Vaspin, a compensatory mechanism against high glucose levels since birth?

Hernandez-Rodriguez et al. Vaspin, a compensatory mechanism since birth?

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What is already known on this topic?
Vaspin, identified as a member of a serine protease inhibitor family, is specifically expressed in visceral adipose tissue and has insulin-sensitizing effects. Elevated vaspin concentrations in serum are associated with obesity and alterations in insulin sensitivity in humans.

What does this study adds?
The present study includes a population of healthy term newborns, excluding mothers with gestational diabetes, pregestational diabetes mellitus, pre-eclampsia, hypertension, or thyroid disease; suggesting that the results found about the negative association between glucose and vaspin levels in umbilical cord blood as well as the predictive nature of glucose levels on vaspin levels support the idea that elevated vaspin levels have a defensive action against insulin resistance from the intrauterine period in populations as the small and large for gestational age, which alterations in fetal nutrition that result in development adaptations that permanently change the insulin-glucose metabolism.

Abstract
Background: Hormones produced by fat tissue known as adipokines, are produced during intrauterine life and have recently been implicated in fetal growth. Vaspin is a adipokine expressed in visceral adipose tissue and has insulin-sensitizing effects. Elevated vaspin concentrations in serum are associated with alterations in insulin sensitivity.

Objective: To determine if vaspin concentrations in cord blood of healthy term newborns differ between small (SGA), appropriate (AGA), and large (LGA) for gestational age. The secondary objective was to determine if there is an association between vaspin and anthropometry, glucose, and insulin in the newborn.

Methods: The study population included healthy term newborns, 30 subjects in the SGA, 12 in the AGA, and 34 in the LGA group. The blood sample was taken from the umbilical cord vein from each child after birth for later analysis of vaspin, insulin and glucose. Anthropometry of the newborns was documented.
Results: Cord blood vaspin, insulin and glucose concentrations were not different between the three study groups. A negative correlation between vaspin and glucose concentrations was demonstrated in the total population ($r^2 = -0.364$, $p = 0.001$). This correlation was observed in the LGA group ($r = -0.482$, $p = 0.004$). Glucose levels significantly predicted vaspin levels ($r^2 = 0.140$, $p = 0.004$).

Conclusion: We found a negative association between glucose and vaspin levels in umbilical cord blood as well as the predictive nature of glucose levels on vaspin levels, suggesting that vaspin can be used as a predictor of alterations in the insulin-glucose metabolism since birth, especially in target populations.

Keywords: Vaspin, insulin, glucose, birth weight, cord blood

Introduction
The understanding that adipose tissue is only an energy reservoir began to change with the discovery of hormones produced by fat tissue known as adipokines, conferring them with endocrine function (1). Adipokines, which are produced during intrauterine life, have recently been implicated in fetal growth; thus the interest in exploring their physiology in early life (2). Vaspin, identified as a member of a serine protease inhibitor family, is specifically expressed in visceral adipose tissue and has insulin-sensitizing effects. Elevated vaspin concentrations in serum are associated with obesity and alterations in insulin sensitivity in humans (3), even in infancy (4).

The administration of vaspin improved glucose tolerance and insulin sensitivity in rodents; moreover, the administration of insulin significantly upregulated vaspin mRNA in subcutaneous adipose tissue, and to a lesser extent, reduced expression in visceral adipose tissue. These authors concluded that elevated vaspin expression could represent a compensatory mechanism of insulin resistance secondary to the metabolic complications of obesity (3, 5). Therefore, vaspin can lead to the development of new treatment strategies for obesity, diabetes and insulin resistance (6).

Alterations in fetal nutrition can result in development adaptations that permanently change the physiology and metabolism of the progeny, specifically, insulin–glucose metabolism (7). This supports the idea that small and large for gestational age (LGA) newborns have an increased risk of developing type 2 diabetes, hypertension and metabolic syndrome later in life (8, 9). The objective of this study was to determine if vaspin concentrations in cord blood of healthy term newborns differ between small for gestational age (SGA), appropriate for gestational age (AGA), and LGA newborns. The secondary objective was to determine if there is an association between vaspin and anthropometry, glucose, and insulin in the newborn.

Material and Methods
The study was approved by the Ethics Committee in Research of the Hospital Universitario “Dr. Jose Eleuterio Gonzalez” with the code PE16-00013. The mother provided written informed consent before enrollment. Seventy-six newborns (>37 weeks gestation) evaluated from December 2012 to January 2015 at the Universidad Autonoma de Nuevo Leon Medical School and the “Dr. Jose E. Gonzalez” University Hospital in Monterrey, Mexico were included.

The study population included healthy term newborns. Exclusion criteria were newborns of mothers with gestational diabetes, pregestational diabetes mellitus, pre-eclampsia, hypertension, or thyroid disease. Elimination criteria were any disease that required inpatient management or intercurrent disease affecting their nutritional status. The blood sample was taken from the umbilical cord vein from each child immediately after birth and centrifuged at 1600 g for 4°C. Aliquots of serum were separated, frozen, and stored at −70°C for later analysis of vaspin, insulin and glucose. Birth weight was documented prospectively using a Torrey scale (Torrey, S.A. de C.V., Monterrey, Mexico) and length was obtained using a SECA 210 infantometer (SECA North America, Chino, CA) at birth. The total sample was classified as SGA (less than 10th. percentile), AGA (percentile between 10 and 90) or LGA (greater than 90th. percentile) according to birth weight for gestational age (10).

Serum vaspin concentrations were analyzed by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Biovendor Human Vaspin ELISA, Brno, Czech Republic) according to the manufacturer’s instructions. Sensitivity of the assay is 0.01 ng/mL, the intrassay and interassay coefficient of variation were 7.6% and 7.7%, respectively. The determination of insulin concentrations was performed by electrochemiluminescence immunoassay (ECLIA) using a commercial kit (Roche Diagnostics, Indianapolis, IN). Sensitivity of the assay is 0.2 μU/mL; the intrassay and interassay coefficient of variation were 3.6% and 3.4%, respectively. Glucose concentrations were determined with the glucose oxidase method using a commercial kit (Pointe Scientific, INC. Canton MI) according to the manufacturer’s instructions.

Statistical analysis
Measures of central tendency are presented as medians (range) and means ± standard deviation (SD) according to the
distribution of the variables. The Kolmogorov-Smirnov test was applied to check the normality of the variables. A non-Gaussian distribution was shown for the data of vaspin and insulin, while glucose data were normally distributed. The Chi squared test was used to compare proportions. For comparison of dimensional continuous variables, a non-parametric Mann-Whitney U test and Kruskall-Wallis test was performed; univariate analysis of variance (ANOVA) was used for normally distributed data. Pearson’s correlation coefficient was applied to detect any positive or negative correlations. For multiple regression analysis, the stepwise forward model was used. A p ≤ 0.05 was considered statistically significant. SPSS Statistics for Macintosh v.22.0 (IBM Corp., Armonk, NY) was used for analysis.

**Results**

The study population (n=76) included 30 subjects in the SGA group, 12 in the AGA group, and 34 in the LGA group. Demographic and anthropometric characteristics are presented in Table I. None of the groups showed significant differences in terms of gender, delivery route, gestational age, and mother’s age. Cord blood vaspin, insulin and glucose concentrations were not different between the three study groups; levels and comparisons are shown in Table II. A significant correlation between vaspin concentration and birth length (cms) was found ($r^2 = 0.277$, $p = 0.016$), but not with birth weight or cephalic perimeter (Table III). Vaspin serum levels were significantly higher in males (0.054 (0.010-5.64) ng/mL) than in females (0.017 (0.010-1.14) ng/mL) when the entire population was analyzed ($p = 0.034$) (Figure 1). Insulin correlated with birthweight in the total population ($r^2 = 0.329$, $p = 0.004$) (Table III) and when this was analyzed by study group, the correlation was only present in the LGA group ($r^2 = 0.507$, $p = 0.002$). Likewise, a positive correlation was found between insulin and glucose only in the SGA group ($r^2 = 0.400$, $p = 0.028$). A negative correlation between vaspin and glucose concentrations was demonstrated in the total population ($r^2 = -0.364$, $p = 0.001$) (Table III). In the analysis by study group, this correlation was observed in the LGA group ($r = -0.482$, $p = 0.004$).

Birth length weakly but significantly predicted vaspin cord blood levels for the total population ($r^2 = 0.07$, $p = 0.016$). In the multivariate analysis, including either a stepwise or an all-at-once approach, the anthropometric variables (birth weight and cephalic perimeter) did not increase prediction of vaspin levels (Table IV). Glucose levels significantly predicted vaspin levels ($r^2 = 0.132$, $p = 0.003$). In the multivariate analysis, including either a stepwise or an all-at-once approach, including insulin in the model did not increase the prediction of vaspin levels (Table IV).

**Discussion**

Vaspin, which has been recently discovered with promising beneficial effects on obesity and diseases related to insulin resistance, could be the basis for future pharmacological treatment (11) although it has not been widely studied. To know its role in window periods such as fetal life becomes necessary. This study includes a sample of healthy term newborns of mothers without diagnosed comorbidities that can interfere with the correct acquisition of weight during intrauterine life, reporting no differences in vaspin levels in umbilical cord serum between the SGA, AGA and LGA study groups and finding differences in these levels according to gender, with these being greater in males than females. It was also found that length at birth and glucose levels are independent variables that predict vaspin in umbilical cord blood.

Vaspin and anthropometric measures

A previous study by Akcay et al (12) reported higher vaspin levels in the SGA group when compared to the AGA and LGA groups concluding that it may be the result of their role in energy homeostasis in intrauterine life since the SGA group presents the total amount of reduced fat mass, altered development of adipose tissue and relatively higher visceral fat deposits, suggesting that greater visceral fat deposits may be the source of higher vaspin concentrations in SGA neonates (9, 13).

With the history of previous studies reporting human vaspin concentrations associated with obesity, insulin resistance, and type 2 diabetes mellitus type 2 (14, 15), Kafalidis et al. (16) reported higher vaspin levels in the LGA group when compared with the AGA group attributing these differences to the accumulation of fat and hyperinsulinemia that occurred in the LGA group.

A previous study compared vaspin concentrations in umbilical cord blood of newborns with intrauterine growth restriction (IUGR) and AGA without reporting statistically significant differences (17). Cekmez et al. (18) studied vaspin levels in LGA and AGA and reported no statistically significant difference between both study groups. The absence of differences in the previous reports, similar to this report, can be due to differences in race, or differences in the definition of SGA or LGA.

Although it has been previously described that vaspin can play a major role in fetal development (19), published studies do not report an association between cord blood vaspin levels and length at birth as we found in this study. It is known that fetal macrosomia is related to hyperinsulinemia during fetal development, which is a result of
elevated glucose levels from the mother that are transferred through the placenta, and levels produced by the fetal pancreas during the second trimester when insulin is secreted autonomously independently of maternal glucose stimulation (Pedersen’s Hypotesis) (20). It is possible that the aforementioned mechanism leads to increased vaspin production to improve utilization of insulin, reducing in this way glucose levels to achieve an optimal intrauterine environment; however, this mechanism needs to be studied more, since in our study length at birth was positively correlated with vaspin levels and not with birth weight, which defines macrosomia.

Vaspin and gender
Korner et al. (21) reported significantly higher serum vaspin levels in girls than boys 7 to 18 years of age. They showed an increase in vaspin levels at puberty in girls while a non-dynamic increase in vaspin with puberty in boys was found. Consequently, the greatest difference between girls and boys was apparently in the adolescent-aged group. This despite not reporting a correlation between sex steroids related to puberty (estradiol and testosterone) and vaspin levels. Briana et al. (17) reported in the IUGR higher vaspin levels in females than in males at postnatal day 1, while no difference was observed in umbilical cord blood. Ackay et al. (12) did not find a difference in vaspin levels in umbilical cord blood according to gender despite differences depending on gender in adipose tissue distribution and mass which could lead to increased vaspin concentrations in females.

In our study, contrary to the aforementioned, we found significantly elevated cord blood vaspin levels in males compared to females. Behavior dependent on gender has been demonstrated for adiponectin (22) and leptin (23); however, if this also applies to vaspin, it needs to be studied.

Vaspin and glucose
A previous report by Kloting et al. (14) mentions that in db/db mice (a mutant that develops insulin resistance) but not in C57BL/6 mice, a single intracerebroventricular injection of vaspin was sufficient to cause a sustained significant improvement in glucose concentrations over at least 6 days. These results indicate, according to the authors, that vaspin reduces plasma glucose only in the presence of elevated blood glucose concentrations and could suggest that treatment with vaspin does not have the potential to cause hypoglycemia. This previously reported mechanism can explain the negative correlation between vaspin and glucose concentrations only observed in the LGA group when the analysis was performed by study group. This seems to indicate that this group probably developed in an abnormal intrauterine environment showing mild maternal hyperglycemia below the diagnostic threshold, because even a limited degree of maternal hyperglycemia, still considered to be in the normal range, may affect fetal weight (24). This finding is also supported by Hida (3) who administered vaspin to diet-induced obese mice, significantly improving insulin sensitivity and glucose tolerance. But the administration of vaspin in in vivo lean mice did not alter glucose tolerance, concluding that the upregulation of vaspin may be a defensive action against insulin resistance.

The predictive nature of glucose levels in vaspin umbilical cord blood levels, represents the mechanism by which vaspin is modified with regard to glucose levels to improve insulin utilization by reducing glucose levels in response to a compensatory mechanism to achieve an optimal intrauterine environment.

Study Limitations
The main limitation of the study is not having another study group of mothers with gestational diabetes, because it would allow comparing the LGA group of mothers without co-morbidities and those with a clear alteration of the insulin-glucose metabolism.

Conclusion
We found a negative association between glucose and vaspin levels in umbilical cord blood as well as the predictive nature of glucose levels on vaspin levels thus supporting the idea that elevated vaspin levels can have a defensive action against insulin resistance from the intrauterine period, suggesting that vaspin can be used as a predictor of alterations in the insulin-glucose metabolism since birth, especially in target populations such as the LGA group.

However, more studies are needed to investigate the role of vaspin in newborns of mothers with a history of insulin resistance to confirm its involvement in pathological processes. This could represent future treatment approaches for alterations of insulin resistance.

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References
Table I. Demographic and anthropometric characteristics of the study groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SGA, n=30</th>
<th>AGA, n=12</th>
<th>LGA, n=34</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td>0.766</td>
</tr>
<tr>
<td>Male</td>
<td>17 (56.7)</td>
<td>6 (50)</td>
<td>21 (61.8)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>13 (43.3)</td>
<td>6 (50)</td>
<td>13 (38.2)</td>
<td></td>
</tr>
<tr>
<td>Delivery Route</td>
<td></td>
<td></td>
<td></td>
<td>0.055</td>
</tr>
<tr>
<td>Vaginal</td>
<td>16 (53.3)</td>
<td>3 (25)</td>
<td>9 (26.5)</td>
<td></td>
</tr>
<tr>
<td>Cesarean Section</td>
<td>14 (46.7)</td>
<td>9 (75)</td>
<td>25 (73.4)</td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>39.10 ± 0.82</td>
<td>38.52 ± 1.37</td>
<td>39.18 ± 1.12</td>
<td>0.181</td>
</tr>
<tr>
<td>Mother’s age (yrs)</td>
<td>23.43 ± 5.23</td>
<td>26.83 ± 7.06</td>
<td>24.82 ± 4.95</td>
<td>0.184</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2608 ± 241.94</td>
<td>3185 ± 519.99</td>
<td>4262.65 ± 324.62</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>48.07 ± 1.85</td>
<td>49.29 ± 2.26</td>
<td>52.91 ± 1.71</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Cephalic perimeter (cm)</td>
<td>33.33 ± 1.10</td>
<td>34.41 ± 1.29</td>
<td>36.10 ± 1,24</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age. Data are given as mean ± SD.

Table II. Vaspin, insulin and glucose concentrations by study groups.

<table>
<thead>
<tr>
<th></th>
<th>SGA, n=30</th>
<th>AGA, n=12</th>
<th>LGA, n=34</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaspin ng/mL</td>
<td>0.021(0.010-1.890)</td>
<td>0.010(0.010-1.520)</td>
<td>0.051(0.010-5.64)</td>
<td>0.266</td>
</tr>
<tr>
<td>Insulin µU/mL</td>
<td>4.51(0.57-20.34)</td>
<td>12.20(1.36-22.41)</td>
<td>7(0.95-114.1)</td>
<td>0.055</td>
</tr>
<tr>
<td>Glucose mg/dL</td>
<td>79.50±22.38</td>
<td>82±34.17</td>
<td>69.67±20.90</td>
<td>0.161</td>
</tr>
</tbody>
</table>

SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age.
Table III. Correlations of vaspin, glucose and insulin with each other and with anthropometric variables in the total population

<table>
<thead>
<tr>
<th></th>
<th>Vaspin ng/mL</th>
<th>Glucose mg/dL</th>
<th>Insulin µU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>r</strong></td>
<td><strong>p</strong></td>
<td><strong>r</strong></td>
<td><strong>p</strong></td>
</tr>
<tr>
<td>Birth weight</td>
<td>0.190</td>
<td>-0.138</td>
<td>0.328</td>
</tr>
<tr>
<td>Birth length</td>
<td>0.277</td>
<td>-0.237</td>
<td>0.171</td>
</tr>
<tr>
<td>Cefalic perimeter</td>
<td>0.014</td>
<td>-0.143</td>
<td>0.216</td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.364</td>
<td>0.129</td>
<td>-0.364</td>
</tr>
<tr>
<td>Insulin</td>
<td>-0.136</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *p ≤ 0.05 is statistically significant*

Table IV. Predictor variables and multivariate analysis using vaspin as a dependent variable

<table>
<thead>
<tr>
<th>Step</th>
<th>Predictor Variable</th>
<th>β±s.e.m.</th>
<th>B±s.e.m.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Birth Length</td>
<td>0.277±2.47</td>
<td>0.089±0.036</td>
<td>0.016*</td>
</tr>
</tbody>
</table>

*Multivariate Analysis, r²=0.132, p=0.018*
Multivariate Analysis. $r^2=0.140$, $p=0.003$

Predictor variables: glucose, insulin

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>1</th>
<th>2</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose</td>
<td>-0.352±3.216</td>
<td>-0.014±0.004</td>
<td>0.002*</td>
</tr>
<tr>
<td>2</td>
<td>Insulin</td>
<td>-0.091±0.829</td>
<td>-0.006±0.007</td>
<td>0.410</td>
</tr>
</tbody>
</table>

*p $\leq 0.05$ is statistically significant

Figure 1. Serum vaspin concentrations in male (n = 44) and female (n = 32) newborns.