Real-world gestational diabetes screening: problems with the oral glucose tolerance test in rural and remote Australia

What do we already know?

Gestational diabetes mellitus (GDM) means high blood glucose in pregnancy. It causes problems in pregnancy and childbirth, like big babies and complicated births. Mums and babies are more likely to have type 2 diabetes later in life. Due to higher obesity levels, regional women are at higher risk of GDM compared to urban women. It is important to screen and detect GDM as early as possible so that it can be managed.

The recommended screening test is a fasted 75g oral glucose tolerance test (OGTT). Also known as the sugar drink test, and OGTT involves collecting three blood samples over two hours. In 2015 new OGTT diagnostic criteria based on the large international *Hyperglycaemia and Adverse Pregnancy Outcome* (HAPO) study were introduced in WA. The HAPO study used a very strict protocol for OGTT collection: blood was collected into fluoride-oxalate (FLOX) tubes, and samples were immediately placed on ice and processed within one hour of test completion ($FLOX^{ICE}$). Ice helped slow down the cells to stop them using glucose (called glycolysis). This is not practical in most clinical settings. FC Mix tubes are used internationally because they stop glycolysis straight away and therefore keep blood sugar results accurate over time, even at room-temperature.

The **ORCHID Study** (*Optimisation of Rural Clinical and Haematological Indicators of Diabetes in pregnancy*) was designed to help simplify screening for GDM. Our first paper showed it can be difficult to get everyone to do the OGTT.[1] This second paper looked at the impact of glycolysis on the accuracy of diagnosis of GDM.[2]

How was this study done?

- 694 (39% Aboriginal) women from 27 clinics across regional, rural and remote WA, from Kununurra to Albany, were recruited from 2015-2018.
- Most routine OGTT samples were analysed more than four hours post fasting collection (median 5.0 h, range 2.3 to 124 h), potentially reducing glucose levels due to glycolysis.
- To measure glucose instability over time we collected matched OGTT samples from 12 participants in a metropolitan clinic that was close to a laboratory. Glucose dropped by over 0.4 mmol/L in FLOX tubes kept at room-temperature ($FLOX^{RT}$) for four hours compared to samples kept on ice ($FLOX^{ICE}$).
- We used this information to adjust ORCHID study OGTT results based on delay to laboratory and how much glucose was likely lost.

What does this research show?

- 600 women completed the ORCHID study and delivered their babies after 30 weeks gestation.
- 83% completed OGTT screening after 24 weeks gestation.
- Compared to the HAPO study, ORCHID glucose levels were significantly lower (mean fasting glucose 4.2 ± 0.40 mmol/L v 4.5 ± 0.38 mmol/L in HAPO, P <0.001) and ORCHID women with large for gestational age (LGA) babies were more likely to be in lower glucose categories (lowest fasting category, 2.5-4.1 mmol/L, 10.8% LGA v 5.3% LGA In HAPO, P = 0.001).
- We estimate 62% of ORCHID women with GDM were misclassified as normal due to glycolysis (GDM incidence, $FLOX^{RT}$ 10.8% v $FLOX^{ICE}$ 28.5% (95% CI, 20.8-29.5%), P <0.001).
- Glucose in FC Mix tubes remained stable for 24 hours. FC Mix tubes gave slightly higher results compared to $FLOX^{ICE}$ (fasting glucose: +0.20 ± 0.05mmol/L). We estimate GDM incidence in ORCHID would be 45% (95% CI, 35.7-55.1%) using FC Mix tubes. As such, use of these tubes in the clinic may require revision of GDM diagnostic thresholds.

Why did the OGTT fail to detect GDM?

Glycolysis is the process cells use to convert glucose into energy. Glycolysis continues in blood samples long after collection. FLOX is added to blood collection tubes to stop glycolysis. However, FLOX is slow to work, taking four hours to stabilise glucose completely. To ensure accurate glucose results, the *World Health Organization* first recommended rapid separation of plasma from cells in FLOX tubes, almost 40-years ago.

Australian OGTT protocols did not change when the HAPO diagnostic criteria were introduced in 2015. Most ORCHID study FLOX samples were kept at room temperature and measured more than four hours after collection. This means glucose levels inside the samples dropped before they were tested.

Changes to clinical practice

This study highlights problems using highly controlled research projects to directly change clinical practice without considering what is feasible in real-world settings. FC Mix tubes offer a practical solution to stabilise glucose, with a small increase in cost ($0.64 per OGTT). The biggest impact of addressing this issue will be felt on the women diagnosed on fasting blood glucose. Most will be managed with diet, exercise and timed delivery at the end of pregnancy. A smaller number will require greater clinical input, insulin or other hypoglycaemic medication and more specialised intervention. Overall the major impact of using the best possible processes is likely to be the need for more comprehensive education programs about sugar control for all pregnant women, as greater numbers will be picked up in the lower risk end of the spectrum.

WA pathology labs have been informed of these findings but at this stage they have not changed any of their own collection processes because they need to follow *Royal College of Pathologists of Australasia* (RCPA) guidelines. However, Pathwest will accept FC Mix tubes from clinics for glucose testing. Now that this paper is published we plan to meet with the RCPA, to discuss updating their guidelines for glucose sample collection.

Kimberley Aboriginal Community Controlled Health Organisations (ACCHO) are now using FC Mix tubes. Please refer to Greiner Bio-One (https://shop.gbo.com/en/row/products/preanalytics/) for more information. If you are interested in obtaining these tubes please contact Interpath (https://www.interpath.com.au/), the Australian distributor for Greiner Bio-One. For further assistance with your enquiries please contact ORCHID Study coordinator, Emma Jamieson.

Future research

We think this is an important finding. From our first paper we know that half of women were not screened for GDM in rural WA in the year following adoption of universal screening. We now know that in the women who are screened, we are missing GDM because of how the test is done. To improve detection of GDM we will:

- Audit GDM incidence and management in Kimberley ACCHO patients, before and after introduction of FC Mix tubes.
- Assess the ability of the adjusted OGTT to pick up ORCHID study women who had poor birth outcomes that were previously missed.
- Compare the adjusted OGTT to other tests in ORCHID study women which are easier for pregnant women to complete.

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ORCHID study publications

1. Kirke AB, Atkinson D, Moore S, Sterry K, Singleton S, Roxburgh C, Parrish K, Porter C, Marley JV. Diabetes screening in pregnancy failing women in rural Western Australia: An audit of oral glucose tolerance test completion rates. *Aust J Rural Health* 2019; 27:64-69.

2. Jamieson E, Spry E, Kirke A, Atkinson D, Marley JV. Real-world gestational diabetes screening: problems with the oral glucose tolerance test in rural and remote Australia. *Int J Environ Res Public Health.* 2019; 16, 4488.

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