Insulin and Leptin Levels in Appropriate-for-Gestational-Age Infants of Diabetic Mother

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Abstract

Objective: Intensified management of gestational diabetes mellitus can normalize birth weight. However, it is still unknown whether intrauterine exposure to maternal diabetes is a risk factor for changing hormone levels involved in the development of insulin resistance in these infants. We compared insulin and leptin levels in appropriate for gestational age (AGA) infants of diabetic and non diabetic mothers.

Methods: We performed a cross-sectional study in the department of Neonatology of the Hospital of Gynecology-Pediatrics, in Leon, Mexico. We evaluated 182 full term AGA newborns (86 infants of diabetic and 96 of non-diabetic mothers). A venous blood sample was taken from cord blood immediately after the separation of the placenta and glucose, insulin and leptin levels were measured. In all diabetic mothers HbA1c was also evaluated immediately post-partum.

Findings: Leptin, insulin and insulin resistance index were significantly higher in infants of diabetic mothers. Leptin levels were positive correlated with insulin, parents' body mass index and age in the entire group. In infants of diabetic mothers only insulin levels showed a significantly correlation, whereas in those of non-diabetic mothers only mothers' age was significantly correlated with leptin levels.

Conclusion: AGA infants of diabetic mothers showed higher leptin, insulin levels and insulin resistance index than those of non-diabetic mothers.

Introduction

The most common and significant neonatal complication clearly associated with gestational diabetes mellitus (GDM) is macrosomia[1], and intensified management of the disease can normalize birth weight[2]. However, it is still unknown whether intrauterine exposure to maternal diabetes is a risk factor for the subsequent development of insulin resistance in appropriate-for-gestational age (AGA) infants.

Leptin, the product of the ob gene, specially secreted from adipose tissue, has brought new insight into the regulation of the energy balance[3]. A relationship has been observed between circulating levels of leptin and the intrauterine growth pattern[4] and between leptin and insulin levels[5]. Furthermore, leptin has been hypothe-

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sized as an insulin counter-regulatory hormone that might be involved not only in obesity and energy partition, but also in the brain development and in the pathogenesis of diabetes[6-7]. Because T2DM and obesity are highly prevalent in Mexican population[8], the aim of this study was to compare insulin resistance and serum leptin levels in AGA infants of diabetic (IDM) and non diabetic mothers (INDM).

Subjects and Methods

This cross sectional study was performed in the Department of Neonatology of the Hospital of Gynecology-Pediatrics, in Leon, Mexico. 182 AGA infants were studied (86 IDM and 96 INDM). The local ethical committee approved the study, and informed consent was obtained from all women. All diabetic women received insulin treatment during pregnancy, and after reclassification we found that 13 (15.1%) corresponded to type 2 DM. All other patients were classified as gestational diabetes.

An infant was considered AGA according to established criteria[9].

A 15 ml venous blood sample was taken from cord blood immediately after the separation of the placenta. Glucose levels were measured during the first hour and serum was frozen at-70°C until we measured leptin and insulin levels (time no longer than 6 months). In all diabetic mothers HbA1c was also measured at the same time that glucose levels.

Blood glucose concentration was measured using the glucose oxidase method by a Vitros 250 analyzer (Ortho Clinical Diagnostics; Johnson-Johnson, Raritan NJ). Serum Insulin was measured with a sold phase radioimmunoassay (Diagnostics Products Corporation. Los Angeles, CA). The intra-assay coefficient of variation (CV) was 5.1%, the interassay CV was 7.1%, and the sensitivity limit was 1.2μU/ml. Serum leptin was measured by inmunoradiometric assay (Diagnostic Systems Laboratories, Inc.,Webster, TX). The intra-assay CV was 3.4%, interassay CV 5.6%, and the sensitivity limit was 0.10ng/ml. Insulin resistance was calculated according to the following formula[10].

Glucose (mmol/L) × insulin (μUI/mL)/22.5

Glycosylated hemoglobin was measured by high-performance liquid chromatography using the D-10 TM Hemoglobin Testing System (Bio-Rad Laboratories, CA, USA).

Descriptive statistics was performed to evaluate variables distribution using the Kilmogorov-Smirnov test for normality. In case of skewed distribution data are showed as mean (95% confidence interval). Unpaired Student's t test was performed to compare insulin, leptin, glucose, insulin resistance, and anthropometric measurements between groups of infants and their parents. Because of non normal distribution of some variables (insulin, leptin and glucose levels), Pearson´s correlation analysis was used to evaluate associations between leptin levels and weight, height, glucose, and insulin levels. All data were analyzed using the STATISTICS software version 6.0 (Statsoft Inc. Tulsa OK, USA).

Findings

Diabetic mothers were older and showed higher BMI than the group of INDM. However, they gained less weight than non diabetic mothers. IDM showed similar birthweight but lower gestational age than INDM without difference in height (Table 1).

Leptin, insulin and HOMA-IR were significantly higher in IDM than in INDM (Table 2). Leptin levels were positive correlated with insulin levels, parents' BMI and their age in the entire group. In IDM only insulin levels showed a significant correlation with leptin levels, whereas in those INDM only mothers' age was correlated with leptin levels (Table 3).

In the reclassification of GDM we found that 13(15.1%) corresponded to T2DM. However, no difference was found in the biochemical variables evaluated when we compared infants born from mothers with GDM or T2DM (data not showed).

Because it has been considered that HbA1c should be below 6% in the third trimester for prevention of congenital malformations and

Table 1: Demographic data of infants of diabetic and non-diabetic mothers

Variables	INDM n= 96	IDM n= 86	*P* value
Mothers' age (yr) [mean (SD)]	26.8 (5.4)	31.3(5.6)	<0.0001
Nº of pregnancies (range)	2 (1-7)	2 (1-7)	0.1
Mothers' BMI [mean (SD)]	24.7 (4.3)	30.2 (4.7)	<0.0001
Fathers' BMI [mean (SD)]	24.9 (2.7)	27.3 (3.4)	<0.0001
Mothers' weight gain (kg) [mean (SD)]	10.7 (3.8)	8.6 (3.3)	<0.0002
Birth weight (g) [mean (SD)]	3268 (315)	3372 (375)	0.06
Height (cm) [mean (SD)]	50.9 (2.0)	50.5 (2.4)	0.3
Gestational age (weeks) [mean (SD)]	39.1 (1.1)	38.1 (0.81)	0.0001
Sex (Male/Female)	44/52	35/51	0.8

IDM: Infants of Diabetic Mothers; INDM: Infant Non-diabetic Mothers

macrosomia[11], we compared infants according to HbA1c ≤6% (n=49) or >6%(n=37), and found that infants' height was lower in those with the higher HbA1c levels (49.6 vs. 51.2 cm; *P*=0.002 respectively), whereas no difference was found in their parents' height. Furthermore, no difference was found in birthweight, glucose or leptin levels between these groups.

Discussion

Despite the selection of only AGA infants, we found that gestational age was lower in IDM than in INDM. Furthermore, IDM with HbA1c >6% in the third trimester showed lower height but similar birth weight when they were compared with those infants whose mothers showed better metabolic control. We also found higher insulin, HOMA-IR, and leptin levels in IDM than in AGA infants with no maternal diabetes mellitus despite mean HbA1c values of 6.2% that have been reported to prevent macrosomia[11]. Our results

are supported by the Summary and Recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus which reports that newborn infants of women with GDM have increased adiposity and reduced fat-free mass even if they are not macrosomic [12].

Excessive fetal growth remains an important perinatal concern in GDM. Maternal hyperglycemia continues to be viewed as the primary determinant of increased fetal growth via delivery of glucose to the fetus, which leads to fetal hyperinsulinemia. Other factors influencing growth include nutrients such as aminoacids and lipids, specific growth factors, placental function, and the fetal response to a given nutrient environment[12]. In addition to classic risk associated with macrosomic infants of women with GDM, there is increasing awareness of childhood risk for obesity, GDM, and T2DM[12].

There is now clear evidence that placental leptin is poorly released into the fetal circulation and that leptin synthesized by fetal adipose tissue can be taken as a marker of fetal adiposity[13].

In two other studies, exposure to GDM was

Table 2: Metabolic variables in AGA infants of diabetic and non-diabetic mothers

Variable	INDM [mean (⁹⁵%CI)] n=96	IDM [mean (⁹⁵%CI)] n=86	*P* Value
Glucose (mmol/L)	3.6 (3.2-3.9)	4.0 (3.6-4.2)	0.08
HbA1 (%)	-	6.2(5.9-6.5)	-
Insulin (μUI/ml)	3.7(2.1-6.9)	10.9(7.2-17.5)	0.0001
HOMA-IR	0.56(0.28-1.0)	1.7(1.1-3.7)	0.0001
Leptin (ng/ml)	18.9 (10.9-23.8)	26.9 (25.2-30.5)	<0.0001

AGA: Appropriate-for-Gestational Age; IDM: Infants of Diabetic Mothers; INDM: Infant Non-diabetic Mothers; CI: Confidence Interval; HbA1: Glycosylated hemoglobin; HOMA-IR: Homeostatic model assessment-insulin resistance

Table 3: Simple correlation analysis between leptin levels and clinical variables
in AGA infants of diabetic and non-diabetic mothers

Grop	Variable	r	P Value
All the group	HOMA-IR	0.14	0.06
	Mothers' BMI	0.24	0.001
	Fathers' BMI	0.14	0.05
	Mothers' age	0.32	0.001
	Insulin	0.20	0.007
Infants of Diabetic Mothers	Gestational age	0.19	0.07
	Insulin	0.28	0.008
Infants of Non-Diabetic Mothers	Mothers' age	0.23	0.02
	Insulin	0.17	0.09

AGA: Appropriate-for-Gestational Age; HOMA-IR: Homeostatic Model Aassessment-Insulin Resistance;
BMI: Body Mass Index

associated with both hyperleptinemia and hyperinsulinemia in large-for-gestational age (LGA) infants[14-15].

Also, a positive correlation between cord leptin and birth weight, all neonatal anthropometric parameters, and placental weight has been observed in AGA infants and confirmed recently by a meta-analysis[16-17]. This suggests that GDM may lead to increase insulin secretion and adiposity in the fetus, despite the rising plasma leptin concentration, which should be studied with a longitudinal follow-up whereas this study is cross-sectional. Fetal insulin, has been correlated with the amount of the circulating glucose from maternal origin and is directly correlated with mothers' diabetes control[12,18]. However, this relationship is modulated by parity and probably by putative genes for type 2 diabetes and obesity[12]. Insulin resistance has been reported in low-birth-weight infants[19] and in LGA IDM[20] but AGA infants are not usually included in these studies.

We also documented a correlation between insulin and leptin levels in IDMs. It is possible that high leptin levels reflect the hyperinsulinemia characteristic of the newborn IDM. The relationship between leptin and insulin is not well defined in humans. Hyperinsulinemia itself may be a cause of leptin resistance. As described, insulin and leptin use many of the same neurons, the same second messengers, and the same distal efferents to effect induction of satiety. In particular, both insulin and leptin receptors use the second messenger insulin receptor substrate 2 (IRS2), a low abundance message. Its absence

confers central nervous system (CNS) insulin resistance and obesity[21]. However, the role of IRS2 in the expression of leptin resistance is controversial[22-23]. It has moreover been shown that CNS insulin induces suppressor of cytokine signaling-3 (SOCS3), which inactivates the leptin receptor inhibits leptin signal transduction[24]. Although confirmation of animal studies are needed, these data suggest that CNS insulin resistance may be proximate cause of leptin resistance, promoting continued weight gain[25].

Furthermore, in fetus it has been reported that leptin functions as an essential factor for brain development, promoting formation of hypo-thalamic pathways that later conveys leptin signs to the brain region regulating food intake in the extrauterine life[7]. Induction of leptin resistance in utero may therefore be hypothesized as potential mechanisms for later development of obesity in offspring exposed to diabetes in utero as part of the phenomenon of hypothalamic obesity[26].

Parents' BMI was significantly higher in the group of IDM and related in the entire group with leptin levels of the newborn. Also leptin levels in the newborn are highly related with birth weight although higher leptin levels have been reported in females since birth[27]. We have not a specific explanation for this relationship, however, the association between father's weight and offspring birth weight has been previously found in other populations[28-29], and could be secondary to genetic regulation. Furthermore, supporting our results, it has been reported that birth weight and parental BMI predict overweight in children from mothers with gestational diabetes[30]. Our study

had some limitations. First, some mothers showed GDM and others type 2DM that could have different influence in insulin and leptin levels in the newborns. Also In the fourth paragraph of the discussion section, some comments were made about role of the leptin. These comments should be made with longitudinal follow-up whereas this study is cross-sectional.   However, we did not include type 1 diabetic mothers. Also gestational age was different between groups.

Conclusion

We found higher insulin resistance index, hyperinsulinism and hyperleptinemia in AGA IDMs compared with AGA infants of non diabetic mothers. It is possible that GDM despite adequate birth weight could lead leptin resistance in fetus and fetal overnutrition may result in a resetting of the adipoinsular axis leading to adiposity during childhood. Thus follow-up studies that involve large number of subjects, that start at birth and continue long term, are needed.

Acknowledgment

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