OBSTETRICS

The role of preanalytical glycolysis in the diagnosis of gestational diabetes mellitus in obese women

Niamh Daly, MD; Mary Stapleton, MSc; Ruth O'Kelly, FRCPath; Brendan Kinsley, MD; Sean Daly, MD; Michael J. Turner, MD

OBJECTIVE: The objective of this prospective observational study was to determine whether the preanalytical management of maternal plasma glucose samples had a significant effect on glucose measurements in obese pregnant women.

STUDY DESIGN: Based on the accurate calculation of body mass index in the first trimester, obese women were recruited at their convenience. In 1 cohort, fasting glucose level was measured in early pregnancy; in the other cohort, an oral glucose tolerance test was performed at 24-28 weeks' gestation. Paired samples were taken from all women in both cohorts. The first sample was transferred to the laboratory in iced water for immediate analysis (fast-tracked analysis). The second sample was not placed on ice and transferred according to established hospital practices (hospital-tracked analysis).

RESULTS: Of the 24 women who had a fasting glucose test in early pregnancy, the result was abnormal (≥5.1 mmol/L) in 7 women (29%) with hospital-tracked analysis compared with 16 women (67%) with fast-tracked analysis ($P < .01$). The mean phlebotomy-analysis interval was 119 minutes for the hospital-tracked samples compared with 23 minutes for the fast-tracked samples ($P < .001$). Of the 24 women who had a glucose tolerance test, the fasting glucose level was abnormal in 4 women (17%) after hospital-tracked analysis compared with 13 women (54%) after fast-tracked analysis ($P < .01$). The hospital-tracked phlebotomy-analysis interval for the fasting sample of the 24-28 week oral glucose tolerance test cohort was 166 minutes compared with 25 minutes for the fast-tracked samples ($P < .001$).

CONCLUSION: Unless maternal fasting glucose samples are transported on ice and analyzed immediately in the laboratory, gestational diabetes mellitus will be underdiagnosed in obese women.

Key words: gestational diabetes mellitus, maternal glucose, maternal obesity, oral glucose tolerance test, preanalytical glycolysis

Cite this article as: Daly N, Stapleton M, O'Kelly R, et al. The role of preanalytical glycolysis in the diagnosis of gestational diabetes mellitus in obese women. Am J Obstet Gynecol 2015;213:84.e1-5.

There is a strong epidemiologic association between maternal obesity and gestational diabetes mellitus (GDM). A metaanalysis concluded that, compared with women with a normal body mass index (BMI), GDM was increased 2-fold in women with mild obesity, 4-fold in women with moderate obesity, and 8-fold in women with severe obesity.[1] There is a consensus that, based on a BMI of >29.9 kg/m^2, maternal obesity is an indication for selective screening for GDM with an oral glucose tolerance test (OGTT) at 24-28 weeks' gestation.[2-4] It has also been recommended that obese women should be screened for GDM soon after they seek prenatal care if they have not been screened recently for type 2 diabetes mellitus[5-7] or that fasting plasma glucose level be measured on all women at first prenatal visit.[8]

The diagnosis of GDM has been transformed after the publication of the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study in women who had mild hyperglycemia during pregnancy.[9] After publication, the criteria for diagnosis of GDM were made more sensitive, and the diagnosis can be made based on 1 abnormal OGTT value and not 2 values as previously.[4,8] These changes are contentious and have been challenged.[10-15] Influential professional bodies, however, have endorsed the new criteria, as has the World Health Organization.[4,8] A key component of the HAPO Study was the standardization of research conditions with particular attention paid to sample handling and centralization of maternal glucose measurements.[16]

Materials and Methods

This study was conducted between April 2014 and August 2014. It was confined to white European obese women, based on a BMI >29.9 kg/m^2 in early pregnancy, after accurate measurement of weight and height. The women were recruited at their convenience after sonographic confirmation of a healthy ongoing

From the University College Dublin Centre for Human Reproduction (Drs N Daly and Turner), Coombe Women and Infants University Hospital (Ms Stapleton, Ms O'Kelly, and Drs Kinsley and S Daly), Dublin, Ireland.

Received Nov. 11, 2014; revised Jan. 14, 2015; accepted March 10, 2015.

Supported by the Coombe Women and Infants University Hospital and by Friends of the Coombe.

The authors report no conflict of interest.

Presented at the 35th annual meeting of the Society for Maternal-Fetal Medicine, San Diego, CA, Feb. 2-7, 2015.

Corresponding author: Niamh Daly, MD. drniamhdaly@gmail.com

0002-9378/$36.00

http://dx.doi.org/10.1016/j.ajog.2015.03.022

TABLE 1
Characteristics of study populations

Variable	Gestation at testing	
	11-18 wk (n = 24)	24-28 wk (n = 24)
Mean age, y (range)	29.0 (18–40)	30.0 (20–36)
Mean body mass index, kg/m^2 (range)	34.6 (30.0–42.0)	34.8 (30.0–43.5)
Mean gestation at time of measurement, wk (range)	14.7 (10.9–18.0)	27.6 (26–28)
Nulliparous, %	29.2 (n = 7)	29.2 (n = 7)

Daly. Preanalytical glycolysis of maternal glucose samples in obese women. Am J Obstet Gynecol 2015.

singleton pregnancy at <18 weeks' gestation. They were given an information leaflet; written consent was obtained. Exclusion criteria were evaluation at >18 weeks' gestation, <18 years old, BMI < 30.0 kg/ m^2, multiple pregnancy, or a preexisting serious medical disorder.

The primary outcome was an elevated glucose level on a fasting sample either alone or at the time of a 75-g OGTT, according to the 2013 World Health Organization (WHO) criteria.[4] A power calculation was made for a secondary outcome of a significant difference of mean maternal fasting glucose level of 0.4 mmol/L similar to that used for the HAPO study.[9] Statistically, it was determined that an adequate power (>0.80) and a 5% significance level would be achieved with 24 paired samples. Paired Student *t* test and McNemar's test for correlated proportions were used to test for statistical significance. A probability value of < .05 was considered statistically significant. The study was approved by the Hospital Research Ethics Committee.

Our established hospital practice is to screen women at risk of GDM selectively, including women with a BMI >29.9 kg/ m^2, between 24 and 28 weeks' gestation with an OGTT. It is standard protocol to advice women to prepare for collection of samples by fasting from midnight. The minimum fasting time recommended is 8 hours. Each woman attends the hospital, where staff members collect 2.7 mL of venous blood into a Sarstedt S-monovette (supplied by Sarstedt Ltd, Drinagh, Co Wexford, Ireland; manufactured in Sarstedt AG and Co, Nümbrecht, Germany) tube that contains fluoride EDTA. This tube uses fluoride as a glycolysis inhibitor (1 mg/ mL blood) and EDTA as an anticoagulant (1.2 mg/mL blood). The woman is asked to drink 75 g of glucose in 410 mL within 5 minutes; time of completion is noted.

A repeat glucose sample is obtained in the same way 1 and 2 hours later. The samples are labeled immediately and stored on a tray at room temperature until all 3 samples are collected. A single blood-form attached to a storage bag that contains all 3 samples for each woman is then delivered to the hospital laboratory. The samples from several women may be delivered together to the laboratory usually between 11:00 AM and 1:00 PM.

For the purpose of this study, pretest preparation by the woman was according to the established hospital practice. Samples were paired. A single researcher collected venous blood into 2 sample tubes that contained the preservative fluoride EDTA. The first sample handling followed rigorously standardized methods similar to those used in the HAPO study (fast-tracked).[16] In brief, glycolysis was minimized in the HAPO study by collecting in tubes that contained sodium fluoride, keeping the samples on ice until the plasma had separated (<1 hour), and by then freezing the plasma. Our fast-tracked samples were held on an ice slurry and were transported to the laboratory and immediately analyzed, not frozen. The second sample was transferred immediately to hospital midwifery staff and handled in the usual way (hospital-tracked sample).

Hospital-tracked sample results were reported as usual to the woman's obstetric team. If the hospital-tracked glucose level was elevated, the woman was referred to the multidisciplinary diabetes team. If the fast-tracked levels were elevated, the multidisciplinary team was notified. Timing of sampling and analysis was documented clearly. Hospital staff was otherwise blinded to the research results, and the primary investigator received all paired results without patient identifiers for analysis.

Our laboratory is accredited to ISO 15189 by the Irish National Accreditation Board. All samples were analyzed with the use of an automated enzymatic (hexokinase) method on the main laboratory analyzer (AU640 random access chemistry analyzer; Beckman Coulter Diagnostics Ltd, Lisemeehan, O'Callaghans Mills, Co Clare, Ireland). Quality control is run three times daily. The assay imprecision was acceptable with coefficients of variation of 2.4% at 1.9 mmol/L, 2.1% at 5.6 mmol/L and 1.8% at 13.5 mmol/L.

RESULTS

Of 48 women studied, 24 women had a fasting glucose sample at 11-18 weeks' gestation, and 24 had a routine OGTT at 24-28 weeks' gestation. The first cohort comprised 24 women who had just presented in early pregnancy for prenatal care and who were given a later appointment for an OGTT at 24-28 weeks' gestation. The second cohort of 24 women comprised women who were attending for a 75-g OGTT at 24-28 weeks' gestation according to the normal hospital policy on screening for GDM, but who had been seen in early pregnancy by the research team for accurate BMI measurement, sonographic dating of the pregnancy, and consent to enter the study. The characteristics of the study population are shown and are analyzed by timing of sampling in Table 1. As expected, the mean BMI was 34.7 kg/m^2; the mean age was 29.5 years, and 29% (n = 14 women) were nulliparous. All samples were paired.