Glycation of Fetal Hemoglobin Reflects Hyperglycemia Exposure in Utero

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OBJECTIVE
The lifetime risk of metabolic diseases in offspring of women with gestational diabetes mellitus (GDM) depends, at least in part, on the impact of glycemic fetal programming. To quantify this impact, we have developed and validated a unique mass spectrometry method to measure the percentage of glycated hemoglobin in cord blood.

RESEARCH DESIGN AND METHODS
This case-control study includes 37 GDM women and 30 pregnant women with normal glucose tolerance (NGT).

RESULTS
Glycation of the α-chain (Gla) was higher in neonates from GDM (2.32 vs. 2.20%, P < 0.01). Gla strongly correlated with maternal A1C measured at delivery in the overall cohort (r = 0.67, P < 0.0001) as well as in each group (GDM: r = 0.66, P < 0.0001; NGT: r = 0.50, P = 0.01).

CONCLUSIONS
Thus, Gla may reflect hyperglycemic exposure during the last weeks of fetal development. Future studies will confirm Gla is a predictive biomarker of prenatally programmed lifetime metabolic health and disease.

The fetal programming theory suggests that maternal hyperglycemia during pregnancy has lifelong consequences for metabolic health in offspring (1,2). However, assessing fetal impacts poses a challenge. Birth weight is often used as a marker but is influenced by multiple determinants (3). Cord blood glucose, C-peptide, and insulin are also measured, albeit only once upon delivery (4–6), despite labor-associated stress and exercise affecting fetal glucose metabolism in opposite ways. A marker of long-term fetal glucose metabolism is needed. Although fetal A1C was a promising candidate, its measurement consistently resisted standard methods because of technical difficulties in face of wide biological variations (7).

We have recently developed an accurate method to measure glycation of hemoglobin (Hb) chains in fetal cord blood (8). Here, we compare results from neonates born to women with gestational diabetes mellitus (GDM) and women with normal glucose tolerance (NGT).

RESEARCH DESIGN AND METHODS
Research Design
This case-control study includes 37 women diagnosed and treated for GDM (all were under insulin therapy) and 30 pregnant women classified as NGT. This case-control study was approved by the Ethics Review Board of Sainte-Foy Hospital, Sherbrooke, QC, Canada.
study included all neonates previously in our method development (8). Additional participants were included in this project aiming at investigating the pathophysiology relevance of measuring fetal A1C, but always with the same inclusion and exclusion criteria as when recruiting for method development. According to Canadian recommendations (9), GDM was defined in this study as two out of three abnormal plasma glucose values during the 75-g oral glucose tolerance test (fasting $\geq$5.3, 1 h $\geq$10.6, and 2 h $\geq$8.9 mmol/L). Exclusion criteria were as follows: multiple pregnancies, mother’s first trimester BMI $\geq$30 kg/m², gestational age $<$37 weeks, and neonate birth weight $<$2,500 g. Gestational age was determined based on ultrasound dating, a standardized clinical procedure at our institution.

Maternal weight and height at first trimester and data related to delivery and neonatal outcomes were retrieved from medical records. Blood for maternal A1C measurements was sampled upon arrival at the Obstetrics Department, before delivery. Cord blood samples for future measurements were centrifuged at 2,500g during 10 min at 4°C. Aliquots of plasma were stored at $-80°C$ until measurement. Glucose was measured using a colorimetric method (Wako Diagnostics, Mountain View, CA); the intra-assay and interassay CVs were both $<$5%; minimum detectable concentration was 0.28 mmol/L. For insulin, C-peptide and proinsulin concentration measurements (radioimmunoassay; Millipore Corp., Billerica, MA), the intra-assay and interassay CVs were all $<$10% for the three analytes; minimum detectable concentrations of insulin, C-peptide, and proinsulin were, respectively, 16.29 pmol/L, 0.033 ng/mL, and 3.055 pmol/L.

**Biomarker Measurements**

Maternal A1C (Bio-Rad VARIANT, Hercules, CA) analysis was performed by the Centre Hospitalier Universitaire de Sherbrooke (CHUS) Clinical Biochemistry laboratory. Cord blood samples for future measurements were centrifuged at 2,500g during 10 min at 4°C. Aliquots of plasma were stored at $-80°C$ until measurement. Glucose was measured using a colorimetric method (Wako Diagnostics, Mountain View, CA); the intra-assay and interassay CVs were both $<$5%; minimum detectable concentration was 0.28 mmol/L. For insulin, C-peptide and proinsulin concentration measurements (radioimmunoassay; Millipore Corp., Billerica, MA), the intra-assay and interassay CVs were all $<$10% for the three analytes; minimum detectable concentrations of insulin, C-peptide, and proinsulin were, respectively, 16.29 pmol/L, 0.033 ng/mL, and 3.055 pmol/L.

**Statistical Analyses**

Continuous variables were assessed for distribution and log transformed to achieve normal distribution as necessary. Variables were compared between groups (GDM vs. NGT) using Student t test or Mann-Whitney U test depending on the normality of the distribution. Frequencies, presented as percentages, were compared using $\chi^2$ tests. Correlations between maternal/neonatal characteristics and the level of glycation of the $\alpha$-chain were assessed by Pearson coefficients. P values $<$0.05 were considered significant. Statistical analyses were performed using SAS version 9 (SAS Institute, Cary, NC).

**RESULTS**

GDM women (Table 1) were heavier at first trimester (BMI = 27.6 $\pm$ 5.7 vs. 24.6 $\pm$ 5.9 kg/m², $P = 0.04$) and had a higher A1C at delivery time (5.8 $\pm$ 0.4 vs. 5.4 $\pm$ 0.3%, $P = 0.001$). Neonate sex distribution and birth weight (3,358 $\pm$ 394 vs. 3,457 $\pm$ 468 g, $P = NS$) were similar in both groups. GDM women delivered earlier (38.5 $\pm$ 0.8 vs. 39.8 $\pm$ 0.9 weeks, $P < 0.001$). GDM neonates were normoglycemic (4.1 $\pm$ 0.9 vs. 4.3 $\pm$ 0.8 mmol/L, $P = NS$) but displayed hyperinsulinemia (89 $\pm$ 104 vs. 41 $\pm$ 32 pmol/L, $P = 0.02$) and impaired insulin sensitivity as estimated by the glucose-to-insulin ratio (0.09 $\pm$ 0.06 vs. 0.14 $\pm$ 0.06, $P = 0.01$).

The mean level of Glx was higher in GDM neonates (2.32 vs. 2.20%, $P = 0.01$) and was correlated with maternal A1C levels in the overall group ($r = 0.67$, $P < 0.0001$) and in each group (GDM: $r = 0.66$, $P < 0.0001$; NGT: $r = 0.50$, $P = 0.01$). Glx was not correlated with birth weight (GDM: $r = 0.16$, $P = 0.34$; NGT: $r = 0.03$, $P = 0.88$) or with gestational age (GDM: $r = 0.01$, $P = 0.96$; NGT: $r = 0.09$, $P = 0.64$).

**CONCLUSIONS**

Glx was higher in neonates exposed to GDM. Our sophisticated M5 method allows for precise Glx measurement, independently of the HbF-to-HbA ratio (8). Standard methods for measuring fetal Hb glycation have consistently failed, due to inherent inaccuracy (7,10) and the wide range of HbF (65–90% in term neonates) and HbA variations attributable to individual characteristics and fetal gestational age (11). An alternative method using electrospray ionization MS has been proposed before but remained with limitations (12); as demonstrated in our methodology paper (8), we were able to clearly establish the linearity of the response, selectivity, and data deconvolution processing in a range that is appropriate for measurement of glycation of $\alpha$- and $\gamma$-chains. Here, we reported associations with clinically relevant maternal and fetal glycemic traits.

Because fetal Hb life span is 60–80 days (11), we assumed that Glx reflects mean fetal glucose exposure over the last 4–6 weeks of pregnancy. Most importantly, the strong correlations with maternal A1C levels suggest that Glx accurately reflects the degree of hyperglycemia exposure. One could argue that maternal A1C may also serve as marker of glycemic exposure; yet given the imperfect, albeit strong, correlation, we believe that direct measurement of Glx captures additional information and offers a more accurate in situ glycemic fetal exposure. Whether Glx is a stronger...
of fetal glucose metabolism. Because even slight maternal hyperglycemia is associated with higher Glx levels in cord blood, we propose Glx as an accurate biomarker of maternal glycemic exposure.

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