



Impact of Implementing Preanalytical Laboratory Standards on the Diagnosis of Gestational Diabetes Mellitus: A Prospective Observational Study

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BACKGROUND: Gestational diabetes mellitus (GDM) is associated with adverse pregnancy outcomes, but risk is reduced with identification and early treatment. Glucose measurements are affected by preanalytical sample handling, such as temperature of storage, phlebotomy–analysis interval, and use of a glycolysis inhibitor. We evaluated glucose concentrations and the incidence of GDM after strict implementation of the American Diabetes Association (ADA) preanalytical guidelines, compared with usual hospital conditions.

METHODS: Women screened selectively for GDM at 24–32 weeks' gestation were recruited at their convenience before a 75-g oral glucose tolerance test. Paired samples were taken: the first sample followed ADA recommendations and was transferred to the laboratory on an iced slurry for immediate separation and analysis (research conditions), and the second sample was not placed on ice and was transferred according to hospital practice (usual conditions).

RESULTS: Of samples from 155 women, the mean fasting, 1-h, and 2-h results were 90.0 (12.6) mg/dL [5.0 (0.7) mmol/L], 142.2 (43.2) mg/dL [7.9 (2.4) mmol/L], and 102.6 (32.4) mg/dL [5.7 (1.8) mmol/L], respectively, under research conditions, and 81 (12.6) mg/dL [4.5 (0.7) mmol/L], 133.2 (41.4) mg/dL [7.4 (2.3) mmol/L], and 99 (32.4) mg/dL [5.5 (1.8) mmol/L] under usual conditions (all $P < 0.0001$). GDM was diagnosed in 38.1% ($n = 59$) under research conditions and 14.2% ($n = 22$) under usual conditions ($P < 0.0001$). The phlebotomy–analysis interval for the fasting, 1-h, and 2-h samples was 20 (9), 17 (10), and 17 (9) min under research conditions and 162 (19), 95 (23), and 32 (19) min under usual conditions (all $P < 0.0001$). All cases of GDM were diagnosed on fasting or 1-h samples; the 2-h test diagnosed no additional cases.

CONCLUSIONS: Implementation of ADA preanalytical glucose sample handling recommendations resulted in higher mean glucose concentrations and 2.7-fold increased detection of GDM compared with usual hospital practices.

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Gestational diabetes mellitus (GDM)² is a common pregnancy complication with potentially serious lifelong consequences for both mother and baby (1–4). The incidence of GDM varies widely, from 1% to 26% (5, 6). Accurate diagnosis is important, because untreated GDM is associated with adverse clinical outcomes (7–10). GDM has also been associated with lifelong risk of metabolic syndrome for both mother and baby (11, 12).

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study of mild hyperglycemia led to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommending that the thresholds used to diagnose GDM be made more sensitive (13, 14). Revised criteria mean that the diagnosis can be made on 1 (rather than 2) increased maternal glucose results, which increases the rate of diagnosis of GDM (13–16).

The guidelines for laboratory standards in the diagnosis of diabetes were revised in 2011 (17). The American Diabetes Association (ADA) recommended that when using fluoride to prevent glycolysis after phlebotomy, “one should place the sample tube immediately in an ice-water slurry, and plasma should be separated from the cells within 30 min” to minimize glycolysis, and not within 60 min as previously recommended (17).

We recently reported on 2 cohorts of obese women and compared the strict implementation of ADA-recommended preanalytical glucose sample management

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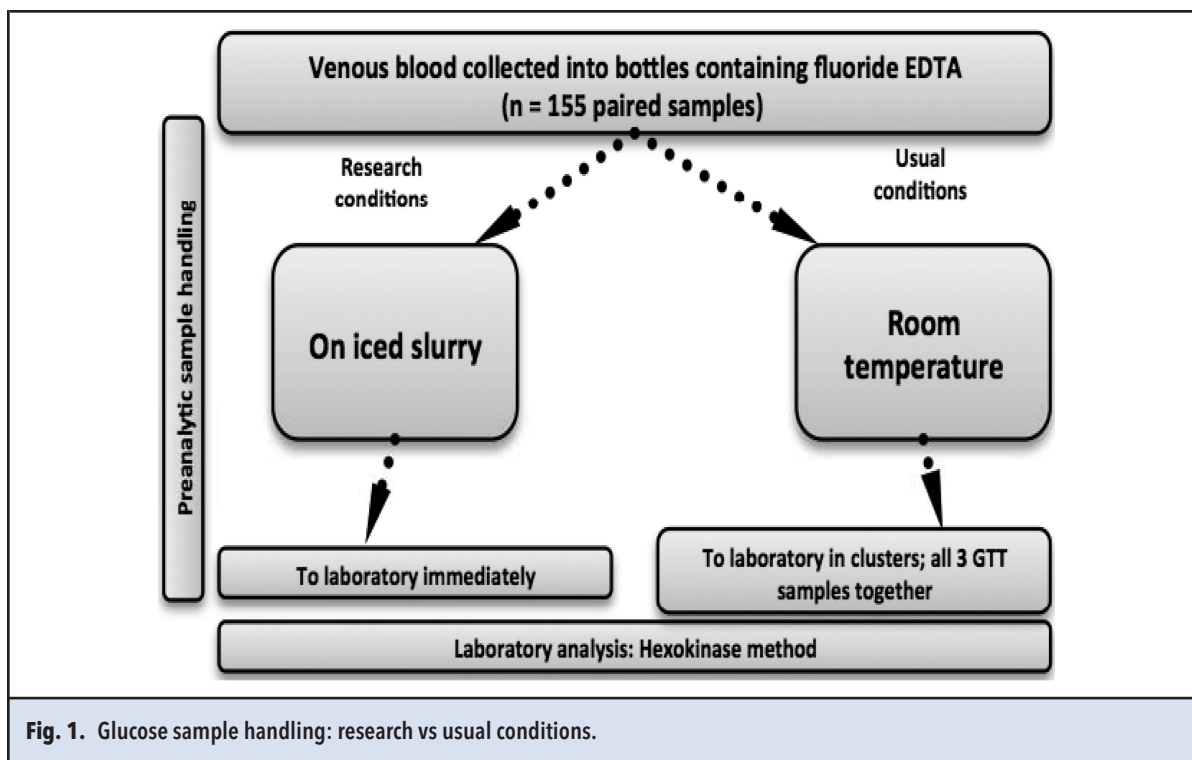
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² Nonstandard abbreviations: GDM, gestational diabetes mellitus; HAPO, Hyperglycemia and Adverse Pregnancy Outcomes; IADPSG, International Association of Diabetes and Pregnancy Study Groups; ADA, American Diabetes Association; OGTT, oral glucose tolerance test.



with usual hospital practice (18). In 24 women who had a fasting glucose result in early pregnancy, the diagnosis of GDM could be made in 16 (66.7%) if the ADA standard was implemented compared with 7 (29.2%) if it was not ($P < 0.01$) (18). In another cohort ($n = 24$) who had an oral glucose tolerance test (OGTT) at 24–32 weeks' gestation, the diagnosis of GDM could be made in 13 (54.2%) if the ADA standard was implemented compared with 4 (16.7%) if it was not ($P < 0.001$) (18). Because obesity is associated with higher leukocyte counts, and because glycolysis is affected by leukocyte count, we further investigated if these findings could be replicated in all women being screened selectively for GDM (19, 20).

In this study, we assessed the impact of strict implementation of ADA-recommended preanalytical sample handling on mean glucose concentrations and the incidence of GDM, compared with paired samples handled under the usual hospital conditions, in all women being screened selectively at 24–32 weeks' gestation.

Methods

We recruited women by convenience when they were selectively screened on the basis of risk factors at 24–32 weeks' gestation with a 75-g OGTT (15, 21). Body mass index had been calculated in early pregnancy. Informed consent was obtained. Exclusion criteria were multiple

pregnancy, age < 18 years, or inability to understand English.

The primary outcome was GDM by IADPSG criteria (13). We aimed to include 160 women with paired samples to determine the effect of sample handling on the incidence of GDM. We used McNemar test for correlated proportions and paired Student t test to test for statistical significance, and a P value of < 0.05 was considered statistically significant. The study was approved by the Hospital Research Ethics Committee.

Sample handling is shown in Fig. 1. Paired samples of maternal blood were collected in fluoride EDTA tubes. Sample handling under research conditions adhered to ADA guidelines (17). The samples were held on an iced slurry, transported to the laboratory, and immediately centrifuged and analyzed (within 30 min of phlebotomy). The samples under usual conditions were labeled immediately and stored at room temperature until all 3 samples were collected. The samples from several women were usually clustered and delivered together to the nearby laboratory. We documented the timing of all sampling and analysis.

All samples were analyzed with a hexokinase method. Assay imprecision was acceptable, with CVs of 2.4% at 34.2 mg/dL (1.9 mmol/L), 2.1% at 100.8 mg/dL (5.6 mmol/L), and 1.8% at 243.0 mg/dL (13.5

Table 1. Comparison of mean glucose concentrations between research and usual conditions for each test.^a

Glucose	Research conditions	Usual conditions	<i>P</i> ^b
Fasting			<0.0001
mg/dL	90.0 (12.6)	81.0 (12.6)	
mmol/L	5.0 (0.7)	4.5 (0.7)	
1-h			<0.0001
mg/dL	140.4 (43.2)	133.2 (41.4)	
mmol/L	7.8 (2.4)	7.4 (2.3)	
2-h			<0.0001
mg/dL	102.6 (32.4)	99.0 (32.4)	
mmol/L	5.7 (1.8)	5.5 (1.8)	

^a Data are mean (SD).
^b Paired Student *t* test.

mmol/L). All sample results were reported as usual to the woman's obstetric team.

Results

Of the 155 women studied, 40.0% were nulliparous, and the mean gestation at OGTT was 27.7 (3.4) weeks (range 24–32 weeks). Table 1 shows maternal glucose concentrations under both research and usual conditions. The diagnosis of GDM was made in 59 (38.1%) cases when preanalytical handling followed ADA standards compared with only 22 (14.2%) cases under usual conditions (*P* < 0.001). Research conditions detected 2.7 times more cases of GDM than usual conditions. All cases of GDM under research conditions were detected by the fasting and 1-h tests; the 2-h test contributed no additional cases (Fig. 2).

Table 2 compares the incidence of GDM between research and usual conditions. The mean phlebotomy–

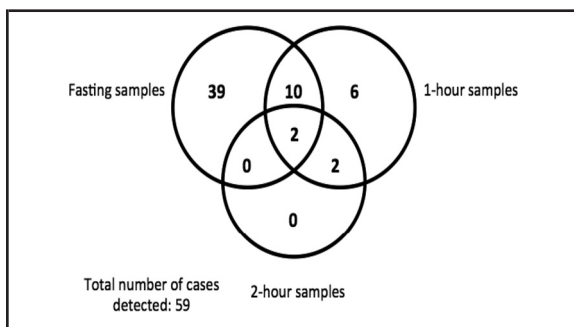


Fig. 2. Venn diagram illustrating number of cases of GDM detected with each of the fasting, 1-h, and 2-h tests of the OGTT.

Table 2. Comparison of the incidence of GDM between research and usual conditions for each test.^a

Glucose	Research conditions	Usual conditions	<i>P</i> ^b
Fasting	51 (32.9)	10 (6.5)	<0.0001
1-h	20 (13.3)	17 (11.0)	NS
2-h	4 (2.6)	4 (2.6)	NS
Total ^c	59 (38.1)	22 (14.2)	<0.0001

^a Data are n (%). NS, not significant.
^b McNemar test of correlated proportions.
^c Some overlap of cases (see Fig. 2).

analysis interval was greater under usual conditions than under research conditions (Table 3). We constructed Bland–Altman plots for each test (see Supplemental Figs. 1–3, which accompany the online version of this article at <http://www.clinchem.org/content/vol62/issue2>) (22). Fig. 3 illustrates an *XY* scatterplot with regression line of absolute fasting glucose measurements under both conditions. All the fasting samples under usual conditions were below the internationally accepted CV of 2.3%.

Discussion

In this prospective observational study of women being screened with an OGTT, we found that the prevalence of GDM was 2.7 times higher if the ADA standards for preanalytic prevention of glycolysis in the maternal samples were strictly implemented compared with the usual hospital conditions. This is consistent with the findings we recently reported in a smaller cohort of obese women being screened for GDM (18). These findings raise the possibility that suboptimal implementation of measures to prevent glycolysis is causing GDM to be underdiagnosed, with potentially serious clinical consequences. Variations in preanalytical standards may also contribute to the wide variations reported in the prevalence of GDM and the poor reproducibility of OGTTs (5, 6, 23, 24).

Another important finding is that when the ADA standards were strictly implemented, the diagnosis of

Table 3. Phlebotomy-analysis interval (min).

Glucose	Research conditions	Usual conditions	<i>P</i> ^a
Fasting	20 (9)	162 (19)	<0.0001
1-h	17 (10)	95 (23)	<0.0001
2-h	17 (9)	32 (19)	<0.0001

^a Paired Student *t* test.

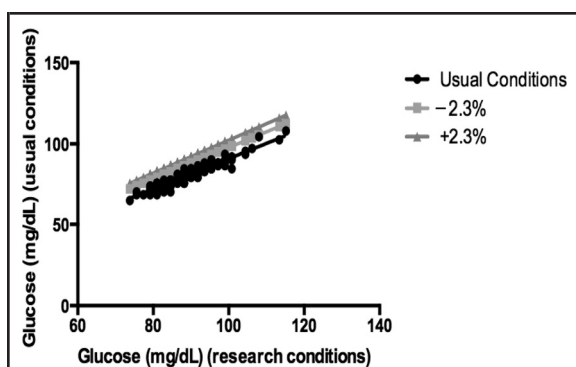


Fig. 3. Scatterplot of fasting glucose measurements (research vs usual conditions). To convert glucose concentrations from mg/dL to mmol/L, multiply by 0.05551.

GDM on the basis of the IADPSG diagnostic criteria could be made on the result of the fasting sample in 86.4% of cases, and on the basis of the 1-h sample alone in the remaining 13.6% of cases. The 2-h sample result was not required for the diagnosis of GDM. If confirmed in other studies, this observation has implications for the need for the 2-h OGTT.

A strength of this study is that because of the size of the cohort and the setting, adherence to ADA standards to prevent glycolysis was fully implemented under close supervision. The effect of glycolysis on the measurement of plasma glucose is well established, and the importance of preanalytical sample management is known to laboratorians (17, 25). Previous reports on glycolysis were usually in small studies of nonpregnant individuals, and the primary outcome was glucose concentrations (25–28). There is a paucity of research examining the impact of glycolysis on the diagnosis of GDM. A Danish study of 60 women compared glucose concentrations and prevalence of GDM under stricter conditions (although they did not use an iced slurry) and routine conditions in early pregnancy (29). They found that 13 cases of GDM and 2 cases of overt diabetes mellitus were missed under routine conditions. The recent revisions in the IADPSG diagnostic criteria also mean that the OGTT has become more sensitive, and therefore the impact of glycolysis on the fasting sample is now more critical.

The implementation of ADA standards was an important component of the HAPO study but was challenging given the international dispersion of centers. All the measurements were undertaken in a central laboratory. Unlike our study, however, it may not have been feasible to bring the same level of close supervision to the preanalytical handling, given the large numbers studied (30).

Conclusions

We believe that the revised recommendations to implement standards to prevent glycolysis are not widely appreciated by clinicians providing obstetric care worldwide. Our findings also suggest that efficiencies may be achieved by omitting the 2-h OGTT sample, which not only makes the test less time-consuming for the patient, but may also potentially shorten the phlebotomy–analysis interval for the fasting sample. Strict implementation of ADA recommendations on preanalytical handling may further increase the number of women being diagnosed with GDM, which may mean that the existing knowledge on the relationship between GDM and pregnancy outcomes may need to be revisited.

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References

- O'Sullivan JB, Mahan CM. Criteria for the oral glucose tolerance test in pregnancy. *Diabetes* 1964;13:278–85.
- Pedersen J. The pregnant diabetic and her newborn: problems and management. Baltimore (MD): William & Wilkins; 1967. p 128–37.
- Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. *Am J Obstet Gynecol* 1982; 144:768–73.
- HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovirindr U, et al. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008;358:1991–2002.
- Hartling L, Dryden DM, Guthrie A, Muise M, Vandermeer B, Aktary WM, et al. (University of Alberta Evidence-based Practice Center). Screening and diagnosing gestational diabetes mellitus. Rockville (MD): Agency for Healthcare Research and Quality. 2012 Oct.
- Buckley BS, Harreiter J, Damm P, Corcoy R, Chico A, Simmons D, et al. Gestational diabetes mellitus in Europe: prevalence, current screening practice and barriers to screening. A review. *Diabet Med* 2012;29:844–54.

7. Turner MJ, Rasmussen MJ, Turner JE, Boylan P, MacDonald D, Stronge JM. The influence of birth weight on labor in nulliparas. *Obstet Gynecol* 1990;76:159-63.
8. Stotland NE, Caughey AB, Breed EM, Escobar GJ. Risk factors and obstetric complications associated with macrosomia. *Int J Gynaecol Obstet* 2004;87:220-6.
9. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med* 2005;352:2477-86.
10. Landon MB, Spong CY, Thom E, Carpenter MW, Ramin SM, Casey B et al. A multicenter, randomized trial of treatment for mild gestational diabetes. *N Engl J Med* 2009;361:1339-48.
11. Lee AJ, Hiscock RJ, Wein P, Walker SP, and Permezel M. Gestational diabetes mellitus: clinical predictors and long-term risk of developing type 2 diabetes: A retrospective cohort study using survival analysis. *Diabetes Care* 2007;30:878-83.
12. Lehnen H, Zechner U, Haaf T. Epigenetics of gestational diabetes mellitus and offspring health: the time for action is in early life. *Mol Human Reprod* 2013;19:415-22.
13. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* 2010;33:676-82.
14. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy. Geneva: WHO; 2013. <http://www.ncbi.nlm.nih.gov/pubmed/24199271> (Accessed October 2014).
15. Ali FM, Farah N, O'Dwyer V, O'Connor C, Kennelly MM, Turner MJ. The impact of new national guidelines on screening for gestational diabetes mellitus. *Ir Med J* 2013;106:57-9.
16. Langer O, Umans J, Miodovnik M. Perspectives on the proposed gestational diabetes mellitus diagnostic criteria. *Obstet Gynecol* 2013;121:177-82.
17. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Diabetes Care* 2011;34:e61-99.
18. Daly N, Stapleton M, O'Kelly R, Kinsley B, Daly S, Turner MJ. The role of preanalytical glycolysis in the diagnosis of gestational diabetes mellitus in obese women. *Am J Obstet Gynecol* 2015;213:84.e1-5.
19. Dixon JB, O'Brien PE. Obesity and the white cell count: changes with sustained weight loss. *Obes Surg* 2006;16:251-7.
20. Ladenson JH. Nonanalytical sources of variation in clinical chemistry results. In: Sonnenwith A, Jaetr L, editors. *Clinical laboratory methods and diagnosis*. St. Louis, MO: CV Mosby; 1980:149-92.
21. Guidelines for the management of pre-gestational and gestational diabetes mellitus from pre-conception to the postnatal period: changing practice to support service delivery. Health Service Executive. <http://www.hse.ie/eng/about/Who/clinical/natclinprog/obsandgynaeprogramme/guide11.pdf> (Accessed October 2014).
22. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307-10.
23. Harlass FE, Brady K, Read JA. Reproducibility of the oral glucose tolerance test in pregnancy. *Am J Obstet Gynecol* 1991;164:564-8.
24. Catalano PM, Avallone DA, Drago NM, Amini SB. Reproducibility of the oral glucose tolerance test in pregnant women. *Am J Obstet Gynecol*. 1993;169:874-81.
25. Chan AYW, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. *Clin Chem* 1989;35:315-7.
26. Bruns DA, Knowler WC. Stabilization of glucose in blood samples: why it matters. *Clin Chem* 2009;55:850-2.
27. Stahl M, Jørgensen LG, Hyltoft Petersen P, Brandslund I, de Fine Olivarius N, Borch-Johnsen K. Optimization of preanalytical conditions and analysis of plasma glucose. 1. Impact of the new WHO and ADA recommendations on diagnosis of diabetes mellitus. *Scand J Clin Lab Invest*. 2001;61:169-79.
28. del Pino IG, Constanso I, Mourín LV, Safont CB, Vázquez PR. Citric/citrate buffer: an effective antiglycolytic agent. *Clin Chem Lab Med* 2013;51:1943-9.
29. Festa R, Carta M, Ceriello A, Testa R. Time is glucose, can't miss gestational diabetes. *Diabetes Technol Ther* 2012;14:444-6.
30. Sacks DB. Diagnosis of gestational diabetes mellitus: it is time for international consensus? *Clin Chem* 2014;60:141-3.