting glucose concentration of >5.5 mol/L or of >8.0 mmol/L 2 hours after a 75 g oral glucose load or both.[17] Maternal FDM was defined as mothers having pregestational diabetes that continued through gestation. The newborns included in the study were subdivided into 3 main groups according to the health state of the mother. The first group consisted of 32 healthy term newborns born to normal healthy mothers (HM) (control). Healthy mothers had normal blood glucose, insulin and lipid profiles. Second group consisted of 26 term infants born to mothers with GDM which were further subdivided into 2 subgroups, subgroup GDM-d (n=18) whose mothers were controlled by low calories diabetic diet (7.5 MJ/day), and subgroup GDM-i (n=8) whose mothers were on insulin treatment. The third group consisted of 21 term infants born to mothers with FDM, they were further subdivided into 2 groups, those on diet control (subgroup FDM-d, n=6) and those controlled by insulin (subgroup FDM-i, n=15). All neonates were clinically normal and well. All neonates with dysmorphic features, major congenital anomalies, intrauterine infections, organic disorders, or chromosomal defects were excluded. Infants born to mothers who received corticosteroid or other hormonal therapy during their pregnancies and those with eclampsia or pre-eclampsia history during pregnancy were also excluded. Clinical and anthropometric measurements including body weight, length, and the head circumference were recorded at birth and body mass index (BMI) was calculated as body weight (grams)/square of length (cm^2) using Euro growth soft ware.

The study was approved by the Ethical Medical Committee of the King Abdul-Aziz University Hospitals, Jeddah, Kingdom of Saudi Arabia. Written informed consent from the parents was obtained before participation.

Blood samples were collected in the morning between 8.00 and 11.00 when the infants were approximately 14 days of age (2 weeks). Blood was collected: 1. in a prechilled EDTA tubes for plasma leptin, insulin, cortisol and FT4; and 2. 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 were aliquot and stored at -20°C until analysis.

Plasma leptin were measured by enzyme linked immunosorbent assay (Diagnostic Systems Laboratories Inc., Texas, USA). Insulin, cortisol, FT4 were measured using electrochemiluminescence immunoassay (ECLIA) (Roche Elecsys 1010/2010 and Modular analytics E170, Roche Diagnostics GmbH, Mannheim, USA) and plasma glucose were determined by the glucose oxidase method using Hitachi 911 analyzer (Boehringer Mannheim GmbH, Mannheim, Germany). Leptin and metabolic hormones were analyzed by the laboratory in a blinded fashion. The complete blood picture, serum calcium, magnesium and total bilirubin were measured routinely in the Central Clinical Pathology Laboratory of King Abdul-Aziz University Hospital.

Of leptin, sensitivity (lower detection limit) was 0.05 ng/ml, interassay and intraassay coefficients of variation were, 4.9% at 4.7 ng/ml and 4.4% at 4.8 ng/ml. Analytic sensitivity was for cortisol <1.00 nmol/L (<0.036 ìg/dL), for FT4 0.30 pmol/L (0.023 ng/dL) and for insulin 0.20 ìu/ml (1.39 pmol/L). The umbilical cord blood thyroid stimulating hormone was routinely measured by the Saudi Arabia Government Laboratory as part of the territory wide neonatal screening for congenital hypothyroidism.

Statistical analysis. The anthropometric parameters and levels of hormones of the studied populations were expressed as mean (SD). The serum leptin and other metabolic hormone concentrations followed a non-Gaussian distribution. One way Analysis of Variance and the Kruskal–Wallis tests were used to compare the parametric parameters, respectively between the 3 groups. Spearman's correlation coefficient was used to evaluate the inter-relations between leptin and glucose and different metabolic hormones and anthropometric measurement. Statistical tests were performed using the Statistical Package for Social Sciences for Windows (Release 10.0, SPSS Inc., Chicago, Illinois). The level of significance was set at $p < 0.05$ in all comparisons.

Results. **Table 1** summarizes the clinical characteristics of the study population. Seventy-nine newborn involved in this study, 32 newborns (male: female, 13:19) were off springs of healthy mothers, 26 newborn (male: female, 16:10) were off springs of GDM and 21 newborns (male: female, 11:10) were to FDM. Gestation age, body weight, length, head circumference, BMI, hemoglobin, platelets and serum

calcium level did not differ significantly between the 3 studied groups. Meanwhile, white blood cell counts were significantly lower in GDM compared to FDM but still within the normal range. Magnesium were significantly lower in both group: GDM (0.78 ± 0.08) and FDM (0.80 ± 0.13), ($p < 0.01$) compared to control group (0.87 ± 0.08 mmol/L) ($p < 0.05$) but also still within normal range. Total bilirubin levels were higher than reference range in all the studied groups. Total bilirubin was significantly elevated in GDM group (116.40 ± 72.86) compared to FDM group (59.53 ± 57.03 μmol/L), ($p < 0.01$).

Table 2 summarizes the results of blood glucose and different metabolic hormones in different subgroups of infant of diabetic mothers. Plasma glucose was significantly lower in GDM-d (2.82 ± 0.92) and FDM-d groups (3.20 ± 0.32), ($p < 0.001$) compared to controls (4.03 ± 0.35 mmol/L), ($p < 0.05$). There were no statistical significant differences found between the studied subgroups.

In addition, there were significant positive correlations between plasma leptin and plasma glucose in GDM ($p < 0.05$, r=0.18) and between leptin and insulin in GDM-d group ($p < 0.01$, r=0.37).

Discussion. Most previous newborn studies concentrated on the association between serum leptin and anthropometric parameters at birth.[18] Very few studies have investigated the relation between serum leptin and insulin[3,9,19,20] and to our knowledge; only 2 studies had shown a significant correlation between serum leptin and insulin in newborn infants at birth.[6,8] Moreover, the influence of maternal diabetes on leptin, and the interaction of leptin with other major metabolic hormones such as that secreted by thyroid and adrenal glands in newborns are not yet fully elucidated. Hence, we sought to determine whether an "adipoinsular" or other leptin related hormonal axis exists in term newborns after 2 weeks of life and whether such relations are affected by maternal diabetes.

Data obtain from this study did not show any significant difference in plasma leptin, insulin between term infants born to diabetic on diet or insulin controls and healthy mothers after 2 weeks of life. However, it is worth noting that infants born to GDM mothers requiring insulin treatment had highest mean serum leptin concentrations. The wide variation in serum leptin and relatively small sample size in individual groups might have contributed to these insignificant differences. More importantly, a significant positive association was found between plasma leptin and insulin in whole group of infants of GDM mothers, support the hypothesis that a functional "adipoinsular

Table 1 - Data of infants born to either mothers with gestational diabetes mellitus (GDM), frank diabetes mellitus (FDM) or healthy mothers (HM).

Variable	Reference range	Infants of HM (n=32)	Infants of GDM mothers (n=26)	Infants of FDM mothers (n=21)
Male/Female		13/19	16/10	11/10
Mode of delivery (n) (Normal: caesarean section)		24:8	20:6	10:11
Gestational age (weeks) range		37.28 ± 0.73 (37-40)	37.38 ± 0.64 (37-39) *p*>0.05	37.48 ± 0.60 (37-39) *p*>0.05
Birth weights (kg) range		3.30 ± 0.59 (2-5.5)	3.64 ± 0.69 (2.20-6.08) *p*>0.05	3.18 ± 0.86 (1.80-5) *p*>0.05
Length (cm) range		53.28 ± 3.44 (45-59)	54.65 ± 3.09 (49-60) *p*>0.05	51.52 ± 2.75 (47-58) *p*>0.05
Head circumference (cm) range		34.56 ± 1.36 (32-38)	35.35 ± 1.29 (33-39) *p*>0.05	34.85 ± 3.41 (22.50-39) *p*>0.05
Body mass index (kg/m^2) range		11.57 ± 1.50 (9.52-16.42)	12.12 ± 1.68 (9.16-16.89) *p*>0.05	11.85 ± 2.64 (8.15-17.01) *p*>0.05
White blood count (Ku/L) range	(9-30)	15.68 ± 3.39 (8.2-20)	12.03 ± 3.58 (7.10-19) *p*<0.01	13.25 ± 6.45 (4.80-31) *p*>0.05
Hemoglobin (gram/ml) range	(16.5-21.5)	17.70 ± 1.73 (12.60-20.50)	17.57 ± 2.81 (11.50-21.80) *p*>0.05	18.36 ± 1.93 (15.20-21.80) *p*>0.05
Platelets (K/μL) range	(150-450)	273.80 ± 72.33 (134.0-406)	231.70 ± 81.63 (113-519) *p*>0.05	278.00 ± 119.50 (49-565) *p*>0.05
Calcium (mmol/L) range	(2.12-2.52)	2.35 ± 0.29 (1.95-2.70)	2.21 ± 015 (1.97-2.46) *p*>0.05	2.14 ± 0.45 (0.86-2.60) *p*>0.05
Magnesium (mmol/L) range	(0.74-0.99)	0.87 ± 0.08 (0.75-0.99)	0.78 ± 0.08 (0.65-0.90) *p*<0.01	0.80 ± 0.13 (0.50-1.10) *p*<0.05
Total bilirubin (μmol/L) range	(0-17)	59.53 ± 57.03 (3.40-169)	116.40 ± 72.86 (13.00-364) *p*<0.01	69.50 ± 67.33 (6.40-178) *p*>0.05

Values are mean (SD) unless otherwise indicated
p=significance versus control

Table 2 - Plasma concentrations of glucose (mmol/L), insulin (uU/mL), insulin: glucose ratio, free thyroxin (FT4) (*pmol/mL*), cortisol (nmol/L) and total leptin (ng/L), in infants born to gestational diabetic mothers (GDM) on diet or insulin control, frank diabetic mothers (FDM) on diet or insulin control or healthy mothers (HM).

Measured parameters	Reference range	Infants of HM (n=32)	Infants of GDM mothers (n=26)		Infants of FDM mothers (n=21)	
			On diet (n=18)	On insulin (n=8)	On diet (n=6)	On insulin (n=15)
Glucose (mmol/L) Range	(3.9-6.7)	4.03 ± 0.35 (3.20-4.7)	2.82 ± 0.92 (1.60-4.40) $p<0.001$	3.23 ± 1.00 (1.70-4.20) $p>0.05$ $^1p>0.05$	3.20 ± 0.32 (2.90-3.60) $p<0.05$ $^2p>0.05$	3.69 ± 1.68 (1.20-9.20) $p>0.05$ $^1p>0.05$ $^2p>0.05$
Insulin (pmol/mL) Range	(2.6-24.9)	4.65 ± 4.72 (0.20-21.65)	6.23 ± 5.98 (0.20-24.56) $p>0.05$	7.84 ± 5.45 (0.53-16.05) $p>0.05$ $^1p>0.05$	4.02 ± 1.90 (0.49-5.86) $p>0.05$ $^2p>0.05$	8.58 ± 11.63 (1.26-40.11) $p>0.05$ $^1p>0.05$ $^2p>0.05$
Insulin: glucose ratio Range	-	1.15 ± 1.15 (0.05-5.04)	2.56 ± 2.73 (0.07-10.19) $p>0.05$	2.91 ± 2.71 (0.13-8.89) $p>0.05$ $^1p>0.05$	1.30 ± 0.65 (0.14-1.89) $p>0.05$ $^2p>0.05$	2.98 ± 4.05 (0.20-11.80) $p>0.05$ $^1p>0.05$ $^2p>0.05$
FT4 (pmol/L) Range	(8.0-18.1)[42]	33.37 ± 12.03 (3.57-58.78)	30.70 ± 9.36 (20.92- 46.84) $p>0.05$	29.89 ± 4.59 (24.33- 36.88) $p>0.05$ $^1p>0.05$	33.23 ± 12.24 (18.87- 46.93) $p>0.05$ $^2p>0.05$	34.77 ± 7.13 (20.68- 48.41) $p>0.05$ $^1p>0.05$ $^2p>0.05$
Cortisol (nmol/L) Range	(298-348)[43]	248.4 ± 187.6 (20.02-910.50)	178.40 ± 142.10 (10.07- 524.20) $p>0.05$	227.40 ± 306.90 (35.14- 906.10) $p>0.05$ $^1p>0.05$	197.90 ± 177.10 (38.83- 485.60) $p>0.05$ $^2p>0.05$	257.70 ± 228.20 (15.26- 700.10) $p>0.05$ $^1p>0.05$ $^2p>0.05$
Leptin (ng/mL) Range	-	6.68 ± 7.90 (0.23-30.24)	8.92 ± 8.30 (1.36- 30.30) $p>0.05$	15.40 ± 11.01 (2.63- 30.90) $p>0.05$ $^1p>0.05$	7.62 ± 4.92 (0.68- 14.33) $p>0.05$ $^2p>0.05$	10.59 ± 8.85 (0.83- 30.96) $p>0.05$ $^1p>0.05$ $^2p>0.05$

Values are mean (SD) unless otherwise indicated
P=significance versus control
^1P=significance infant of diabetic mother on diet control versus on insulin control
^2P=significance GDM versus FDM of the same control group either on diet or insulin control

axis" might exist in term newborns of infant of diabetic mother. These results might be explained by that, it is most likely that in partial controlled mothers excess maternal glucose crosses the placenta and stimulates an increase in insulin production of the fetus. Fetal insulin regulates up the ob gene expression and induces leptin production by adipocytes. Leptin functions as a counter regulatory hormone and inhibits insulin production by activating adenosine triphosphate (ATP) sensitive potassium channels in the pancreatic β cells.[21] This negative feedback mechanism has been extensively studied in rodents both in vivo and in vitro[22] and has also been observed in human adults after prolonged administration of insulin.[23] Three previous studies[21,24,25] had 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. None of these studies,[24,25] however, showed a significant correlation between serum leptin and insulin or insulin: glucose ratio. Only the subgroup analysis by Maffei et al[21] revealed a significant association between serum leptin and insulin in 8 neonates born to insulin dependent

diabetic mothers. On contrary to our results, Ng et al[6] found a significant elevation of serum insulin levels and insulin: glucose ratios in infants born to mothers depend on insulin for control of diabetes compared to infants of healthy mothers. The difference of their results from ours can be explained by that they carried their study immediately after birth meanwhile ours carried after 2 weeks of life. In vivo insulin increases total leptin concentration, which appears to be mediated at the adipocyte level, at least in part, by insulin stimulated glucose uptake and metabolism[26,27] As recently reviewed,[26,27] the interaction between leptin and insulin secretion is complex and in many aspects it remains to be clarified. Maffei et al[21] reported that although insulin-dependent diabetic mothers (IDDM) were clinically well controlled they still gave birth to newborns with significantly higher levels of leptin and insulin at birth. Moderate increase in circulating leptin was recently reported to decrease considerably the expression of the gene for its synthesis in adipose tissue.[28] Whether such a mechanism could affect later regulation of these hormones remains to be explored.

In this respect, Schubring et al[3] reported that leptin levels were high in the fetus but decline rapidly and dramatically after birth in healthy neonates. Also, a recent research study showed that, at the first day of age, the differences in leptin concentrations between infants of healthy mothers and those with either GDM or FDM were no longer significant.[6] The researchers hypothesize that high leptin levels could represent an important feed back indicator of nutrient supply and subsequently for adipose tissue status during late gestation and at birth. The rapid decrease of leptin levels after birth could be mediated by hormonal changes after birth, example, the fall in insulin or cortisol levels. The rapid fall of leptin levels might be important for the stimulation of feeding behavior and the acquisition of energy homeostasis in the neonate. Also, immediately after birth, the newborn is able to react to cold stress by an increase of thermogenesis via oxygen uptake.[29] High leptin levels in the newborn may stimulate the sympathetic nervous system which triggers a beta-3-adrenoceptor response mediating nonshivering thermogenesis in brown adipose tissue. The sympathetic stimulation in turn inhibits leptin expression.[30] On the other hand, and probably most likely, the rapid decline of leptin serum concentrations could also be due to the fact that the fetoplacental unit is cut off during birth; since the placenta is a rich source of leptin then sudden removal of the placenta might cause a fall in neonatal serum leptin concentrations.[31] Therefore, this result favors the idea

that the differences in leptin concentration observed at birth is a transient phenomenon, which normalizes as fetal adaptation proceeds.[32]

In contrary to others,[9,24,25] this study showed no correlation between plasma leptin and birth weight or BMI in both infants of healthy and diabetic mothers. However, a disturbed BMI and leptin relationship could play a role in the higher incidence of obesity in the offspring of diabetic mothers.

The results of the present study showed a significant hypoglycemia in infants of GDM and FDM on diet control after 2 weeks of life compared to infants of healthy mothers. Infants of GDM had a significant positive correlation between plasma leptin and glucose. On contrary to our study, Berg[33] reported more glycemia in off springs of diabetic mother than control group of infants of healthy mothers with similarity of plasma insulin levels in both groups. The explanation of different sensitivity in insulin response to glucose stimulation should also be considered. Leptin has an anti-diabetic effect in rodents[33] achieved through both an insulin-independent and an insulin-sensitizing mechanism. It was confirmed in humans that increased plasma leptin in the offspring of DM could also represent a compensatory mechanism in a preclinical disturbance of glucose metabolism. This possibility is supported by the analysis of a healthy population which revealed a negative correlation between plasma leptin and the measurement of insulin sensitivity[34] and the report that an insulin-resistant phenotype is associated with high serum leptin levels in the offspring of patients with NIDDM.[35] The study by Vauhkonen et al[36] found a defective insulin secretion in non-diabetic adult offspring of patients with type 2 diabetes mellitus and believe that latent autoimmune diabetes mellitus is a familial disease involving gene defects leading to a progressive beta-cell destruction.[37] On the other hand, Simmons[38] hypothesized that dysregulation within the adipoinsular axis induced by fetal hyperglycemia in mothers with GDM may be the trigger for permanent resetting of a positive feedback loop that leads to hyperinsulinemia and further adiposity and hyperleptinemia during postnatal life.

In contrary to our study, Ng et al[6] found a significant association between serum leptin and cortisol in infants of GDM mothers. It has been reported that pharmacological doses of dexamethasone induce leptin production,[25,39] and insulin could block the dexamethasone stimulating effect on leptin release.[40] However, it would be unlikely that cortisol could act directly by stimulating the ob gene transcription[41] but cortisol might interact indirectly with other hormonal

systems such as the insulin/glucose pathway in affecting serum leptin.[6]

In conclusion, leptin and insulin levels are normal in infants of both healthy and diabetic mothers after 2 weeks of age. However, there is hypoglycemia in infants of GDM and FDM on diet controls, which may indicate a disturbance in glucose metabolism in those infants. The rapid decline in leptin levels after birth might be an important stimulus for the onset of feeding or energy uptake. There were significant associations between serum leptin and insulin in infants of GDM, supporting the hypothesis that a functional adipoinsular axis might be present in term newborns at 2 week age. Despite there were no associations between leptin and cortisol or thyroxin hormones level, further experimental and clinical investigations with large number of participants are required to determine their physiological role in controlling fetal and neonatal growth at this crucial stage of human development in high risk pregnancies.

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