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# Effects of maternal glucose tolerance, pregnancy diet quality and neonatal insulinemia upon insulin resistance/sensitivity biomarkers in normoweight neonates

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Abstract

**Introduction:** Differences in neonatal insulin sensitivity/resistance markers due to the maternal impaired glucose tolerance (IGT) have not been tested. The Healthy Eating Index (HEI) score has been employed for evaluating pregnancy diet quality.

**Aims:** To study, the effect of neonatal insulinemia, maternal IGT and diet HEI score upon insulin sensitivity/resistance at birth.

**Methods:** 176 singleton, normoweight, full-term, Caucasian Spanish neonates, delivered without fetal distress whose mothers were screened for gestational IGT were studied. Quantitative Insulin Sensitivity Check Index (QUICKI) and Homeostatic Model Assessment (HOMA-IR) were calculated. Diet followed during the third month of pregnancy was recorded and the respective HEI score calculated in a sample of 29 mothers.

**Results:** As quartile for cord blood insulin levels increased, glucose, the insulin/cortisol ratio and HOMA-IR (all  $p < 0.001$ ) and IGF-I ( $p < 0.01$ ) increased while QUICKI and the glucose/insulin ratio (both  $p < 0.001$ ) and GH ( $p < 0.05$ ) decreased. Neonates from IGT mothers had higher insulin, HOMA-IR (both  $p < 0.01$ ) and insulin/cortisol ratio ( $p < 0.05$ ) and lower GH, QUICKI (both  $p < 0.01$ ) and glucose/insulin ratio ( $p < 0.05$ ) than their normal maternal glucose tolerance (NGT) counterparts. Neonatal insulinemia influences more than IGT on the insulin resistance/sensitivity markers at birth. Mothers of hyperinsulinemic neonates showed lower HEI scores ( $p < 0.05$ ).

**Conclusion:** A large percentage of full-term normoweight infants with hyperinsulinemia showed altered insulin resistance markers. Their mothers consumed low quality diets. Screening strategies focused on neonatal glycemia and insulinemia together with maternal nutritional assessment and advice during pregnancy should be considered.

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Key words: Biomarkers. HOMA-IR. Newborns. QUICKI. Insulin resistance. Pregnancy diet.

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## EFFECTOS DE LA TOLERANCIA A LA GLUCOSA Y CALIDAD DE LA DIETA MATERNA DURANTE EMBARAZO Y DE LOS NIVELES DE INSULINA AL NACIMIENTO SOBRE BIOMARCADORES DE RESISTENCIA/SENSIBILIDAD A LA INSULINA EN NEONATOS NORMOPESO

Resumen

**Introducción:** Los efectos de la disminución de la tolerancia a la glucosa (DTG) materna sobre la insulinorresistencia neonatal han sido poco estudiados. La puntuación del Índice de Alimentación Saludable (HEI) es útil para evaluar la calidad de la dieta en gestantes.

**Objetivos:** Estudiar la asociación de insulinemia neonatal y DTG materna con la resistencia/sensibilidad a la insulina al nacimiento y con el HEI materno.

**Métodos:** Se estudiaron 176 neonatos españoles, caucásicos, a término, normopeso, de embarazo único y sin distrés fetal, cuyas madres fueron cribadas para DTG en el embarazo. Se calcularon el Quantitative Insulin Sensitivity Check Index (QUICKI) y el Homeostatic Model Assessment (HOMA-IR). En 29 madres se estudió la dieta consumida durante el tercer mes de gestación y se calculó su respectivo HEI.

**Resultados:** Al aumentar el cuartil de insulina en sangre de cordón, aumentaron glucosa, cociente insulina/cortisol, HOMA-IR ( $p < 0,001$ ) e IGF-I ( $p < 0,01$ ), y disminuyeron QUICKI y cociente glucosa/insulina ( $p < 0,001$ ) y GH ( $p < 0,05$ ). Los neonatos de madres con DTG tuvieron mayores niveles de insulina, HOMA-IR ( $p < 0,01$ ) y cociente insulina/cortisol ( $p < 0,05$ ) y menores de GH, QUICKI ( $p < 0,01$ ) y el cociente glucosa/insulina ( $p < 0,05$ ) que los de madres con tolerancia a la glucosa normal. La insulinemia neonatal influye más en los marcadores de resistencia/sensibilidad a la insulina que la DTG. Las madres de los niños hiperinsulinémicos tuvieron HEI más bajos ( $p < 0,05$ ).

**Conclusiones:** Un gran porcentaje de neonatos con hiperinsulinemia muestran marcadores de insulinorresistencia alterados. Sus madres ingirieron dietas de baja calidad. Debe considerarse la necesidad del cribado de glucosa e insulina en estos neonatos y el asesoramiento y evaluación nutricional durante el embarazo.

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Palabras clave: Biomarcadores. Dieta en gestación. HOMA-IR. Neonatos. QUICKI. Insulinorresistencia.

## Abbreviations

BMI: Body Mass Index.  
GDM: Gestational Diabetes Mellitus.  
GH: Growth Hormone.  
HEI: Healthy Eating Index.  
HOMA: Homeostatic Model Assessment.  
IGF-I: Insulin-like Growth Factor I.  
IGT: Impaired Glucose Tolerance.  
MS: Metabolic Syndrome.  
NGT: Normal Glucose Tolerance.  
PI: Ponderal Index.  
Q1: 1<sup>st</sup> Quartile.  
Q4: 4<sup>th</sup> Quartile.  
QUICKI: Quantitative Insulin Sensitivity Check Index.

## Introduction

Insulin resistance describes an impaired biological response to insulin.<sup>1</sup> The “thrifty phenotype” hypothesis proposed by Hales and Barker<sup>2</sup> suggests that type-2 diabetes is due in part to the action of unknown factors that reduce fetal growth, islet  $\beta$ -cell ontogeny and insulin sensitivity during the prenatal period. Insulin fasting levels above 15  $\mu$ IU/mL have been considered to be a marker for hyperinsulinemia, and thus for insulin resistance.<sup>3</sup> Various studies have reported insulin levels at birth.<sup>4-7</sup>

The Homeostatic Model Assessment (HOMA) is widely employed as a marker of insulin resistance.<sup>8-10</sup> The Quantitative Insulin Sensitivity Check Index (QUICKI) is also considered useful for evaluating insulin sensitivity.<sup>11</sup> As neonates from mothers who screened positive for gestational diabetes mellitus (GDM) in the O’Sullivan glucose tolerance test<sup>12</sup> also displayed alterations in glucose metabolism parameters,<sup>13</sup> our research group has recently published the normal range of neonatal serum insulin levels and biomarkers of insulin resistance/sensitivity (e.g. HOMA-IR, QUICKI), determined from strictly selected neonates whose mothers did not show impaired glucose tolerance (IGT).<sup>6</sup>

To date, few studies testing HOMA-IR or QUICKI have been published<sup>14,15</sup> in neonates. None of these studies has tested QUICKI at birth. Moreover, to the best of our knowledge, differences in insulin sensitivity/resistance markers (such as HOMA-IR and QUICKI) due to the presence of maternal IGT have not been tested.

Dietary quality in the first trimester may also be a harbinger of diet quality throughout the pregnancy.<sup>16</sup> Several approaches have been used to define diet quality. Among them the Healthy Eating Index (HEI), reflecting the complexity of the dietary patterns based on 10 components, has been employed.<sup>17</sup> Each of its 10 components contributes to a total score of 100. A cut-off point < 70 for the HEI has been proposed for distinguish adequate from inadequate diets.<sup>17</sup> The validity of

HEI score has been demonstrated in studies using plasma biomarkers.<sup>18</sup> The HEI score or slight modifications, based on national recommendations, have resulted to be a useful tool for evaluating the diet quality of pregnant women.<sup>17,19,20</sup>

Present study hypothesizes that neonatal hyperinsulinemia at birth, even in normoweight neonates, is caused by insulin resistance during pregnancy and this circumstance is aggravated when maternal IGT was associated. This paper aims a) to establish the differences in glucose, GH, cortisol, IGF-I, QUICKI, HOMA-IR, levels between full-term normoweight neonates with low (< 1.8  $\mu$ IU/mL) and high ( $\geq$  6.0  $\mu$ IU/mL) insulin levels; b) to compare the insulin sensitivity/resistance markers levels of neonates from mothers having IGT, normal glucose tolerance (NGT), and mothers presenting GDM; c) to find out the possible interaction between these both previous factors. As information of diet followed during the first three months by some participant mothers was available, an approach was made to ascertain the effect of these diets on neonatal insulin sensitivity/resistance biomarkers.

## Methods

### *Sample characteristics*

The study was performed in the Mérida Hospital (Badajoz, Spain) in 176 Caucasian, singleton, full-term, normoweight neonates born without fetal distress whose mothers were screened for GDM using the O’Sullivan glucose tolerance test.<sup>12</sup> The study was performed in accordance with the Helsinki Declaration and approved by the Management and Ethical Committee of the Mérida Hospital.

### *Protocol*

Between weeks 24 and 28 of pregnancy, future mothers were screened for IGT and GDM using the O’Sullivan glucose tolerance test.<sup>12</sup> Shortly, mothers received 50 g of glucose and after one hour were tested for glycemia. They were considered to have IGT when glycemia was over 140 mg/dL ( $\geq$  7.78 mmol/L). When mothers were diagnosed of IGT, a second test was done. Mothers received 100 g of glucose and were tested after one, two and three hours for GDM.<sup>12</sup>

Data concerning delivery (type, primiparity/multiparity), mothers (age and gestational weight gain) and neonates (weight, length, gender, gestational age, Apgar at 1<sup>st</sup> and 5<sup>th</sup> minutes scores) were obtained from hospital records. Anthropometrical measurements were taken by the Obstetric Department personnel following the routine Mérida Hospital protocols.

After delivery, the umbilical cord was cut and blood was obtained by arterial puncture and collected in BD Vacutainer<sup>®</sup> SST II tubes with separation gel reference

number 8019381 (Becton Dickinson, Plymouth, UK) and adequately identified for its processing. Once in the laboratory, the blood was centrifuged to obtain serum (3,500 rpm for 5 minutes). Aliquots were made and frozen at -18°C until processed.

#### *Dietary data collection*

In 29 future mothers (approx. 16% of participating mothers), following the first fetal ultrasonography, information of the diet followed during the third month of pregnancy was obtained. Maternal dietary intake was calculated according to a 72 h recall, followed by a food frequency questionnaire of one month<sup>21</sup> and validated by the Nutrition Department of the Facultad de Farmacia Universidad Complutense de Madrid. The amount consumed was calculated by means of photographs of sample ratios.<sup>22</sup> Daily energy and nutrient intakes per head were calculated using a computer program compiled from food tables.<sup>23</sup>

The HEI slightly modified for Spanish population<sup>23</sup>, was used to evaluate mothers' diet quality. This HEI measures diet quality of a 100-point scale with each of the following 10 components contributing 10 possible points: grains and legumes, vegetables, fruits, dairy products, meat (including eggs and fish), total fat, saturated fat, cholesterol, sodium and variety of the diet; and considering the portion sizes. A diet was considered inadequate when its HEI score was  $\leq 70$  and adequate when the HEI score was  $> 70$ .<sup>17</sup>

#### *Assays*

Serum glucose was measured by the glucose hexokinase method (Gluco-quant<sup>®</sup>, Roche Diagnostics) using the reagent kit reference number 11929534 in a Roche/Hitachi Modular P (Roche Diagnostics, Basel, Switzerland) analyzer. Cortisol and insulin concentrations were determined by electrochemiluminescence immunoassay (ECLIA), using the kit reference number 11875116 for cortisol and the reagent kit reference number 12017547 for insulin; both were supplied by Roche Diagnostics in a Roche/Hitachi Modular Analytics E 170 analyzer (Roche Diagnostics, Basel, Switzerland). According to the manufacturer, major steroid molecules show slight cross-reactivity in the cortisol assay (e.g. cortisone, 0.3%).

IGF-I and GH concentrations were determined by chemiluminescent immunometric assays using the kit reference number LKGF1 for IGF-I and LKGH1 for GH; both were supplied by Diagnostic Products Corporation. An Immulite 1000 (Diagnostic Products Corporation, Flanders, New Jersey) analyzer was used.

Our laboratory participates in the Spanish Clinical Chemistry Society (SEQC) External Quality Evaluation Program, which follows UNE-EN-ISO 9001:2000 standards and is certified by AENOR. All the assays

were properly calibrated and performed under internal and external quality control provided by the manufacturers and SEQC, respectively. Intraassay and interassay variation coefficients were 1% and 1.7% for glucose; 1.7% and 2.8% for cortisol; 1.5% and 4.9% for insulin; 5% and 5.8% for GH, and 4.3% and 6.6% for IGF-I, respectively.

The indexes used to test insulin resistance or sensitivity were QUICKI, calculated by the formula  $1/[(\log \text{Insulin})(\mu\text{IU/mL})+(\log \text{Glucose}) \text{ mg/dL}]$ ; HOMA-IR, calculated as:  $\text{Glucose} (\mu\text{mol/L}) \times \text{Insulin} (\mu\text{IU/mL}) / 22.5$ .<sup>24</sup> In addition, the glucose/insulin and insulin/cortisol ratios were calculated. According to Gesteiro et al.<sup>6</sup> the cut-off point for high insulin level (4<sup>th</sup> quartile, Q4) was set at  $\geq 6.0 \mu\text{IU/mL}$  while for low insulin level (1<sup>st</sup> quartile, Q1) at  $< 1.8 \mu\text{IU/mL}$ .

#### *Statistical studies*

The Kolmogorov-Smirnov test was used for assessing the normal data distribution. The number of neonates in Q1 and Q4 groups was adequate to test absolute differences of 2 in HOMA-IR with a power of 95% (nominal alpha = 0.05) between neonates belonging to the Q1 and Q4 groups. A standard deviation of 0.45 and of 4 for HOMA-IR in the Q1 and Q4 respectively was assumed for this calculation. One way ANOVA followed by Bonferroni *post hoc* was performed to compare children belonging to Q1 and Q4 for insulin born from NGT and IGT mothers. A stepwise multiple regression procedure was used to identify variables that explained insulin variability at birth. Explicative variables considered were the insulin at birth, the maternal age, basal glucose and glucose tolerance by O'Sullivan test, neonatal gender, gestational age, bodyweight, length, body mass index (BMI), ponderal index (PI), Apgar test scores at 1<sup>st</sup> and 5<sup>th</sup> minutes, glucose, cortisol, GH, and IGF-I. HOMAs and ratios where insulin was included were not considered in the model to prevent multicollinearity and influential cases. Due to the reduced number of GDM neonates found, the Mann-Whitney U test was employed for Q1 vs Q4 and GDM offspring vs. NGT offspring comparisons. The HEI score comparison was also performed using the Mann-Whitney U test. The Kruskal-Wallis non-parametric comparison test followed by multiple comparison non parametric test were used. Percentage distribution between groups was compared by the Chi square test or the Fisher test. Statistical significance was set at  $p < 0.05$ . Statistical analyses were performed using the SPSS statistical software package (version 15.0) and the SAS (version 9.0).

#### **Results**

Of the 176 neonates studied, 56 (30%) had insulin levels  $\geq 6.0 \mu\text{IU/mL}$ , while 33 (15%) presented insulin

**Table I**  
Maternal age and glucose levels and anthropometric, hormone and metabolic parameters and insulin resistance/sensitivity marker data of neonates according to reference quartiles for insulin levels<sup>1</sup>

	Q1 (n = 33)	Q2 (n = 43)	Q3 (n = 44)	Q4 (n = 56)	ANOVA p
Maternal Age (years)	29.67 ± 4.21	29.86 ± 5.62	30.20 ± 5.49	31.00 ± 4.77	NS
Maternal Glucose (mg/dL)	80.76 ± 6.39a	82.38 ± 5.59ab	85.27 ± 6.83b	84.59 ± 7.03b	**
Gestational Age (weeks)	39.85 ± 1.21	39.84 ± 1.04	39.88 ± 1.07	39.92 ± 1.07	NS
Birth weight (g)	3,223.3 ± 353.4	3,263.5 ± 320.3	3,333.9 ± 317.3	3,382.4 ± 321.3	NS
Length (cm)	49.94 ± 1.04	49.72 ± 1.48	50.09 ± 1.51	50.18 ± 1.45	NS
BMI (kg/m <sup>2</sup> )	12.91 ± 1.23	13.20 ± 1.17	13.29 ± 1.17	13.42 ± 0.96	NS
PI (kg/m <sup>3</sup> )	25.86 ± 2.44	26.59 ± 2.67	26.57 ± 2.61	26.76 ± 2.03	NS
Glucose (mg/dL)	59.64 ± 20.16a	68.60 ± 32.15a	64.39 ± 18.59a	99.65 ± 47.95b	***
Cortisol (µg/dL)	7.36 ± 2.87	7.10 ± 3.26	7.54 ± 3.84	8.23 ± 4.00	NS
GH (ng/dL)	17.87 ± 7.88a	16.14 ± 10.24ab	17.81 ± 13.18ab	12.82 ± 8.91b	*
IGF-I (ng/mL)	46.82 ± 19.92a	52.62 ± 23.28ab	64.20 ± 23.36b	63.65 ± 32.33b	**
QUICKI	0.56 ± 0.19a	0.45 ± 0.08b	0.43 ± 0.07b	0.36 ± 0.07c	***
Glucose/insulin	74.63 ± 75.08a	29.49 ± 15.35b	15.27 ± 5.05bc	8.49 ± 4.44c	***
Insulin/cortisol	0.18 ± 0.11a	0.39 ± 0.14ab	0.71 ± 0.35b	2.27 ± 2.14c	***
HOMA-IR	0.16 ± 0.09a	0.42 ± 0.18ab	0.68 ± 0.24b	4.07 ± 4.25c	***

<sup>1</sup>Reference quartile values, Q1 < 1.8 mIU/mL, Q2: 1.8-3.2 mIU/mL, Q3: 3.2-6 mIU/mL, Q4: > 6 mIU/mL. For more details see Gesteiro et al.<sup>6</sup> Data are Mean ± SD. Values bearing different letters are significantly different. BMI: Body Mass Index; PI: Ponderal Index; GH: Growth Hormone; IGF-I: Insulin-Like Growth Factor I; QUICKI: Quantitative Insulin Sensitive Check Index; HOMA: Homeostatic Model Assessment for Insulin Sensitivity. For details see text. \*p < 0.05; \*\*\*p < 0.001; NS = Not Significant.

levels < 1.8 µIU/mL (cut-off points to define Q4 and Q1 respectively, reference values for neonates<sup>6</sup>). Almost 10% of the neonates in the present study had insulin values ≥ 15 µIU/mL, figure proposed as cut-off point for hyperinsulinemia.<sup>3</sup> In the Q4 neonates group, 30.4% of them presented insulin levels ≥ 15 µIU/mL; 85.7% had HOMA-IR ≥ 1.03 and 71% showed QUICKI values < 0.38 at birth. As quartile for cord blood insulin levels increased, glucose, the insulin/cortisol ratio and HOMA-IR (all p < 0.001) and IGF-I (p < 0.01) increased while QUICKI, the glucose/insulin ratio (all p < 0.001) and GH (p < 0.05) decreased. Glucose, IGF-I, the insulin/cortisol ratio, and HOMA-IR were significantly higher while GH, QUICKI, and the glucose/insulin ratio were significantly lower in Q4 newborns with respect to their Q1 counterparts (table I). These results were not affected when data were corrected for maternal glucose, maternal age, gestational age, gender and neonatal bodyweight.

One hundred and sixteen mothers displayed NGT while 60 were IGT. Nine of the 60 IGT mothers were diagnosed of GDM. Neonates from IGT mothers had higher insulin, HOMA-IR (both p < 0.01), and insulin/cortisol ratio (p < 0.05) and lower GH, QUICKI (both p < 0.01), glucose/insulin ratio (p < 0.05) when compared with NGT mothers (table II). The percentage of neonates with high or low insulin levels in the NGT and IGT mothers groups tended to be significantly different (p = 0.093) (fig. 1A). Five of the nine mothers with GDM delivered neonates with

high insulin levels while one had an infant with low insulin levels.

Comparisons for anthropometrical data and insulin sensitivity/resistance markers in Q1 and Q4 neonates whose mothers presented NGT or IGT are shown in table III. There were not significant differences in any of the studied parameters between Q1-NGT and Q1-IGT neonates. Only maternal age and maternal glycemia were significantly different (both p < 0.05) between Q4-NGT and Q4-IGT neonates. Data shown in table III were not affected after neonatal body weight, gestational age, gender, maternal age, and basal glucose adjustments.

According to the stepwise multiple regression procedure maternal age and glucose tolerance, and neonatal gender, bodyweight and gestational age do not contribute significantly to explain data variability of insulin levels at birth. Neonatal glycemia and GH levels explain 21% of insulin variability at birth (R<sup>2</sup> = 0.209; p < 0.001) (data not shown).

With exception of IGF-I (p < 0.05), that was higher in the GDM offspring, no significant differences were found between the GDM offspring and the non-GDM-IGT offspring for any insulin sensitivity/resistance markers studied (data not shown).

GDM offspring insulinemia was 57% higher (p = 0.07) than that of their non-GDM counterparts (fig. 1B). Significantly higher IGF-I and HOMA-IR (both p < 0.05) were found for GDM offspring (fig. 2A and 2B).

**Table II**

Maternal age and glucose levels and anthropometric, hormone and metabolic parameters and insulin resistance/sensitivity marker data of neonates whose mothers had normal glucose tolerance (NGT) or impaired glucose tolerance (IGT) by O'Sullivan glucose tolerance test

	NGT (n = 116)	IGT (n = 60)	Significance NGT vs IGT
Maternal Age (years)	29.39 ± 4.88	31.95 ± 4.77	**
Maternal Glucose (mg/dL)	81.86 ± 6.00	87.05 ± 6.84	NS
Gestational Age (weeks)	39.58 ± 1.10	39.35 ± 1.40	NS
Birth weight (g)	3,304.6 ± 325.0	3,284.5 ± 335.7	NS
Length (cm)	50.06 ± 1.39	49.79 ± 1.45	NS
BMI (kg/m <sup>2</sup> )	13.18 ± 1.13	13.24 ± 1.09	NS
PI (kg/m <sup>3</sup> )	26.36 ± 2.45	26.60 ± 2.32	NS
Glucose (mg/dL)	75.29 ± 37.90	76.69 ± 36.26	NS
Cortisol (μg/dL)	7.41 ± 3.04	8.11 ± 4.45	NS
GH (ng/dL)	16.74 ± 9.81	14.36 ± 11.55	*
IGF-I (ng/mL)	55.17 ± 22.94	62.27 ± 32.20	NS
Insulin (μIU/mL)	5.50 ± 7.46	8.64 ± 10.70	**
QUICKI	0.45 ± 0.14	0.41 ± 0.10	**
Glucose/insulin	33.63 ± 48.98	17.72 ± 13.82	**
Insulin/cortisol	0.83 ± 1.19	1.36 ± 1.90	*
HOMA-IR	1.36 ± 2.82	2.03 ± 3.08	**

Data are Mean ± SD. BM: Body Mass Index; PI: Ponderal Index; GH: Growth Hormone; IGF-I: Insulin-Like Growth Factor I; QUICKI: Quantitative Insulin Sensitive Check Index; HOMA: Homeostatic Model Assessment for Insulin Sensitivity. For details see text. \*p < 0.05; \*\*p < 0.001; NS = Not Significant.

Non significant differences were found between diet characteristics of NGT and IGT mothers (data not shown). Figure 3A shows the diet differences of mothers whose neonates had high insulin or low insulin levels at birth. Diets of Q4 newborns had higher HEI score (p = 0.031) but lower lipid energy contribution (p = 0.031) and cholesterol content (mg) (p = 0.024) (data not shown). Moreover, Q4 neonates whose mothers were dietary assessed had higher glucose (p < 0.040), insulin (p < 0.001), and HOMA-IR (p < 0.001) but

lower QUICKI (p = 0.004) and glucose/insulin ratio (p = 0.001) than their Q1 counterparts (fig. 3B).

## Discussion

To the best of our knowledge this is the first report studying the effect of the newborn insulinemia and maternal glucose tolerance on the level of sensitivity/resistance biomarkers. Moreover, these markers were

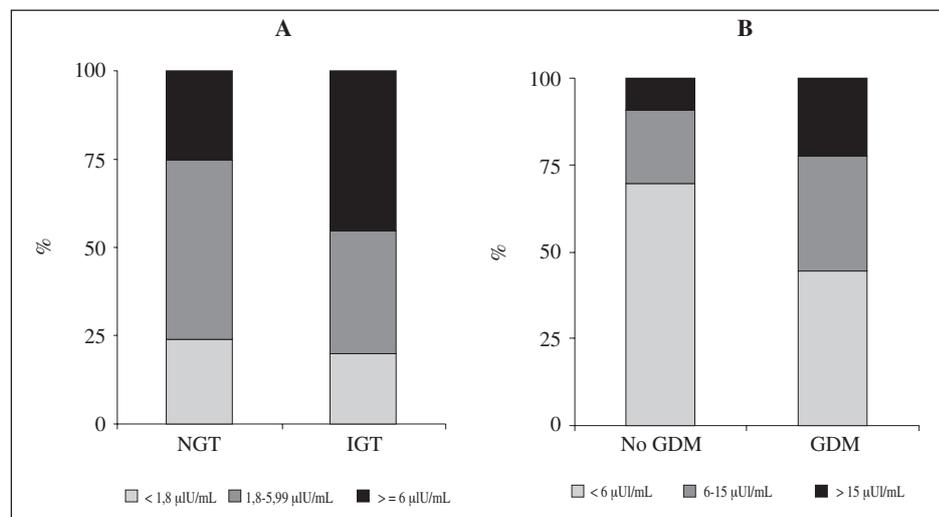


Fig. 1.—Neonatal insulinemia distribution. A) According to the presence or absence of Gestational Diabetes Mellitus (GDM). B) According to the impaired glucose tolerance (IGT) test.

**Table III**

Maternal age and glucose levels and anthropometric, hormone and metabolic parameters and insulin resistance/sensitivity marker data according to the insulin quartile of neonates whose mothers had normal glucose tolerance (NGT) or impaired glucose tolerance (IGT) by the O'Sullivan glucose tolerance test

	Insulin, Quartile 1		Insulin, Quartile 4		ANOVA p
	NGT (n = 26)	IGT (n = 7)	NGT (n = 29)	IGT (n = 27)	
Maternal age	29.5 ± 4.6ab	30.1 ± 2.7ab	29.3 ± 4.5a	32.8 ± 4.4b	*
Maternal Glucose (mg/dL)	80.4 ± 6.2 a	82.0 ± 7.3 ab	80.6 ± 5.7 a	88.5 ± 6.1 b	***
Gestational Age (weeks)	40.0 ± 1.1	39.4 ± 1.5	39.7 ± 1.2	39.5 ± 1.3	NS
Birth Weight (g)	3,255.8 ± 376.5	3,102.9 ± 232.8	3,340.3 ± 305.1	3,384.8 ± 346.3	NS
Length (cm)	50.2 ± 0.9 ab	48.9 ± 0.9 a	50.2 ± 1.1 b	50.1 ± 1.7 b	*
BMI (kg/m <sup>2</sup> )	12.9 ± 1.3	12.9 ± 0.7	13.2 ± 1.0	13.5 ± 0.9	NS
PI (kg/m <sup>3</sup> )	25.7 ± 2.6	26.5 ± 1.5	26.3 ± 2.1	26.9 ± 2.0	NS
Glucose (mg/dL)	61.3 ± 19.3 a	53.4 ± 23.7 a	102.0 ± 52.5 b	93.4 ± 42.9 b	***
Cortisol (µg/dL)	7.3 ± 3.1	7.5 ± 1.9	7.6 ± 2.9	8.9 ± 4.9	NS
GH (ng/dL)	16.5 ± 6.9 ab	22.8 ± 9.7 a	15.7 ± 10.8 ab	11.7 ± 7.0 b	*
Insulin (µIU/mL)	1.09 ± 0.47 a	1.23 ± 0.27 a	14.0 ± 10.9 b	15.2 ± 12.8 b	***
IGF-I (ng/mL)	46.7 ± 19.5a	47.15 ± 22.9ab	60.5 ± 23.5ab	68.6 ± 39.2b	*
QUICKI	0.58 ± 0.20 a	0.52 ± 0.18 a	0.36 ± 0.07 b	0.37 ± 0.08 b	***
Glucose/insulin	83.31 ± 82.5 a	42.6 ± 14.7 a	9.3 ± 5.1 b	7.8 ± 3.5 b	***
Insulin/cortisol	0.18 ± 0.12 a	0.17 ± 0.05 a	2.1 ± 1.8 b	2.3 ± 2.4 b	***
HOMA-IR	0.16 ± 0.09 a	0.17 ± 0.09 a	4.1 ± 4.6 b	3.8 ± 3.8 b	***

Values are mean ± SD. Values bearing different letters are significantly different. BMI: Body Mass Index; PI: Ponderal Index; GH: Growth Hormone; IGF-I: Insulin-Like Growth Factor I; QUICKI: Quantitative Insulin Sensitive Check Index; HOMA, Homeostatic Model Assessment for Insulin Sensitivity. For details see text. \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; NS = Not Significant. Values bearing different letters are significantly different. BMI: Body Mass Index; PI: Ponderal Index; GH: Growth Hormone; IGF-I: Insulin-Like Growth Factor I; QUICKI: Quantitative Insulin Sensitive Check Index; HOMA: Homeostatic Model Assessment for Insulin Sensitivity. For details see text. \*p < 0.05; \*\*\*p < 0.001; NS = Not Significant.

assessed in full-term, normoweight, without fetal distress neonates presenting high or low insulin concentrations at birth.

Metabolic and hormonal modifications, including those related to glucose metabolism and insulin secretion, occur during pregnancy to insure adequate glucose metabolism, fetal growth and survival.<sup>25</sup> However, fetal insulin levels increase under certain adverse circumstances (e.g. hyperglycemia, GDM).<sup>26</sup>

Present data suggest that increases in neonatal insulinemia were not able to normalize neonatal glycemia in Q4 neonates, as those newborns presented significantly

higher cord blood insulin levels. Despite the fact that all studied infants were normoweight and full-term, approximately one third of them presented very high insulin levels ( $\geq 15$  µIU/mL), 6 of 7 presented HOMA-IR values over the 75<sup>th</sup> percentile described by Gesteiro et al.<sup>6</sup> and almost three quarters had QUICKI values under the 25<sup>th</sup> percentile described by Gesteiro et al.<sup>6</sup> Present data suggest that high HOMA-IR values (indicative of insulin resistance at birth) were more prevalent in Q4 neonates.

The non significant differences in BMI and body weight contrast with the higher IGF-I levels found in

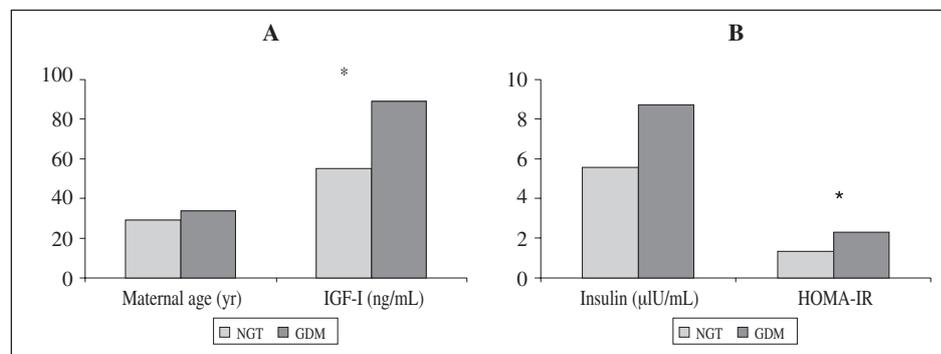


Fig. 2.—Differences between normal glucose tolerance (NGT) and Gestational Diabetes Mellitus (GDM) offsprings. A) Maternal age and neonatal IGF-I. B) Insulin, HOMA-IR, \*p < 0.05

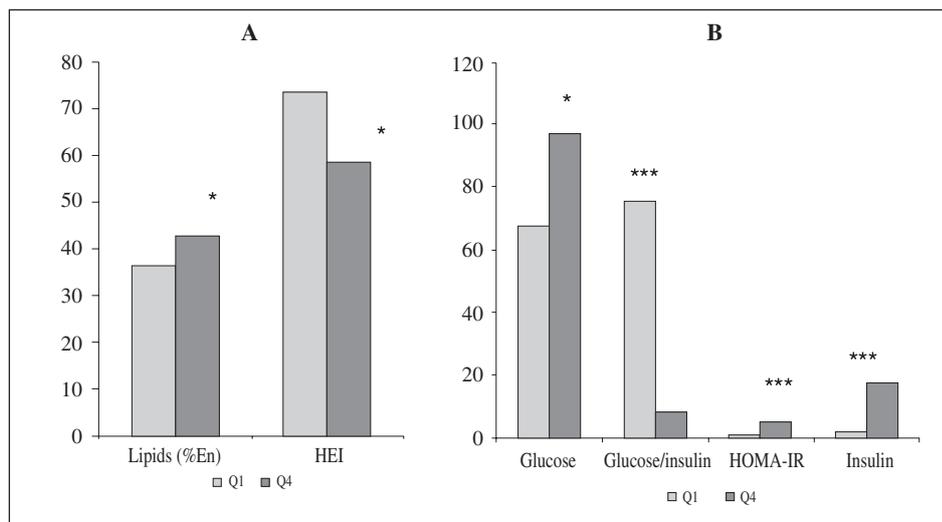


Fig. 3.—Comparison between Q1 and Q4 neonates groups whose mothers were nutritionally surveyed. A) Differences in lipid energy contribution and Healthy Eating index (HEI) score of first trimester pregnancy diets. B) Differences in insulin sensitivity/resistance biomarkers in neonates \* $p < 0.05$ , \*\*\* $p < 0.001$ . Q1 (n = 9); Q4 (n = 9).

Q4 neonates. IGF-I and GH values display an inverse relationship because GH secretion is under a negative feed-back influence by IGF-I,<sup>27</sup> explaining, at least partially, the present results. Alterations in insulin secretion may reflect higher IGF-I levels in those children who grow more rapidly than others. IGF-I enhances b-cell mass and function *in vivo*, but its relevance for normal islet development in humans remains to be determined<sup>4</sup>. The mechanism whereby IGF-I levels are linked to insulin secretion is not completely known, but IGF-I activity may directly regulate not only childhood growth but also the maintenance of b-cell mass and insulin secretory response to glucose.<sup>4,28</sup> In fact, IGF-I was higher in IGT and GDM offspring than in their NGT counterparts.

Q1 neonates presented significantly lower glycemia than their Q4 counterparts. Near 50% of Q1 neonates were under the 25<sup>th</sup> glucose percentile, while approximately 50% of Q4 were over the 75<sup>th</sup> glucose percentile.<sup>6</sup> Levels of several markers of insulin resistance increased in neonates with high insulin levels.

Low insulin/cortisol ratio has been defined in starvation and malnutrition.<sup>29</sup> The insulin/cortisol ratio of Q4 infants was 10 times higher than that of Q1 neonates, suggesting that a rise in insulin levels does not necessarily signify an increase in cortisol levels and that among Q4 neonates there was a large percentage showing high serum insulin/cortisol ratios. Thus, according to this ratio, the nutritional status in Q1 neonates seems to be impaired respect to that of their Q4 counterparts, explaining, at least in part, the lower birthweight of Q1 neonates. Present data suggest that maternal IGT impaired several of the sensitivity/resistance markers studied.

Maternal hyperglycemia can impair glycemic metabolism in infants, as neonates from IGT mothers display a high prevalence of hyperinsulinemia.<sup>15</sup> Pregnancy is a “diabetogenic” physiological situation characterized among others changes by the maternal insulin

resistance, hyperinsulinemia and hyperlipemia.<sup>30</sup> When insulin resistance during pregnancy is exacerbated, the mother develops GDM.<sup>31</sup> In the present study 34% of the mothers were IGT while 5% were diagnosed of GDM. Figure 1A shows that more neonates with insulin levels  $\geq 6.0 \mu\text{IU/mL}$  than those with levels  $< 1.8 \mu\text{IU/mL}$  were born from IGT mothers. Most insulin resistance/sensitivity markers were impaired in neonates whose mothers had IGT.

When the insulinemia and maternal glucose tolerance association was studied, the highest absolute differences were found between hyperinsulinemic neonates from IGT mothers and hypoinsulinemic infants from NGT mothers. Thus, it should be accepted that neonatal insulinemia much more than maternal IGT affects the sensitivity/resistance markers of the normoweight children studied.

Dietary information of mothers whose newborns were Q1 or Q4 suggests a clear influence of maternal diet in neonatal glucose homeostasis. In fact the diet of mothers whose neonates were Q4 for insulin level presented a lower HEI score and was richer in lipids and cholesterol. Epidemiological data suggest that subjects with higher fat intake are more prone to develop glucose metabolism disturbances than subjects with low fat intake.<sup>32,33</sup> However, the low number of mother with available dietary information is a limitation of this paper and the possible relationship with data can be carefully interpreted. The HEI score for the Q1 mothers average diet was 73.5, overlapping with the average HEI score (mean  $\pm$  SD) found for the Spanish women ( $73.7 \pm 10.5$ ) and the Extremadura Autonomous Community ( $70.5 \pm 10.9$ ). Nonetheless the average HEI score for Q4 mothers was 58.7, a value out of these intervals,<sup>34</sup> suggesting that a high percentage of Q4 mothers would need dietary changes and nutritional advice.

Insulin resistance, which is primarily related to contra-insular pregnancy hormone activity, may lower

glucose tolerance or cause GDM in susceptible pregnant women or impair metabolic control in diabetic women.<sup>26</sup> GDM *per se* significantly increases the offspring's subsequent risk of obesity and type-2 diabetes later in life<sup>3</sup>. Maternal GDM reflects a metabolically altered fetal environment in which maternal hyperglycemia leads to excess fetal insulin production.<sup>26,35</sup> Neonates from GDM mothers presented very high mean values for different insulin resistance markers. According to HOMA-IR these neonates seem insulin resistant. Nonetheless, a limitation of the study is the low number of GDM mothers, suggesting that data of GDM offspring should be carefully interpreted. *In utero* glycemia has been associated with insulin resistance in children of high-risk populations such as the Pima Indians and African-Americans<sup>36,37</sup> but limited data regarding this association are available in low-risk populations. Results of a cohort study of 7-11 year-old Caucasian children from mothers with GDM, have led Keely et al.<sup>35</sup> to conclude that metabolic markers of insulin resistance may be present in children in the absence of abnormal fasting glucose or 2-h glucose overload test values.

In conclusion, hyperinsulinemia at birth and pregnancy diet, but not maternal IGT status, highly affected several insulin resistance/sensitivity markers in full-term, normoweight infants. A follow-up study of this children population appears necessary to clearly demonstrate the utility of a neonatal screening overall in these normoweight infants with insulin levels > 6.0 µIU/mL at birth. Nutritional assessment and advice seem necessary during pregnancy to avoid insulin resistance at birth.

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## References

- American Diabetes Association (ADA) Consensus Development conference on insulin resistance. *Diabetes Care* 1998; 2: 310-314.
- Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992; 5: 595-601.
- Ten S, Maclaren N. Insulin resistance syndrome in children. *J Clin Endocrinol Metab* 2004; 89: 2526-2539.
- Bazaes RA, Alegria A, Pittaluga E, Ávila A, Iñiguez G, Mericq V. Determinants of insulin sensitivity and secretion in very-low-birth-weight children. *J Clin Endocrinol Metab* 2004; 89: 1267-1272.
- Christou H, Connors JM, Ziotopeoulou M, Hatzidakis V, Papanthassoglou E, Ringer SA et al. Cord blood leptin and insulin-like growth factor levels are independent predictors of fetal growth. *J Clin Endocrinol Metab* 2001; 86: 935-938.
- Gesteiro E, Bastida S, Sánchez-Muniz FJ. Insulin resistance markers in term, normoweight neonates. The Merida cohort. *Eur J Pediatr* 2009; 168: 281-288.
- Kirimi E, Cesur Y, Gül A. Normal levels of insulin, growth hormone and cortisol levels in venous cord blood of healthy full-term infants: correlation with birthweight and placental weight. *Eastern J Med* 2000; 6: 14-17.
- Miras M, Ochetti M, Martín S, Silvano L, Sobrero G, Castro L et al. Serum levels of adiponectin and leptin in children born small for gestational age: relation to insulin sensitivity parameters. *J Pediatr Endocrinol Metab* 2010; 23: 463-71.
- Lemos JO, Rondó PH, Pereira JA, Oliveira RG, Freire MB, Sonsin PB. The relationship between birth weight and insulin resistance in childhood. *Br J Nutr* 2010; 103: 386-392.
- Mericq V, Ong KK, Bazaes R, Peña V, Avila A, Salazar T et al. Longitudinal changes in insulin sensitivity and secretion from birth to age three years in small- and appropriate-for-gestational-age children. *Diabetologia* 2005; 48: 2609-2614.
- Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; 85: 2402-2410.
- O'Sullivan BA, Henderson ST, Davis JM. Gestational diabetes. *J Am Pharm Assoc (Wash)* 1998; 38: 364-371; quiz 372-373.
- Gesteiro E, Fernández de los Ríos A, Bastida S, Sánchez-Muniz FJ. Relationship between the maternal O'Sullivan tests and the neonatal insulin sensitivity and insulinemia. Workshop on Glycaemic Response and Health. ILSI Europe. Nice (France), 6-8 December 2006. Abstract book, p. 58.
- Setia S, Sridhar MG, Bhat V, Chaturvedula L, Vinayagamoorti R, John M. Insulin sensitivity and insulin secretion at birth in intrauterine growth retarded infants. *Pathology* 2006; 38: 236-238.
- Yadda KK, Gupta R, Gupta A, Gupta M. Insulin levels in low birth weight neonates. *Indian J Med Res* 2003; 118: 197-203.
- Rifas-Shiman SL, Rich-Edwards JW, Willet WC, Kleinman KP, Oken E, Gillman MW. Changes in dietary intake from the first to the second trimester of pregnancy. *Pediatr Perinat Epidemiol* 2006; 20: 35-42.
- Kennedy ET, Ohls J, Carlson S, Fleming K. The Healthy Eating Index: design and applications. *J Am Diet Assoc* 1995; 95: 1103-1108.
- Hahn CS, Rock CL, King I, Drewnowski A. Validation of the healthy Eating Index with use of plasma biomarkers in a clinical sample of women. *Am J Clin Nutr* 2001; 74: 479-489.
- Rifas-Shiman SL, Rich-Edwards JW, Kleinman KP, Oken E, Gillman MW. Dietary quality during pregnancy varies by maternal characteristics in Project Viva: a US cohort. *J Am Diet Assoc* 2009; 109: 1004-1011.
- Bodnar LM, Siega-Riz AM. A Diet Quality Index for Pregnancy detects variation in diet and difference by sociodemographic factors. *Public Health Nutr* 2002; 5: 801-809.
- Bastida S, Sánchez-Muniz FJ, Cuenca C, Perea S, Aragonés A. High density lipoprotein-cholesterol changes in children with high cholesterol levels at birth. *Eur J Pediatr* 2002; 161: 94-98.
- Mataix Verdú J, Llopis González J. Manual gráfico e contenido nutricional de platos galegos. Jiménez Contreras JF, Lendoiro Otero RM, Meniño Olivera MJ (eds). Consellería de Sanidade, Carrefour, Galicia. 1993.
- Ortega RM, López-Sobaler AM, Andrés P, Requejo AM, Aparicio A, Molinero LM. DIAL software for assessing diets and food calculations. Departamento de Nutrición (UCM) and Alce Ingeniería SA Madrid, 2004. <http://www.alceingenieria.net/nutricion.htm>. Accessed Apr 2011.
- Livesey G. A systematic review of the glycaemic response to foods and health. ILSI Europe workshop. Glycaemic response on health. Nice, France. 6-8 Dec 2006. Abstract book, pp. 82-127.
- Beardsall K, Dunger D. The physiology and clinical management of glucose metabolism in the newborn. *Endoc Dev* 2007; 12: 124-137.

26. Vannini P. Pregnancy and diabetes: physiopathological aspects. *Minerva Endocrin* 1994; 19: 42-50.
27. Ochoa R, Zárate A, Hernández M, Galván R, Basurto L. Serum leptin and somatotropin components correlate with neonatal birth weight. *Gynecol Obstet Invest* 2001; 52: 243-247.
28. Ong KK, Petry CJ, Emmett PM, Sandhu MS, Kiess W, Hales CN et al. The ALSPAC study team. Insulin sensitivity and secretion in normal children related to size at birth postnatal growth and plasma Insulin growth factor I levels. *Diabetologia* 2004; 47: 1064-1070.
29. Torún B and Chew F. Protein-Energy Malnutrition. En: *Modern nutrition in health and disease*. Shils ME, Olson JA, Shike M. Ed. Lea & Febiger. 8th ed, pp. 950-976. Philadelphia, PA, 1994.
30. Montelongo A, Lasunción MA, Pallardo LF, Herrera E. Longitudinal study of plasma lipoproteins and hormones during pregnancy in normal and diabetic women. *Diabetes* 1992; 41: 1651-1659.
31. Herrera E, Ramos Álvarez P. Papel del tejido adiposo, sensibilidad insulínica e ingesta lipídica en la gestación y su implicación en el riesgo de padecer diabetes en la edad adulta. En: *Desarrollo perinatal: Origen de patologías adultas*. Pascual-Leone AM, Medina JM. Ed. Instituto de España. Real Academia de Farmacia. Monografía XXIII. Madrid, 2008.
32. Martín de Santa Olalla L, Sánchez-Muniz FJ, Vaquero MP. N-3 fatty acids in glucose metabolism and insulin sensitivity. *Nutr Hosp* 2009; 24: 113-127.
33. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC et al. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU study. *Diabetología* 2001; 44: 312-319.
34. Norte Navarro AI, Ortiz Moncada R. Spanish diet quality according to the Healthy Eating Index. *Nutr Hosp* 2011; 26: 330-336.
35. Keely EJ, Malcolm JC, Hadjiyannakis S, Gaboury I, Lough G, Lawson ML. Prevalence of metabolic markers of insulin resistance in offspring of gestational diabetes pregnancies. *Ped Diabetes* 2008; 9: 53-59.
36. Dabelea D, Pettitt DJ, Hansen RL, Imperatore G, Benett PH, Knowler WC. Birth weight, type 2 diabetes, and insulin resistance in Pima Indian children and young adults. *Diabetes Care* 1999; 22: 944-950.
37. Sobngwi E, Boudou P, Mauvais-Jarvis F. Effect of a diabetic environment *in utero* on predisposition to type 2 diabetes. *Lancet* 2003; 361: 1861-1865.