Elevated Pregnancy Losses at High and Low Extremes of Maternal Glucose in Early Normal and Diabetic Pregnancy

Evidence for a protective adaptation in diabetes

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FOR THE DIABETES IN EARLY PREGNANCY STUDY GROUP

OBJECTIVE — Early pregnancy losses increase with marked hyperglycemia in diabetic pregnancy. However, mean loss rates do not differ from those of nondiabetic pregnancy. This observation might be explained by increased fetal losses at the extremes of glycemia in diabetic and nondiabetic pregnancy. To test this hypothesis, we examined relationships of proximate measures of prior glycemia, glycated protein and fructosamine, to pregnancy loss.

RESEARCH DESIGN AND METHODS — A total of 389 diabetic and 429 nondiabetic pregnant subjects participated in the Diabetes In Early Pregnancy study. Glycated protein and fructosamine measurements were standardized as multiples of control values for each center (*Z* score). The logarithm of odds of pregnancy loss were plotted against *Z* scores and tested by logistic models.

RESULTS — Mean pregnancy loss rates were 12% in diabetic and 13% in normal pregnancies. However, over six intervals of glycated protein in diabetic pregnancy, fetal loss rates at the upper and lower extremes (24 and 33%, respectively) were approximately threefold higher than the four intervening rates (8–14%). The odds ratio of pregnancy loss for these extreme intervals to the intervening intervals is 3.0 ($P = 0.01$). Nondiabetic losses showed a similar pattern. In confirmation, logit pregnancy losses were increased in a J-shaped curve at the glycemic extremes in normal ($P < 0.019$) and diabetic ($P < 0.015$) pregnancy. The upper glycemic extreme in diabetic pregnancy was two- to fivefold higher than in control pregnancy.

CONCLUSIONS — Pregnancy losses are increased at the extremes of glycemia in both normal and diabetic pregnancy but at higher levels in diabetic pregnancy. The data suggest defensive adaptations against hyperglycemia in diabetic pregnancy.

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Abbreviations: DIEP, Diabetes In Early Pregnancy; NICHD, National Institute of Child Health and Human Development.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

The National Institute of Child Health and Human Development (NICHD)-sponsored Diabetes In Early Pregnancy (DIEP) study found similar early pregnancy loss rates in nondiabetic and diabetic pregnancies (odds ratio [OR] 0.91 in diabetic vs. nondiabetic pregnancy adjusted for other risk factors associated with spontaneous abortion) (1). However, diabetic women who had a spontaneous abortion had higher fasting and postprandial glucose levels than those delivering a live infant at term (1). Further, a 1-SD increase in first trimester glycated hemoglobin from normal was associated with a 3.1% increase in the rate of pregnancy loss, and a 4-SD increase was associated with a >40% pregnancy loss rate compared with a normal pregnancy mean of 14% (1). The association of pregnancy loss with poor glycemic control in diabetic pregnancy has been observed previously (2,3).

Other studies have shown increased perinatal morbidity associated with low blood glucose levels in nondiabetic and diabetic pregnancies (4–6). However, in the initial DIEP report, low glycated hemoglobin levels were not associated with pregnancy losses in normal or diabetic pregnancy. Since the vulnerable period for first trimester glycemic abnormality is a shorter interval than that represented by glycated hemoglobin, we reasoned that shorter-term measures of integrated glycemic status of 2–3 weeks' duration as reflected in plasma glycated protein and fructosamine levels (7–18) might provide a more proximate and accurate (16) indication of the glycemic association of pregnancy loss.

We therefore measured levels of glycated protein and fructosamine in stored plasma from the diabetic and normal pregnant subjects participating in the DIEP in order to answer the following questions: *1*) Are early pregnancy losses associated with low as well as high levels of glycemia in diabetic pregnancy? 2) Are pregnancy losses in nondiabetic preg-

Table 1—*Fetal loss rates across Z score intervals for glycated protein*

	Nondiabetic pregnancy		Diabetic pregnancy	
Z score intervals	Fetal losses/ live births	Fetal loss (%)	Fetal losses/ live births	Fetal loss (%)
<−1.0	6/38	16	4/12	33
−1.0 to <1.0	40/337	12	8/102	8
1.0 to <2.0	6/48	13	14/99	14
2.0 to <3.0	3/6	50	12/108	11
3.0 to <4.0	—	—	5/47	11
≥4.0	—	—	5/21	24

nancy associated with low or high glucose concentrations? *3*) Are the levels of glycemia associated with perinatal loss similar or different in nondiabetic and diabetic pregnancy?

RESEARCH DESIGN AND METHODS

— The DIEP was conducted in five academic centers, Cornell University Medical College, Harvard Medical School, Northwestern University Medical School, the University of Pittsburgh Medical School, and the University of Washington School of Medicine. The sponsor of the study and data-coordinating center was the NICHD, Bethesda, Maryland. The study design has been described in detail previously (1,17–19). Briefly, insulin-dependent diabetic women contemplating pregnancy were recruited by public advertising, news releases, and contacting clinics specializing in diabetes between the years of 1980 and 1985. The diagnosis of pregnancy was made during the 1st week of missed menses by plasma human chorionic gonadotropin determination. Diabetic subjects were then admitted to a metabolic ward for monitoring and educated on home glucose monitoring, diary keeping, and insulin adjustment. Fixed goals of glycemic management were not mandated. Nondiabetic control subjects were screened for gestational diabetes at 26 weeks' gestation (20) and were excluded from the control cohort if they were positive. Glycated protein measurements were performed in 429 control and 389 diabetic pregnancies and fructosamine in 386 control and 358 diabetic pregnancies. The methods of early pregnancy diagnosis, pregnancy dating, and assessment of pregnancy loss have been described in detail previously (1). Early pregnancy loss was defined as <20 weeks' gestation (1).

Plasma or serum samples were collected weekly from diabetic subjects from gestational weeks 4–12. Nondiabetic subjects had blood samples drawn at biweekly intervals from weeks 4 through 12. Frozen samples were initially shipped to the NICHD and stored at −20° C and then to analytical sites and stored at −80° C.

Glycated protein assay. Glycated serum and plasma proteins were assessed with use of boronate affinity high-performance liquid chromatography methodology (CLC 330 Glycated Hemoglobin Analyzer; Primus, Kansas City, MO) in the laboratories at Sansum Diabetes Research Institute. The normal ranges for glycated serum and plasma protein levels are 16.1–23.0% and 20.1–25.0%, respectively. The interassay coefficient of variation (CV) for glycated protein in serum was 0.87% and in plasma 1.73%.

Fructosamine assay. This assay was performed on thawed and refrozen samples after the glycated protein assay was shipped on dry ice to the reference laboratory. The assay is based on the reduction of nitroblue tetrazolium by the ketoamine bond of glycated protein (15). The analysis was performed using a Ro-TAG kit (Hoffmann-La Roche, Nutley, NJ) adapted to a COBAS BIO centrifugal analyzer (21) (Roche Biomedical Laboratories, Indianapolis, IN). The normal range is 1.0–2.7 mmol/l with a CV of 3.6% in replicate analyses.

Statistical methods. Samples obtained in the first trimester were grouped into three intervals: 4–6, 7–9, and 10–12 weeks of gestation. The mean and maximal values of glycated protein and fructosamine measurements within each of these intervals were standardized to adjust for between-center and plasma versus serum differences by subtracting the value for the corresponding control group and dividing by the SD (standardized value or *Z* score). The data from individual subjects from all five centers were then combined for analysis. The analysis was carried out using both the mean and maximal measurements in each time interval. However, little difference was seen between the analyses of mean and maximal values; maximal values are therefore presented hereafter.

Descriptive statistics were produced and ordinary and chain logistic regression methods applied to assess the relationship between the protein level and early pregnancy loss. The chain logistic model (22) was used based on the rationale that pregnancy loss in a given time interval might be affected by the values of the glycated protein and fructosamine levels in the preceding interval as well as that interval. The chain logistic model allows a life-table analysis structure between pregnancy loss and covariates as used previously for survival data analysis (22).

RESULTS

— Mean standardized maximal glycated protein values were similar between the nondiabetic control pregnancies that resulted in live births and those that resulted in fetal losses (0.0 ± 0.8 and 0.1 ± 1.0, $P = 0.64$). Mean standardized maximal glycated protein values were also similar in the diabetic pregnancies that resulted in live births and fetal losses (1.7 ± 1.4 and 1.9 ± 1.8, $P = 0.53$), despite the fact that mean values in the diabetic group were nearly twofold greater than in the control group.

Pregnancy loss rates at different levels of standardized glycated protein *Z* scores are presented in Table 1. Diabetic pregnant women with a *Z* score ≥4.0 above the mean for normal subjects had a pregnancy loss rate of 24% (5 of 21). Those with a *Z* score <1.0 below the mean had a pregnancy loss rate of 33% (4 of 12). Pregnancy loss rates in the intervening *Z* score intervals were 8% in the −1.0 to <1.0 interval, 14% in the 1.0 to <2.0 interval, and 11% in the 2.0 to <3 and 3.0 to <4.0 intervals for an average of 11% (39 of 356) in the −1.0 to <4.0 interval. When two extreme *Z* score intervals are collapsed (i.e., ≥4.0 and <−1.0) and compared with the intermediate *Z* score intervals (i.e., −1.0 to 4.0), the difference in pregnancy loss rate is significant (OR 3.0 [95% CI 1.3–7.0], $P = 0.01$ using two-sided Fisher's exact test). A similar pattern across strata was observed for

Table 2—*Relationships of glycated protein values to fetal loss by two methods of logistic regression*

	Nondiabetic pregnancy (n = 429)			IDDM Pregnancy (n = 389)		
	β ± SE	χ^{2}*	P	β ± SE	χ^{2}*	P
Ordinary logistic regression†						
Intercept	−2.16 ± 0.18			−2.00 ± 0.23		
Glycated protein (x)	−0.04 ± 0.17	0.32	0.57	−0.26 ± 0.16	0.65	0.42
Glycated protein (x^2)	0.31 ± 0.13	5.48	0.019	0.09 ± 0.04	5.89	0.015
Chain logistic regression‡						
Intercept	−2.83 ± 0.58			−2.83 ± 0.79		
Glycated protein (x)	−0.20 ± 0.16	1.49	0.22	−0.32 ± 0.17	2.88	0.09
Glycated protein (x^2)	0.22 ± 0.07	6.56	0.01	0.07 ± 0.04	3.23	0.07

*Likelihood ratio test; †n = 429 nondiabetic and 389 type 1 diabetic pregnancies; ‡n = 332 nondiabetic and 251 type 1 diabetic pregnancies.

fructosamine values and pregnancy losses (data not shown).

Normal control subjects had a similar pattern of pregnancy loss rates, higher at the extremes and lower for the middle Z score values but over a narrower range than in the diabetic subjects, ranging from <–1.0 to <3.0 Z score values. Loss rates were 50, 13, 12, and 16%, respectively, in the nondiabetic control subjects at the four intervals of Z score values shown in Table 1. Over the entire range of values, the loss rates were nearly identical in the diabetic and nondiabetic pregnancies at 12 and 13%, respectively, as seen previously (1). Again, a similar pattern was seen for standardized fructosamine values and pregnancy losses (data not shown).

To analyze the data as a continuum, ordinary logistic regression and chain logistic regression analyses were performed. As presented in Table 2, sample sizes are lower in the chain logistic model than in the ordinary logistic model because not all subjects had values at all intervals, causing a break in the chain logistic regression model for those subjects. Regardless, both analyses indicate an excess of early pregnancy loss at the higher and lower extremes of glycemic status in both diabetic and nondiabetic pregnancies. In the ordinary logistic regression model, the quadratic term (x^2) testing the J-shaped curve of standardized glycated protein level versus pregnancy losses was statistically significant (P = 0.019 in nondiabetic subjects and P = 0.015 in the diabetic subjects). In the chain logistic model, the value for x^2 shows a P value of 0.07 in the diabetic subjects and 0.01 in the control subjects. Values for fructosamine were not statistically significant and are not shown.

Logit plots of pregnancy loss versus individual value of glycated protein and fructosamine (i.e., fitted logistic models) are presented in Fig. 1*A* and *B*, respectively, obtained from ordinary logistic regression analysis along with the 95% CI. As seen in Fig. 1, diabetic women with a pregnancy loss and a Z score <–1.0 or >4.0 have higher loss rates. For example, for a Z score of −2, the predicted logit is −1.13 and the corresponding predicted probability of pregnancy loss 0.32. For a Z score of 4.5, the predicted logit is −1.36 and the corresponding predicted probability of pregnancy loss 0.26. For a Z score of 0, the predicted probability of pregnancy loss is 0.14. Nondiabetic control pregnant women have a similar pattern but over a narrower range of elevated values. For example, for a Z score of −1.5, the predicted logit is −1.40 and the corresponding predicted probability of pregnancy loss 0.25. For a Z score of 2, the predicted logit is −1.00 and the corresponding predicted probability of pregnancy loss is 0.37. For a Z score of 0, the predicted probability of pregnancy loss is 0.12. The 95% CIs parallel the logit plots and confirm the curvilinearity of the J-shaped plots. The fructosamine values confirm the same trends, though by an entirely separate and analytical method.

CONCLUSIONS — The DIEP addressed the following two questions: *1*) what is the relationship between glucose levels and malformations? and *2*) what is the relationship between these levels and the risk of spontaneous abortion in pregnancies complicated by insulin dependent diabetes (1,18)? The first reports from this study used glycated hemoglobin as a marker of glycemic control (23). The analysis of the relationship of glycated hemoglobin to spontaneous abortion revealed an indistinguishable pregnancy loss rate in the diabetic and control groups (1). However, successive SDs in glycated hemoglobin above the normal range were associated with progressive increases in pregnancy losses. Other investigators have also shown a relationship between glycemia and pregnancy losses in diabetic women (2,3), but none have determined whether the relationship extends to normal pregnancy and whether there is risk for fetal loss if the glycemic levels are too low. The latter possibility is supported by the early observations of Abell et al. (4) who observed increased perinatal losses in pregnant mothers with a flat glucose tolerance test curve, a risk that was exaggerated by the presence of other indexes of fetal distress. This observation implies a role for fetal malnutrition based on impaired delivery of maternal nutrients to the fetus that could extend to the first trimester as well, which could also be aggravated by additional risk factors (24). To our knowledge, no studies have investigated the potential for a deleterious effect of low glycemic status in the first trimester on spontaneous abortion in normal and diabetic pregnancy.

Additional evidence of a deleterious effect of glycemic deficiency has been reported. Glycemic deficiency was implicated in teratogenicity and fetal resorption in rodents (25). Fetal growth retardation was associated with hypoglycemia or low-normal glycemic levels in a diabetic population (26). The DIEP study may not have found a relationship between low-level glycated hemoglobin and first trimester loss (1) because of the prolonged time constant for change dictated by the 120-day lifespan of red cells. This time constant is well in excess of the short-term events relating glycemic status

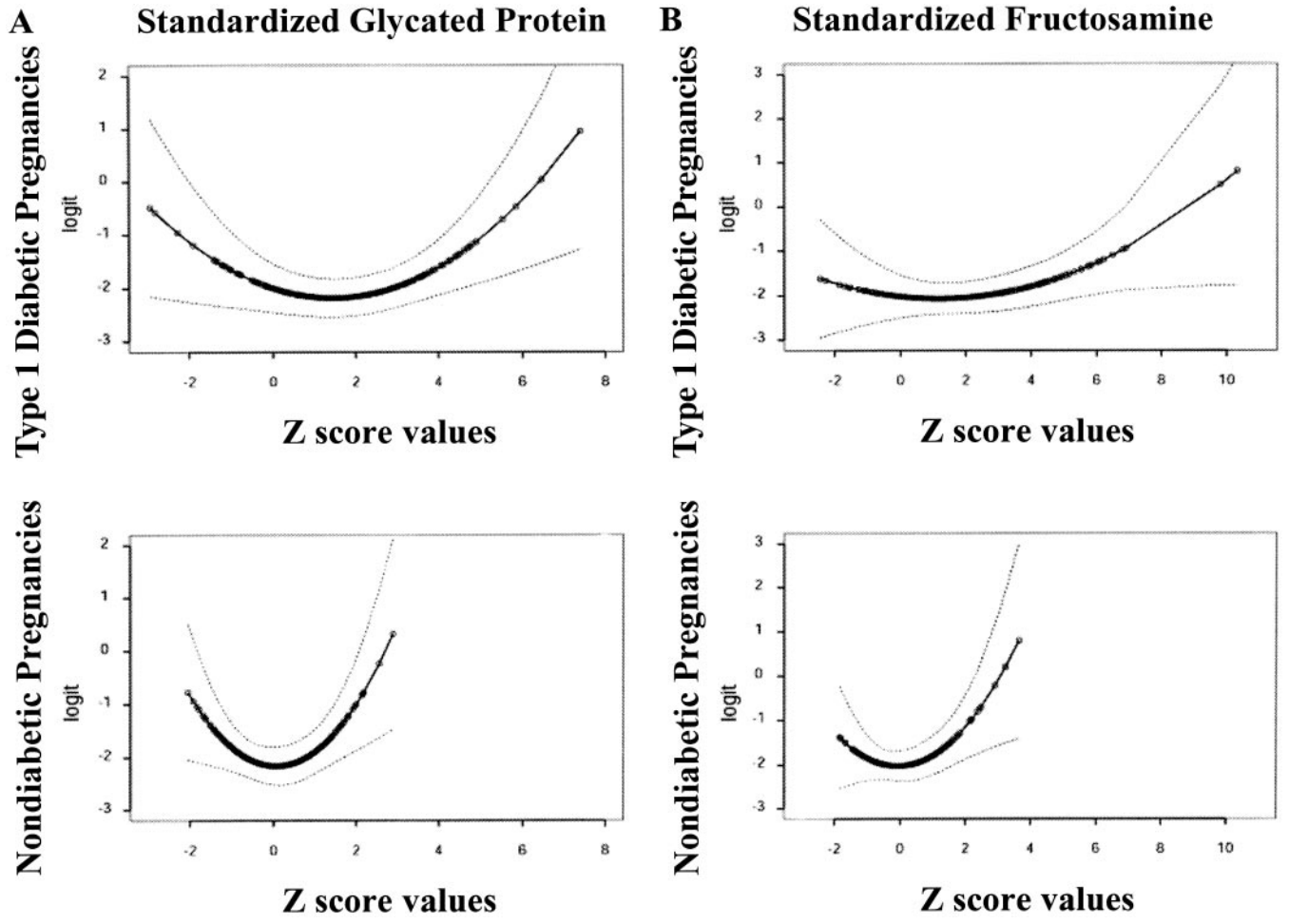

Figure 1—A*: Logit plots of pregnancy loss versus standardized glycated protein values in type 1 diabetic and nondiabetic pregnancy.* B*: Logit plots of pregnancy loss versus standardized fructosamine values in type 1 diabetic and nondiabetic pregnancy. Dashed lines represent the 5th and 95th percentile CIs.*

to fetal well being in the 8 weeks that can be feasibly monitored in the first trimester. In this light, the measurement of protein glycation is a more serviceable research monitor of glycemic status related to first trimester pregnancy loss and teratogenesis.

Our observations showing a relationship of pregnancy loss to protein glycation are in keeping with other studies showing that glycated proteins are a more sensitive measure of fluctuations of blood glucose in the short term compared with glycated hemoglobin (13,16). Further research is justified to understand adverse fetal and neonatal outcome at glycemic extremes, using protein glycation as an index of intermediate-term glycemic status in both diabetic and nondiabetic pregnancy.

A provocative observation in this study is the finding that early pregnancy loss rates in diabetic pregnancy do not increase until glycated protein *Z* scores are >4.0, whereas loss rates in nondiabetic pregnancies increase when glycated protein *Z* scores exceed 2.0. This finding suggests that adaptation may occur to chronic hyperglycemia in the diabetic pregnancy, allowing the embryo or fetus of the diabetic mother to resist the deleterious effect of hyperglycemia. Such adaptations could be anti-inflammatory, osmotic, or involving antioxidant and vasodilatory defense mechanisms that have been observed in in vitro systems under oxidant stress (27–30). For instance, aortic endothelial cells exposed to hydrogen peroxide showed an initial thromboxane and prostacyclin rise, then a decline over a 24-h incubation period (R.H.K. and X.-D.Z., unpublished data). Understanding these mechanisms may provide an approach to the prevention or treatment of some diabetes complications.

This investigation is the first to show increased pregnancy losses at glycemic extremes of both normal and diabetic pregnancies in the largest cohort of normal and diabetic subjects studied to date. Limitations are the smaller numbers of subjects at the extremes and the hypothesizing rather than a priori hypothesis testing nature of this report. The speculation of nutritive deficiency on the one hand and glycemic injury on the other requires further confirmation and investigation.

In conclusion, we have shown that both high and low extremes of glycemia as measured by protein glycation are associated with increased risk for early pregnancy loss. The J-shaped curve relating high and low glycemic status to first trimester pregnancy loss applies to both diabetic and nondiabetic pregnancies. At the high extreme of glycemia, even short-term toxicity of severe hyperglycemia

may impact fetal viability. At the low extreme of glycemia, impaired fetal nutrition is implicated. However, the level of hyperglycemia tolerated in the diabetic pregnancy before reaching the threshold of fetal vulnerability is higher than that in nondiabetic pregnancy, implying maternal, placental, or fetal defenses to the stress of hyperglycemia. Important clues to the prevention of diabetes complications may be embedded in this interesting observation and deserve future research.

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References

1. Mills JL, Simpson JL, Driscoll SG, Jovanovic-Peterson L, Van Allen M, Aarons JH, Metzger B, Bieber FR, Knopp RH, Holmes LB, et al.: Incidence of spontaneous abortion among normal women and insulin-dependent diabetic women whose pregnancies were identified within 21 days of conception. *N Engl J Med* 319:1617–1623, 1988
2. Greene MF, Hare JW, Cloherty JP, Benacerraf BR, Soeldner JS: First-trimester hemoglobin A1 and risk for major malformation and spontaneous abortion in diabetic pregnancy. *Teratology* 39:225–231, 1989
3. Miodovnik M, Mimouni F, Tsang RC, Ammar E, Kaplan L, Siddiqi TA: Glycemic control and spontaneous abortion in insulin-dependent diabetic women. *Obstet Gynecol* 68:366–369, 1986
4. Abell DA, Beischer NA, Papas AJ, Willis MM: The association between abnormal glucose tolerance (hyperglycemia and hypoglycemia) and estriol excretion in pregnancy. *Am J Obstet Gynecol* 124:388–392, 1976
5. Raman L, Rao VA, Kumar S: Influence of maternal levels of blood glucose on fetal outcome. *Int J Gynaecol Obstet* 20:363–369, 1982
6. Langer O, Damus K, Maiman M, Divon M, Levy J, Bauman W: A link between relative hypoglycemia-hypoinsulinemia during oral glucose tolerance tests and intrauterine growth retardation. *Am J Obstet Gynecol* 155:711–716, 1986
7. Beisswenger PJ, Healy JC, Shultz EK: Glycosylated serum proteins and glycosylated hemoglobin in the assessment of glycemic control in insulin-dependent and non-insulin-dependent diabetes mellitus. *Metabolism* 42:989–992, 1993
8. Tahara Y, Shima K: Kinetics of HbA_{1c}, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. *Diabetes Care* 18:440–447, 1995
9. Kennedy L, Mehl TD, Riley WJ, Merimee TJ: Non-enzymatically glycosylated serum protein in diabetes mellitus: an index of short-term glycaemia. *Diabetologia* 21: 94–98, 1981
10. Schleicher ED, Gerbitz KD, Dolhofer R, Reindl E, Wieland OH, Edelmann E, Haslbeck M, Kemmler W, Walter H, Mehnert H: Clinical utility of nonenzymatically glycosylated blood proteins as an index of glucose control. *Diabetes Care* 7:548–556, 1984
11. Schleicher ED, Mayer R, Wagner EM, Gerbitz KD: Is serum fructosamine assay specific for determination of glycated serum protein? *Clin Chem* 34:320–323, 1988
12. Shima K, Abe F, Chikakiyo H, Ito N: The relative value of glycated albumin, hemoglobin A1c and fructosamine when screening for diabetes mellitus. *Diabetes Res Clin Pract* 7:243–250, 1989
13. Lee PD, Sherman LD, O'Day MR, Rognerud CL, Ou CN: Comparisons of home blood glucose testing and glycated protein measurements. *Diabetes Res Clin Pract* 16:53–62, 1992
14. Johnson RN, Metcalf PA, Baker JR: Fructosamine: a new approach to the estimation of serum glycosylprotein: an index of diabetic control. *Clin Chim Acta* 127:87–95, 1983
15. Fluckiger R, Woodtli T, Berger W: Evaluation of the fructosamine test for the measurement of plasma protein glycation. *Diabetologia* 30:648–652, 1987
16. Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A: Correlation of glucose regulation and hemoglobin A1c in diabetes mellitus. *N Engl J Med* 295:417–420, 1976
17. Jovanovic L, Singh M, Saxena BB, Mills JL, Tulchinsky D, Holmes LB, Simpson JL, Metzger BE, Labarbera A, Aarons J, et al.: Verification of early pregnancy tests in a multicenter trial. *Proc Soc Exp Biol Med* 184:201–205, 1987
18. Mills JL, Knopp RH, Simpson JL, Jovanovic-Peterson L, Metzger BE, Holmes LB, Aarons JH, Brown Z, Reed GF, Bieber FR, et al.: Lack of relation of increased malformation rates in infants of diabetic mothers to glycemic control during organogenesis. *N Engl J Med* 318:671–676, 1988
19. Mills JL, Fishl AR, Knopp RH, Ober CL, Jovanovic LG, Polk BF: Malformations in infants of diabetic mothers: problems in study design. *Prev Med* 12:274–286, 1983
20. Proceedings of the Second International Workshop-Conference on Gestational Diabetes Mellitus, October 25–27, 1984, Chicago, Illinois. *Diabetes* 34 (Suppl. 2): 1–130, 1985
21. Cefalu WT, Prather KL, Chester DL, Wheeler CJ, Biswas M, Pernoll ML: Total serum glycosylated proteins in detection and monitoring of gestational diabetes. *Diabetes Care* 13:872–875, 1990
22. Hosmer DW, Lemeshow S: *Applied logistic regression*. New York, Wiley, 1989
23. Pecoraro RE, Graf RJ, Halter JB, Beiter H, Porte D Jr.: Comparison of a colorimetric assay for glycosylated hemoglobin with ion-exchange chromatography. *Diabetes* 28:1120–1125, 1979
24. Mills JL, Jovanovic L, Knopp R, Aarons J, Conley M, Park E, Lee YJ, Holmes L, Simpson JL, Metzger B: Physiological reduction in fasting plasma glucose concentration in the first trimester of normal pregnancy: the diabetes in early pregnancy study. *Metabolism* 47:1140–1144, 1998
25. Buchanan TA, Schemmer JK, Freinkel N: Embryotoxic effects of brief maternal insulin-hypoglycemia during organogenesis in the rat. *J Clin Invest* 78:643–649, 1986
26. Combs CA, Gunderson E, Kitzmiller JL, Gavin LA, Main EK: Relationship of fetal macrosomia to maternal postprandial glucose control during pregnancy. *Diabetes Care* 15:1251–1257, 1992
27. Panayiotidis M, Tsolas O, Galaris D: Glucose oxidase-produced H2O2 induces Ca2+-dependent DNA damage in human peripheral blood lymphocytes. *Free Radic Biol Med* 26:548–556, 1999
28. Maziere C, Dantin F, Dubois F, Santus R, Maziere J: Biphasic effect of UVA radiation on STAT1 activity and tyrosine phosphorylation in cultured human keratinocytes. *Free Radic Biol Med* 28:1430–1437, 2000
29. Meilhac O, Ramachandran S, Chiang K, Santanam N, Parthasarathy S: Role of arterial wall antioxidant defense in beneficial effects of exercise on atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 21: 1681–1688, 2001
30. Nakamura J, Purvis ER, Swenberg JA: Micromolar concentrations of hydrogen peroxide induce oxidative DNA lesions more efficiently than millimolar concentrations in mammalian cells. *Nucleic Acid Res* 31: 1790–1795, 2003