

Longitudinal Assessment of Glycosylated Blood Protein Concentrations in Normal Pregnancy and Gestational Diabetes

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Longitudinal changes in glycosylated hemoglobin concentration (GlyHb) and glycosylated serum protein concentration (GSP) in both normal pregnancy and pregnancy complicated by gestational diabetes were determined using affinity chromatography, a method in which nonenzymatically glycosylated proteins are specifically measured. At 7–10 wk gestation, GlyHb in women who developed diabetes ($N = 21$) was higher than GlyHb in normal women ($N = 49$) ($6.7 \pm 0.2\%$ versus $5.7 \pm 0.2\%$, respectively, $P < 0.001$) and remained elevated throughout gestation. In normal pregnancy, GlyHb decreased to a nadir at 23–26 wk and returned to baseline concentration by 31–34 wk. In gestational diabetes, there was an initial increase in GlyHb to $7.1 \pm 0.5\%$ at 11–14 wk followed by a steady decrease. At 7–10 wk, GSP in women who developed diabetes was not elevated compared with normal concentration, although at 11–14 wk there was significant difference between the two groups ($P < 0.02$). In normal women, GSP remained constant throughout gestation. In gestational diabetes, GSP decreased to early pregnancy values ($P < 0.02$). Glycosylated blood proteins were elevated in early gestation in women who developed gestational diabetes and may have predictive value in identifying women who will develop diabetes in pregnancy. *DIABETES CARE* 1986; 9:107–10.

Assessment of the glycosylated blood proteins in pregnancy is of potential clinical utility, since euglycemia is associated with a significant reduction in fetal morbidity and mortality in diabetic pregnancy.¹ Although nonenzymatically glycosylated hemoglobin measured as hemoglobin A₁ (HbA₁) is a reliable indicator of blood glucose control in the nonpregnant diabetic woman,² its interpretation in diabetic pregnancy is limited by inadequate and conflicting data in normal pregnancy.^{3–5} In a cross-sectional study of 377 nondiabetic women, Phelps et al. observed significant biphasic changes, with HbA₁ concentrations decreasing to a nadir at 24 wk gestation and then increasing until term.⁶

No longitudinal studies of protein glycosylation in women with gestational diabetes have been performed. HbA₁ values in diabetic pregnancy are reportedly higher than those in normal pregnancy, but lower than those in the nonpregnant diabetic women.³ Elevation of maternal HbA₁ in the first and second trimester is correlated with an increased incidence of significant fetal anomalies.^{7,8}

In the present study, longitudinal changes in glycosylated hemoglobin concentration (GlyHb) and total glycosylated

serum protein concentration (GSP) in both normal pregnancy and pregnancy complicated by gestational diabetes were determined using affinity chromatography, a method in which nonenzymatically glycosylated proteins are specifically measured.

SUBJECTS AND METHODS

Seventy women aged 18–38 yr who received prenatal care at Duke University Medical Center between April 1983 and October 1984 participated in a longitudinal study of protein glycosylation in pregnancy. Women with a past history of gestational diabetes were specifically invited to participate in the study, and other subjects were chosen at random. Women who presented with GlyHb concentrations above the normal nonpregnant range ($>8.2\%$) or who had preexisting diabetes were excluded from the study as were women with cardiovascular or renal disease.

Informed consent was obtained from all study patients. All women were screened for diabetes by measuring the plasma glucose concentration 1 h after a 50-g oral glucose load at 10–15 wk gestation and by repeating the glucose challenge

screen after 23 wk gestation. Women whose plasma glucose screen was >134 mg/dl or who demonstrated glycosuria or excessive weight gain were evaluated by a 100-g oral glucose tolerance test (OGTT). Gestational diabetes was diagnosed if two or more values exceeded the criteria of the National Diabetes Data Group: fasting, 105 mg/dl; 1 h, 190 mg/dl; 2 h, 165 mg/dl; and 3 h, 145 mg/dl.⁹ Twenty-one women developed gestational diabetes and 49 remained normal.

All women were enrolled before 15 wk gestation. Fifteen normal women and six women who developed gestational diabetes were enrolled before 11 wk. In 10 women, diabetes was treated with diet therapy alone. In 11 women, diet therapy was supplemented with insulin therapy when fasting plasma glucose concentration was repeatedly >105 mg/dl. Treatment was begun when gestational diabetes was diagnosed by OGTT; therefore, most women were studied several weeks before therapy. Subjects were studied at intervals of 4 wk, at which time GlyHb and GSP concentrations were determined. In all subjects with gestational diabetes, glucose tolerance or postprandial glucose concentrations returned to normal by 6 wk postpartum.

GlyHb was measured by affinity chromatography¹⁰ using a modification of GlycoTest (Pierce Chemicals, Rockford, Illinois), and the mean normal nonpregnant value was $6.4 \pm 0.2\%$. Intraassay variation was 3.6% and interassay variation was 6.4%. Blood was collected in tubes containing EDTA and red cells were packed by centrifugation at $1500 \times g$. The packed cells were hemolyzed with water and stored at -70°C for 4–10 days before assay. Glycosylated and nonglycosylated hemoglobins were separated by chromatography

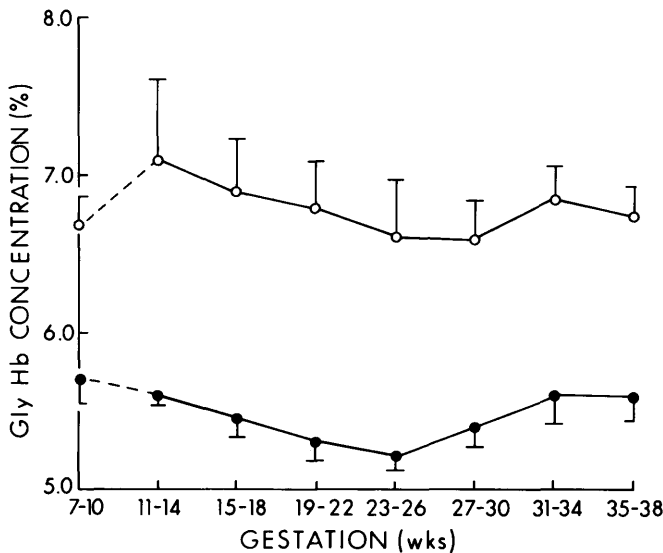


FIG. 1. Longitudinal assessment of GlyHb in pregnancy (mean \pm SEM). Nondiabetic women ($N = 49$) are designated \bullet and women who developed gestational diabetes ($N = 21$) are designated \circ . The dashed line connecting the 7–10- and 11–14-wk data points indicates the smaller sample size at 7–10 wk (15 nondiabetic women and 6 women who developed diabetes).

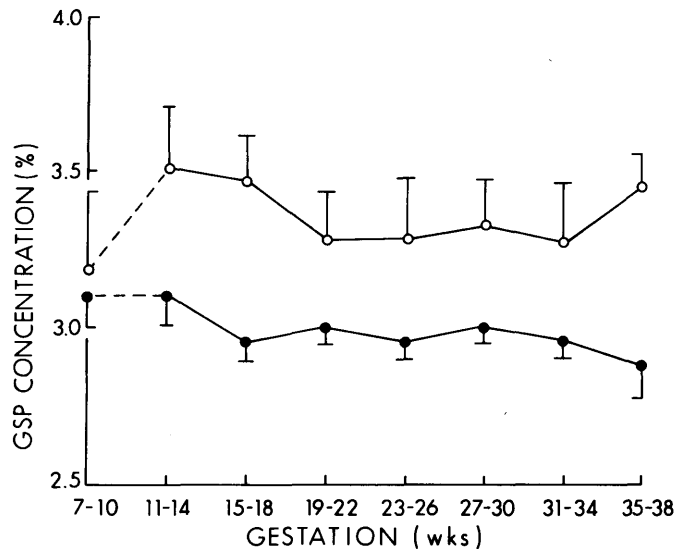


FIG. 2. Longitudinal assessment of GSP in pregnancy (mean \pm SEM). Nondiabetic women ($N = 49$) are designated \bullet and women who developed gestational diabetes ($N = 21$) are designated \circ . The dashed line connecting the 7–10- and 11–14-wk data points indicates the smaller sample size at 7–10 wk (15 nondiabetic women and 6 women who developed diabetes).

on columns of 0.5-ml bed volume packed with immobilized *m*-aminophenylboronic acid on a support of crosslinked 6% agarose. Nonglycosylated hemoglobin was eluted in a 0.25 M ammonium acetate, 0.5 M magnesium chloride buffer (pH 8.0), and glycosylated hemoglobin in a 0.2 M sorbitol, 0.1 M tris buffer (pH 8.5). To standardize the GlyHb assay, frozen aliquots of hemolysates with GlyHb concentrations of 2.7%, 5.2%, 7.0%, and 12% (which were stable through the 18-mo study period) were employed. GSP was measured by affinity chromatography using GlycoGel (Pierce). Using this method, the mean normal nonpregnant GSP was $3.6 \pm 0.2\%$. Intraassay variation was 4.1% and interassay variation was 6.4%. To standardize the assay, sera with GSP concentrations of 2.0%, 4.2%, 6.5%, and 9.0% were used. Statistical analyses were made by Student's paired and nonpaired *t*-tests. Results are presented as \pm SEM.¹¹

RESULTS

GlyHb was determined longitudinally in all women as shown in Figure 1. At 7–10 wk gestation, GlyHb in normal women was $5.7 \pm 0.2\%$. In women who developed gestational diabetes, GlyHb was significantly increased to $6.7 \pm 0.2\%$ ($P < 0.001$) and remained significantly elevated throughout gestation compared with normal values ($P < 0.001$ at 11–14, 15–18, 19–22, 23–26, 27–30, 31–34, and 35–38 wk).

In normal pregnancy, GlyHb decreased progressively from the 11–14-wk value, reaching a nadir of $5.2 \pm 0.1\%$ at 23–26 wk ($P < 0.001$). It returned to baseline concentration ($5.6 \pm 0.2\%$) by 31–34 wk gestation.

In gestational diabetes, there was an initial increase in GlyHb to $7.1 \pm 0.5\%$ at 11–14 wk gestation, which was significant in the six women studied at both 7–10 wk and 11–14 wk ($P < 0.01$, paired *t*-test). After the initial increase, GlyHb decreased steadily to $6.6 \pm 0.4\%$ at 23–26 wk, there was no further change through 35–38 wk.

As shown in Figure 2, the concentration of total glycosylated serum proteins at 7–10 wk gestation was $3.1 \pm 0.1\%$ in normal women and $3.2 \pm 0.3\%$ in women who developed gestational diabetes (NS). At 11–14 wk, GSP in women who developed gestational diabetes was elevated compared with values in normal women ($3.5 \pm 0.2\%$ versus $3.1 \pm 0.1\%$, respectively, $P < 0.02$).

In normal women, GSP remained constant throughout gestation. In women who developed gestational diabetes, concentrations in midgestation were decreased from values at 11–14 wk ($P < 0.02$ at 19–22 wk, 23–26 wk, 27–30 wk, and 31–34 wk, paired *t*-test).

DISCUSSION

In the present study, concentrations of GlyHb and GSP were determined at 4-wk intervals throughout gestation in 21 women who developed gestational diabetes and compared with values in 49 nondiabetic control women. This is the first report of serial determinations of GlyHb made longitudinally in gestational diabetic women and of GSP concentrations throughout pregnancy.

GlyHb concentration in nondiabetic women decreases until midpregnancy and subsequently increases to early pregnancy values by 34–38 wk, consistent with changes in HbA_{1c} noted by Phelps et al.⁶ Other investigators have reported that HbA_{1c} does not vary with the stage of gestation¹² or decreases sequentially from 12 to 40 wk.⁵ However, these studies were done by ion-exchange chromatography, a technique of limited sensitivity as hemoglobin F co-elutes with HbA₁ and changes in temperature and pH affect assay results.

GlyHb in women who develop gestational diabetes is elevated by 7–10 wk gestation and remains elevated throughout pregnancy. By 7–10 wk, GlyHb is already increasing in women who develop gestational diabetes and decreasing in nondiabetic women. In gestational diabetes, GlyHb increases or remains constant in all patients before the onset of therapy. Peak concentrations are reached by 11–14 wk followed by decreases until 23–26 wk. This decrease is due in part to therapeutic intervention started once the diagnosis of gestational diabetes has been established. Whereas increased HbA₁ has been previously reported in 25 diabetic women in the first trimester, only 1 of these women had gestational diabetes.¹³

GSP concentrations, which remain constant throughout gestation in normal women, are not consistently elevated in women who develop gestational diabetes. In nonpregnant subjects, GSP is less sensitive in predicting glucose intolerance than is GlyHb,¹⁴ possibly because it reflects blood glucose regulation over a shorter period of time; therefore, GSP may

not be as effective as GlyHb in detecting minor changes in blood glucose concentrations in pregnancy.

In conclusion, glycosylated blood proteins are elevated in early gestation in women who develop gestational diabetes and may have predictive value in identifying those women who will develop gestational diabetes. Longitudinal changes of GlyHb and GSP in pregnancy are a reflection of changes in blood glucose concentrations. The finding of increased GlyHb by 7–10 wk in women who develop gestational diabetes suggests that GlyHb in pregnancy, as in the nonpregnant state, reflects glycemia over approximately a 4-wk period. In interpreting GlyHb or GSP in pregnancy, it is necessary that a discrete gestational interval be specified. The current study provides normative data for GlyHb and GSP in both normal pregnancy and pregnancy complicated by gestational diabetes.

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