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 (Glα) was higher in neonates from GDM (2.32 vs. 2.20%, $P < 0.01$). Glα 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, Glα may reflect hyperglycemic exposure during the last weeks of fetal development. Future studies will confirm Glα 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

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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 ≥5.3, 1 h ≥10.6, and 2 h ≥8.9 mmol/L). Exclusion criteria were as follows: multiple pregnancies, mother's first trimester BMI ≥30 kg/m^2, 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 glycation measurements were collected within 15 min after delivery, in BD Vacutainer tubes with sodium fluoride/Na_2-EDTA as an additive (8). Insulin sensitivity was assessed by the glucose-to-insulin ratio in cord blood (6).

Principles

Hb in cord blood is a composite of HbF ($\alpha_2\gamma_2$ chains) and HbA ($\alpha_2\beta_2$). According to our previous analyses (8), only glycation of the α-chain (Glα) was specifically assessed in this study because of the following: *1*) the α-chain is common to HbF and HbA and represents the sum of both forms, which are produced by the fetus; *2*) the α-chain is not a function of the HbF-to-HbA ratio, an important consideration since this ratio is not 1:1 and varies substantially at birth; and *3*) the number of γ-chain variants and β-chain methodological interferences make the measurement of these chains unsuitably long.

Glycation of Hb chains was measured using a quadrupole orthogonal acceleration time-of-flight mass spectrometer (SYNAPT MS; Waters Corporation, Milford, MA) with an electrospray ionization source working in positive mode and W-Optics (8). The intra/interday coefficients of variation (CVs) for Glα levels were 2.10/3.72%. The linearity (r^2) of the α-chain glycation response, measured from 1.97 to 3.11%, was 0.9990. In the current samples, the minimum value that we measured was 1.9% and the maximum value was 2.8%; the interquartile range was 2.12–2.40%.

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 2,500*g* 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 χ^2 tests. Correlations between maternal/neonatal characteristics and the level of glycation of the α-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 ± 5.7 vs. 24.6 ± 5.9 kg/m^2, *P* = 0.04) and had a higher A1C at delivery time (5.8 ± 0.4 vs. 5.4 ± 0.3%, *P* = 0.001). Neonate sex distribution and birth weight (3,358 ± 394 vs. 3,457 ± 468 g, *P* = NS) were similar in both groups. GDM women delivered earlier (38.5 ± 0.8 vs. 39.8 ± 0.9 weeks, *P* < 0.001). GDM neonates were normoglycemic (4.1 ± 0.9 vs. 4.3 ± 0.8 mmol/L, *P* = NS) but displayed hyperinsulinemia (89 ± 104 vs. 41 ± 32 pmol/L, *P* = 0.02) and impaired insulin sensitivity as estimated by the glucose-to-insulin ratio (0.09 ± 0.06 vs. 0.14 ± 0.06, *P* = 0.01).

The mean level of Glα 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). Glα 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

Glα was higher in neonates exposed to GDM. Our sophisticated MS method allows for precise Glα 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 α- and γ-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 Glα reflects mean fetal glucose exposure over the last 4–6 weeks of pregnancy. Most importantly, the strong correlations with maternal A1C levels suggest that Glα 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 Glα captures additional information and offers a more accurate in situ glycemic fetal exposure. Whether Glα is a stronger

Table 1—Characteristics of mothers and neonates

	NGT (n = 30)	GDM (n = 37)	P value
Mother clinical characteristics			
Age (years)	28.9 ± 5.2	29.6 ± 5.1	NS
First trimester BMI (kg/m^2)*	24.6 ± 5.9	27.6 ± 5.7	0.04
Gestational age at delivery (weeks)	39.8 ± 0.9	38.5 ± 0.8	<0.001
Maternal A1C level at delivery (%)	5.4 ± 0.3	5.8 ± 0.4	0.001
Delivery (% of caesarian section)	1 (3.3%)	9 (24.3%)	0.02
Neonate clinical characteristics			
Sex (% female)	11 (36.7%)	15 (40.5%)	NS
Weight (g)	3,457 ± 468	3,358 ± 394	NS
Cord blood			
HbF (%)	77.0 ± 4.7	79.9 ± 4.3	0.01
HbA (%)	16.1 ± 5.0	13.0 ± 4.6	0.01
Glucose (mmol/L)	4.3 ± 0.8	4.1 ± 0.9	NS
Insulin (pmol/L)	41 ± 32	89 ± 104	0.02
C-peptide (ng/mL)	0.26 ± 0.22	0.40 ± 0.47	NS
Proinsulin (pmol/L)	31.7 ± 20.3	35.2 ± 25.2	NS
Glucose-to-insulin ratio	0.14 ± 0.06	0.09 ± 0.06	0.01
Glycated α-chain (%)	2.20 ± 0.15	2.32 ± 0.21	0.01

Variables are given as n (%) or mean ± SD. P value <0.05 was considered statistically significant.
*First trimester medical record: n = 29 in NGT and n = 37 in GDM.

predictor of short- and long-term complications compared with maternal A1C at delivery will necessitate larger studies and longitudinal follow-up. We cannot exclude that contamination of maternal blood in the cord blood samples might influence our results, but this is usually limited; blood from a heel prick could be considered as a source of neonatal blood for future studies.

High birth weight is considered a classical marker of fetal glucose exposure (13,14), although it is a function of multiple factors and is an admittedly imperfect reflection of maternal hyperglycemia (3). Interestingly, we found no correlation between Glα and birth weight, arguing again that birth weight is a relatively crude measurement of exposure to maternal hyperglycemia.

In contrast to the long-term significance of Glα, other common surrogates of in utero hyperglycemic exposure, such as cord blood glucose, insulin, or C-peptide (4–6), have drawbacks limiting reliability; they are measured only once, i.e., at birth, despite labor-associated fetal stress, exercise, and hypoxia, which affect fetal glycemia in opposite ways.

The method proposed here is both accurate and enables detection of fetal in situ glycemic exposure. Importantly, our study was conducted under stringent conditions, i.e., in neonates developing in the healthiest of maternal environments. Indeed, none of the mothers were obese at conception and all were intensively monitored and efficiently treated throughout pregnancy, as shown by their predelivery A1C, which, albeit slightly higher than in control subjects, was well within the normal range for pregnant women at delivery (15). Based on these results, we are confident that our method is capable of detecting subtle differences in glycemic exposure, even within the "controlled" range. On the other hand, we admit that the strict selection of our participants limits the generalizability of our results; future studies should include women of a larger range of BMI, different levels of glycemic control, and other ethnicities.

Further investigation into Glα may open up two entirely new fields of research. First, Glα may help refine the glycemic targets of treatment of diabetes in pregnancy. This is an important issue for care that should translate into normal Glα levels at birth. However, current glycemic treatment targets for GDM are admittedly (16) far from glucose levels observed in normal pregnancy and should be redefined. Our titer of higher Glα values in neonates born from ideally treated GDM pregnancies clearly shows this is now within grasp. Second, Glα may help screen newborns and identify those at risk for future metabolic diseases, thus providing reliable quantification of the impact of fetal programming. Such investigations will require longitudinal studies.

Current technology now enables accurate measurement of Glα and thus of fetal glucose metabolism. Because even slight maternal hyperglycemia is associated with higher Glα levels in cord blood, we propose Glα as an accurate biomarker of maternal glycemic exposure.

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Author Contributions. F.O.D. conceived the MS study design, performed clinical data collection and MS data analysis and interpretation, and wrote the manuscript. M.-F.H. provided assistance with the statistical analysis, actively participated in data interpretation, and wrote the manuscript. C.A. performed acquisition of data and data analysis and interpretation and revised and edited the manuscript. J.M. participated in data collection and data analysis and interpretation and revised and edited the manuscript. P.P., L.B., J.R., and J.-C.P. participated in data analysis and interpretation and revised and edited the manuscript. C.A.-B. conceived the MS study design, performed data analysis and interpretation, and revised and edited the manuscript. Measuring glycation of Hb chains is J.-L.A.'s brain child. J.-L.A. also conceived the study design, received funding, participated in data analysis and interpretation, and wrote the manuscript. M.-F.H., C.A.-B., and J.-L.A. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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