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Underestimation of risk for large babies in rural and remote Australia: Time to change plasma glucose collection protocols

Emma L. Jamieson [a,*], Erica P. Spry [b,c], Andrew B. Kirke [a], Carly Roxburgh [d], David N. Atkinson [c], Julia V. Marley [b,c]

[a] The University of Western Australia, The Rural Clinical School of Western Australia, Building 3 Edith Cowan University Campus, Robertson Drive, PO Box 412, Bunbury 6230, Australia
[b] Kimberley Aboriginal Medical Services, 12 Napier Terrace, Broome 6725, Australia
[c] The University of Western Australia, The Rural Clinical School of Western Australia, 12 Napier Terrace, PO Box 1377, Broome 6725, Australia
[d] The University of Western Australia, The Rural Clinical School of Western Australia, 35 Stirling Terrace, Albany 6330, Australia

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ABSTRACT

Aims: Preanalytical glycolysis in oral glucose tolerance tests (OGTT) leads to substantial underestimation of gestational diabetes mellitus (GDM) and hence risk for large-for-gestational-age (LGA) babies. This paper quantified the impact of glycolysis on identification of LGA risk in a prospective rural and remote Australian cohort.

Methods: For 495 women, OGTT results from room temperature fluoride-oxalate (FLOX) tubes were algorithmically corrected for estimated glycolysis compared to 1) the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study protocol (FLOX tubes in ice-slurry); and 2) room temperature fluoride-citrate (FC) tubes. GDM was defined by International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria. Unadjusted and corrected OGTT were related to LGA outcome.

Results: Correction for FC tubes increased GDM incidence from 9.7% to 44.6%. After correction for HAPO protocol, GDM incidence was 27.7% and prediction of LGA risk (RR 1.82, [1.11–2.99]) improved compared to unadjusted rates (RR 1.12, [0.51–2.47]). To provide similar results for FC tube correction (29.3% GDM; RR 1.81, [1.11–2.96]) required + 0.2 mmol/L adjustment of IADPSG criteria.

Conclusions: FC tubes present a practical alternative to the HAPO protocol in remote settings but give + 0.2 mmol/L higher glucose readings. Modification of IADPSG criteria would reduce perceived 'overdiagnosis' and improve LGA risk-assessment.

Introduction

Maternal obesity and gestational diabetes mellitus (GDM) are independent risk factors for excess fetal growth, with an additive effect [1,2]. If GDM is identified and intensively managed, excess fetal growth and associated adverse perinatal outcomes can be modified [3,4]. Almost half of Australian mothers are overweight (21%) or obese (26%) at first presentation and 15% have GDM [5]. Compared to their urban counterparts, women from rural and remote Australia of childbearing age are at higher risk for GDM with a third in the obesity category [6,7]. Two-thirds of Australian Aboriginal mothers reside outside of major cities and are more likely to have GDM (1.3-fold) and obesity compared to non-Indigenous women [8].

The International Association of the Diabetes and Pregnancy Study Groups (IADPSG) recommends that all women without known diabetes are screened for GDM with a 75 g oral glucose tolerance test (OGTT) using diagnostic criteria based on Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study data [9]. The IADPSG also recommends '*proper sample collection and processing to minimize glycolysis*' [9]. In the HAPO study, collection tubes containing fluoride-oxalate (FLOX) were immediately immersed in ice and processed within one hour of test completion [10].

HAPO ensured consistent glucose measurements across recruitment sites [11], however in remote Australia this method is impractical. In

* Corresponding author at: The Rural Clinical School of WA, PO Box 412, Bunbury 6230, Western Australia.
E-mail addresses: emma.jamieson@rcswa.edu.au (E.L. Jamieson), research@kamsc.org.au (E.P. Spry), andrew.kirke@rcswa.edu.au (A.B. Kirke), carly.roxburgh@rcswa.edu.au (C. Roxburgh), david.atkinson@rcswa.edu.au (D.N. Atkinson), julia.marley@rcswa.edu.au (J.V. Marley).

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Western Australia (WA), FLOX samples are generally stored at room temperature without rapid processing. As FLOX tubes only result in complete cessation of glycolysis after four hours, this leads to variably lower plasma glucose (PG) levels being reported (up to 0.4 mmol/L lower v ice) [12]. This difference is generally not clinically important, however does significantly impact GDM diagnosis. Early centrifugation (<10 min) of FLOX tubes almost doubled GDM incidence in a large (12, 317), predominantly urban Australian cohort (20.6% v 11.6% delayed centrifugation) [13]. Larger distances to laboratory in rural and remote settings render OGTT samples more susceptible to glycolysis [12,14]. It is critical that sources of error for PG measurement are reduced to ensure accurate diagnosis of, and management for, GDM [15].

An alternative collection tube containing fluoride and citrate (FC), resulting in virtually immediate cessation of glycolysis, is recommended by European (2008) and American Diabetes Association (ADA) (2011) guidelines [16,17]. Citrate obviates the need for rapid cooling and processing. However, direct substitution of FC tubes for use with pregnancy OGTT is not straightforward. PG measurements from FC tubes are on average +0.2 mmol/L higher than by the HAPO preanalytical protocol [12,18,19]. For fasting PG (FPG) this is about half a standard deviation and has significant impact on GDM incidence [20]. In a Danish study using FC tubes, substantially more women had FPG ≥ 5.1 mmol/L, compared to HAPO (40% v 8.3%) [21], however they had significantly fewer large-for-gestational-age (LGA) babies. Use of FC tubes may have contributed to differences in results for this Danish cohort.

The IADPSG derived diagnostic thresholds were based on increased (1.75-fold) risk for LGA and neonatal abdominal fat mass and hyperinsulinaemia above the 90th centile in the HAPO study [9]. In a meta-analysis of data from 25 antenatal cohorts, including HAPO, FPG showed stronger association with LGA compared to post-load PG [22]. FPG values are more tightly clustered and hence small differences are more likely to affect diagnosis of GDM [12]. Modified diagnostic thresholds for use with FC tubes may be required to ensure risk for LGA is correctly identified [19].

The Optimisation of Rural Clinical and Haematological Indicators for Diabetes in pregnancy (ORCHID) study was a prospective cohort study of pregnant women in regional, rural and remote WA designed to investigate screening methods for GDM. In WA, birthweight is the only IADPSG outcome, selected to derive the diagnostic criteria, that is routinely collected. The main aim of this paper was to estimate the effect of different OGTT sample processing on identification of risk for excess fetal growth in rural and remote Australia.

Subjects, materials and methods

Study setting

In WA one-fifth of pregnant women reside regionally [23]. Maternity services are facilitated by public hospital maternity care, midwifery group practice care, community midwifery/shared care, general practitioner obstetric care or team midwifery care. Pathology collection centres are not available in many rural and remote towns and communities.

Participants

Pregnant women at first antenatal presentation at a participating site, aged 16 years or older, singleton pregnancy and no documented pre-existing diabetes, were invited to participate. Data were collected from 9 January 2015 to 31 May 2018 at 27 sites in the Kimberley, Mid-West, Goldfields, Southwest and Great Southern regions of WA.

OGTT

Local procedures were relied on for OGTT. Most sites (16) performed OGTT in the clinic, with the remaining sites referring participants to dedicated pathology collection centres. Venous whole blood samples were collected into FLOX tubes (BD Biosciences, Australia), batched and sent to local pathology laboratories. Distances between study sites and laboratory ranged from 0.35 km to 650 km. Median time to analysis was 5 h from FPG collection [12]. PGs were measured by the hexokinase method [24].

As previously described, 11 ORCHID participants from an urban site had multiple samples at each OGTT timepoint: FLOX tube stored at room temperature ($FLOX^{RT}$); FLOX tube placed immediately in crushed-ice (HAPO method); and FC tube (Greiner Bio-one, Austria) stored at room temperature [12]. Glucose was measured at multiple points up to 24 h and linear regression used to estimate PG for each measurement from the ORCHID cohort had the HAPO method (HAPO preanalytical protocol correction) or FC tubes (FC tube correction) been used. Equations for HAPO preanalytical protocol correction, using delay to analysis in hours, were:

$$\text{FPG} = 0.999(\text{FPG FLOX}^{\text{RT}}) + 0.108(\text{delay}) - 0.004(\text{delay}^2) + 0.125$$

$$\text{1-h PG} = 0.981(\text{1-h FLOX}^{\text{RT}}) + 0.107(\text{delay}) - 0.004(\text{delay}^2) + 0.279$$

$$\text{2-h PG} = 0.949(\text{2-h FLOX}^{\text{RT}}) + 0.091(\text{delay}) - 0.003(\text{delay}^2) + 0.426$$

Equations for FC tube correction, using delay to analysis in hours, were:

$$\text{FPG} = 0.986(\text{FPG FLOX}^{\text{RT}}) + 0.106(\text{delay}) - 0.004(\text{delay}^2) + 0.386$$

$$\text{1-h PG} = 1.043(\text{1-h FLOX}^{\text{RT}}) + 0.112(\text{delay}) - 0.004(\text{delay}^2) + 0.146$$

$$\text{2-h PG} = 1.098\ (\text{2-h FLOX}^{\text{RT}}) + 0.105(\text{delay}) - 0.004(\text{delay}^2) - 0.182$$

95% CI for beta-coefficients and Z-value estimates are listed in Supplementary Table 1.

Only ORCHID participants who did not meet HAPO study OGTT unblinding criteria were included in this analysis: unadjusted FPG ≥ 5.9 mmol/L or unadjusted 2-h PG ≥ 11.2 mmol/L; any unadjusted OGTT PG < 2.5 mmol/L. Unadjusted and corrected PG were stratified into the categories used in HAPO (Fig. 1 (FPG); Supplementary Fig. 1 (1-h PG); and Supplementary Fig. 2 (2-h PG)) [20]. GDM was defined by IADPSG criteria [9]. For estimated FC results, modified IADPSG criteria were also used (FC protocol: +0.2 mmol/L higher) [12,18,19].

Birth outcomes

Birth outcome data were recorded from hospital discharge summaries, primarily STORK reports [25]. Adverse outcomes were defined by HAPO study definition [20], or modified as below:

- LGA newborn (birthweight >90th centile): birthweight centiles calculated using Global bulk centile calculator, GROW v8.0.1, adjusting for gestational age, maternal height, maternal weight at first antenatal visit, parity, ethnicity and infant sex. Adjustment for maternal weight made within BMI limits of 18.5–30 kg/m^2 only.
- Clinical neonatal hypoglycaemia: documented neonatal hypoglycaemia and/or treatment with glucose or dextrose infusion.

Statistical analysis

Study data were collected and managed using secure REDCap electronic data capture tools hosted at The University of WA [26]. All analyses performed with Stata, version 15 (Statacorp). Differences in characteristics between ORCHID and HAPO cohorts and between ORCHID management groups were compared using χ^2 tests for categorical data, and t-tests for continuous data. Wilcoxon rank-sum test was used to compare between GDM and estimated GDM for continuous data. Differences in proportions within PG categories between ORCHID and HAPO cohorts were compared using χ^2 tests within ordinal logistic regression. Risk ratios for LGA newborn were calculated before and after

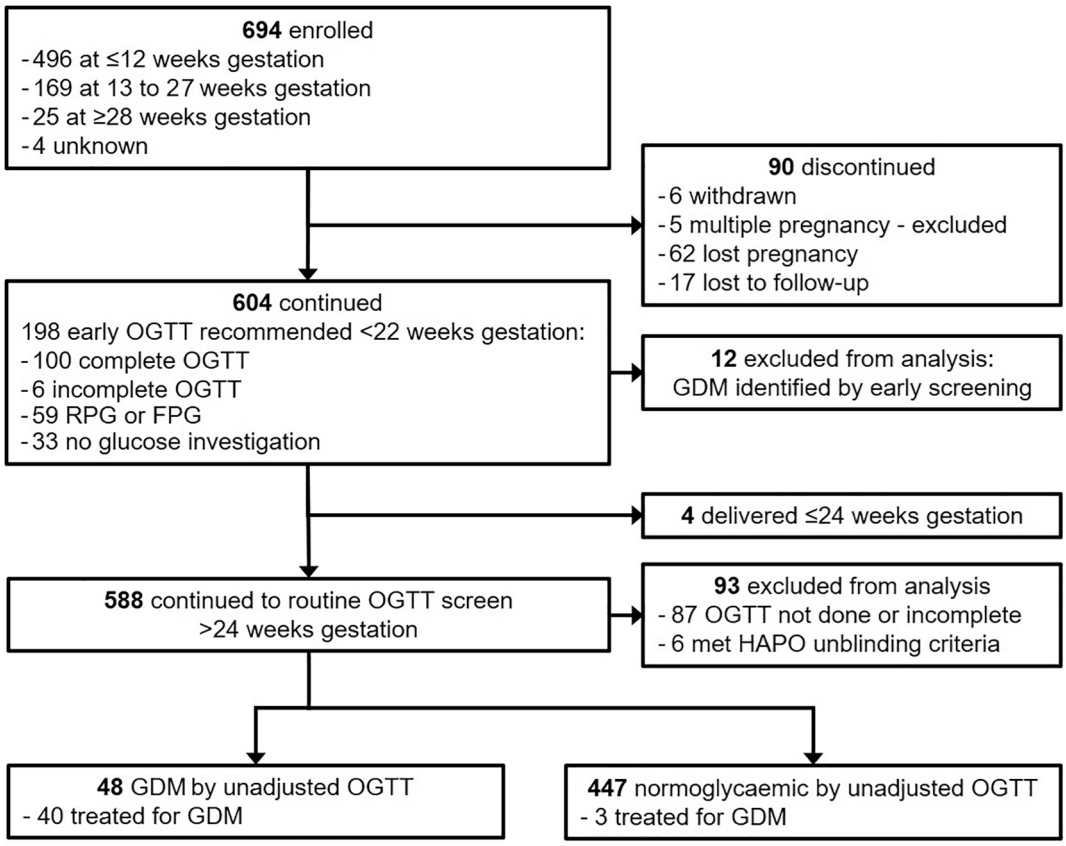

Fig. 1. Flowchart for prospective ORCHID cohort participation and GDM screening outcomes. ORCHID = Optimisation of Rural Clinical and Haematological Indicators for Diabetes in pregnancy study; GDM = gestational diabetes mellitus; OGTT = 75 g oral glucose tolerance test; RPG = random plasma glucose; FPG = fasting plasma glucose; HAPO = Hyperglycaemia and Adverse Pregnancy Outcomes study. Local procedures relied on for OGTT. Recommendations for early screening and GDM management at the discretion of the antenatal care provider. HAPO participants were unblinded if OGTT met one or more of the following criteria: FPG ≥ 5.9 mmol/L; 2-h plasma glucose (PG) ≥ 11.2 mmol/L; any unadjusted OGTT PG < 2.5 mmol/L. GDM defined by International Association of the Diabetes and Pregnancy Study Groups criteria; one or more OGTT PG values equal to or above the following thresholds: FPG 5.1 mmol/L; 1-h PG 10.0 mmol/L; 2-h PG 8.5 mmol/L.

logistic regression (model: GDM, maternal age, BMI at OGTT, antenatal smoking [binary], gestation at OGTT and management for GDM [binary]). Sensitivity, specificity and positive predictive value calculated for accuracy of unadjusted; HAPO preanalytical protocol corrected; and FC protocol corrected; OGTT for LGA newborn. $P < 0.05$ was defined as statistically significant.

Ethics approval

Ethics approval obtained from the WA Aboriginal Health Ethics Committee (2014-007) and WA Country Health Service Human Research Ethics Committee (RGS2924). The Kimberley Aboriginal Health Planning Forum Research Subcommittee supported this project.

Results

Of 604 ORCHID study participants, described previously [12], 495 had complete routine OGTT results after 24-weeks gestation below HAPO unblinding criteria (Fig. 1). Forty of 48 participants who met IADPSG criteria and three with borderline results were treated for GDM (43 self-blood-glucose-monitoring, dietary and lifestyle intervention with five having additional pharmacotherapy with metformin (2) or insulin (3)).

ORCHID participant ethnicity varied from Australian HAPO cohorts (Brisbane, Newcastle): ORCHID had a lower proportion of Caucasian participants (60.4% v 91.4%, $P < 0.001$)[27] as recruitment was designed to overrepresent Aboriginal women (33.1%). Supplementary Table 2 shows baseline characteristics, OGTT and perinatal outcomes for the 495 ORCHID participants and blinded HAPO cohort.

After stratifying by HAPO glucose category, unadjusted ORCHID OGTT PG were more likely to be in a lower category, at all three OGTT samples, compared with the HAPO cohort, however the largest differences were clearly in FPG:

- FPG: OR 3.34 [95% CI, 2.82–3.95], $P < 0.001$ (Fig. 2A)
- 1-h PG: OR 1.63 [95% CI, 1.39–1.91], $P < 0.001$ (Supplementary Fig. 1A)
- 2-h PG: OR 1.41 [95% CI, 1.20–1.65], $P < 0.001$ (Supplementary Fig. 2A).

Following HAPO preanalytical protocol correction, ORCHID FPG shifted up a median of two FPG categories (range 0–4, Fig. 2B). As expected, the change in 1-h PG and 2-h PG was less pronounced; less than a third of participants shifted one glucose category (Supplementary Figs. 1B and 2B). Results corrected for FC tubes were a further 0.2 (±0.02) mmol/L higher.

GDM rates were 9.7% unadjusted, 27.7% by HAPO preanalytical protocol correction and 44.6% with FC tube correction. With modified IADPSG criteria (FC protocol: IADPSG criteria + 0.2 mmol/L) the latter reduced to 29.3%. Most (72.2%, 70/97) women reclassified to GDM by FC protocol correction were based on FPG alone.

The effect of GDM reclassification on the association between GDM and LGA was explored. For FPG prior to correction, most ORCHID participants with an LGA newborn were in lower HAPO glucose categories (categories one to four: 52/56, 92.9% v 77.9% in HAPO, $P = 0.007$; Fig. 2A). Following HAPO preanalytical protocol correction and FC

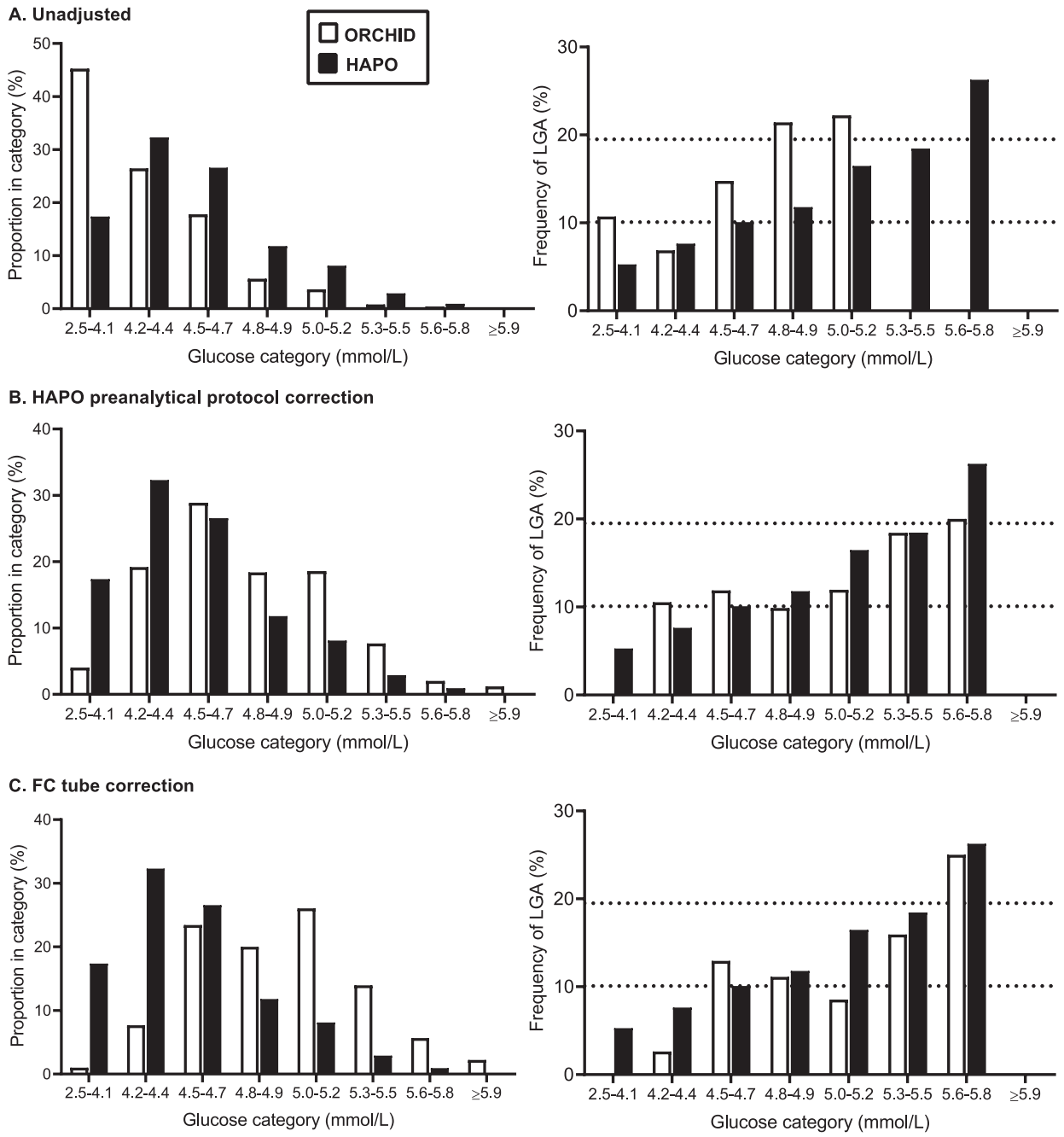

Fig. 2. Proportion of ORCHID participants (N = 495)* and frequency of LGA newborn by FPG category before and after correction for glycolysis, compared to blinded HAPO participants (N = 23,217). ORCHID = Optimisation of Rural Clinical and Haematological Indicators for Diabetes in pregnancy study; LGA = large-for-gestational-age (>90th centile for newborn birthweight); FPG = fasting plasma glucose; HAPO = Hyperglycaemia and Adverse Pregnancy Outcomes study; FC = fluoride-citrate. *Data include 495 ORCHID study participants with complete 75 g oral glucose tolerance test (OGTT) after 24 weeks gestation who did not meet HAPO study OGTT unblinding criteria; one or more OGTT: FPG ≥ 5.9 mmol/L; 2-h PG ≥ 11.2 mmol/L; any OGTT PG < 2.5 mmol/L. Reported HAPO data for 23,217 blinded participants with complete OGTT after 24-weeks gestation and birthweight data.(21) Data in left hand panel are proportion (%) of participants within each FPG category. Data in right hand panel are frequency (%) of LGA newborn within each FPG category. Low dotted line represents 10% LGA frequency in HAPO study reported for participants with mean FPG of 4.5 mmol/L. High dotted line represents 19.5% LGA frequency in HAPO study reported for participants with FPG ≥ 5.1 mmol/L. A: Unadjusted ORCHID FPG by local procedures; fluoride-oxalate (FLOX) tube stored at room temperature until processing. B: ORCHID FPG corrected for glycolysis compared to HAPO preanalytical protocol; FLOX tube immediately stored on crushed ice and processed within 1 h; correction by $FLOX^{ICE}$ algorithm. C: ORCHID FPG corrected for glycolysis compared to FC tube stored at room temperature; correction by FC Mix algorithm. Linear regression β-coefficients for FPG + delay-to-analysis + delay-to-analysis^2 + constant for $FLOX^{ICE}$ and FC Mix algorithm are reported in Supplementary Table 1.

protocol correction, frequency of LGA by glucose category had a similar distribution to the HAPO cohort (Fig. 2B and C), and identification of risk for excess fetal growth with GDM improved (from 12.5% to 16.8% and 16.6%, respectively) (Table 1). This was despite insignificant improvement to the total area under the receiver operating characteristics (ROC) curve (AUC) of individual OGTT samples for detection of LGA newborn (Supplementary Fig. 3).

Obesity was more common in those with FC protocol corrected GDM compared to women below modified IADPSG thresholds (39/145, 26.9% v 18.3%, 64/350, P = 0.032). A significantly increased risk for

Table 1
GDM* diagnosis and associated risk for LGA newborn in ORCHID participants before and after correction for glycolysis; blinded HAPO cohort provided for reference.

	LGA infants of non-GDM mothers % (*n*/*N*)	LGA infants of GDM mothers % (*n*/*N*)	Risk ratio for LGA (95% CI)
HAPO cohort (*N* = 23,217)			
Unadjusted PG (IADPSG criteria)	8.3%	16.2%	1.95 *P* < 0.001
ORCHID participants (N = 495)†			
Unadjusted PG (IADPSG criteria)	11.2% (50/447)	12.5% (6/48)	1.12 (0.51 to 2.47) *P* = 0.784
HAPO preanalytical protocol correction‡ (IADPSG criteria)	9.2% (33/358)	16.8% (23/137)	1.82 (1.11 to 2.99) *P* = 0.017
FC tube correction§ (IADPSG criteria)	9.8% (27/274)	13.1% (29/221)	1.33 (0.81 to 2.18) *P* = 0.254
FC tube correction§ (modified IADPSG criteria)	9.1% (32/350)	16.6% (24/145)	1.81 (1.11 to 2.96) *P* = 0.018

Data are proportion (%) of group with LGA (number with LGA/total number in group). Risk ratio and *P*-value reported for risk of LGA newborn relative to the non-GDM group.
LGA = large-for-gestational-age (>90th centile for newborn birthweight); ORCHID = Optimisation of Rural Clinical and Haematological Indicators for Diabetes in pregnancy study; HAPO = Hyperglycaemia and Adverse Pregnancy Outcomes study; PG = plasma glucose; IADPSG = International Association of the Diabetes in Pregnancy Study Groups; FC = fluoride–citrate; CI = confidence interval. Reported HAPO data for 23,217 blinded participants with complete 75 g oral glucose tolerance test (OGTT) after 24 weeks gestation and birthweight data [39].
Linear regression β-coefficients for FPG + delay-to-analysis + delay-to-analysis^2 + constant for FLOX^{ICE} and FC Mix algorithm are reported in Supplementary Table 1.
* Gestational diabetes mellitus (GDM) was defined by IADPSG criteria; one of more PG equal to or above the following thresholds: FPG, 5.1 mmol/L; 1-h PG, 10.0 mmol/L; 2-h PG, 8.5 mmol/L. Where indicated, thresholds were modified + 0.2 mmol/L.
† Data include 495 ORCHID participants with complete OGTT after 24 weeks gestation who did not meet HAPO study OGTT unblinding criteria; one or more OGTT PG: FPG ≥ 5.9 mmol/L; 2-h PG ≥ 11.2 mmol/L; any OGTT PG < 2.5 mmol/L.
‡ OGTT corrected for glycolysis compared to HAPO preanalytical protocol; fluoride-oxalate (FLOX) tube immediately stored on crushed ice and processed within 1 h; correction by FLOX^{ICE} algorithm.
§ OGTT corrected for glycolysis compared to FC tube stored at room temperature; correction by FC Mix algorithm.

LGA newborn remained after accounting for BMI and other maternal factors (adjusted RR 2.16, 95% CI; 1.28–3.65, *P* = 0.004). Estimated sensitivity for predicting LGA quadrupled compared with unadjusted ORCHID results (Table 2). The positive predictive value for LGA in 102 participants with untreated GDM based on FC protocol correction was 19.6% (95% CI; 12.4–28.6).

Discussion

ORCHID is the first study to assess the ability of the OGTT to identify pregnancies at risk of excess fetal growth in a high risk population from rural and remote Australia, where distances to laboratory are up to 650 km and impact of glycolysis on OGTT results is not uniform. Despite similar rates of LGA (11.3% v 9.5%) the distribution of unadjusted ORCHID PGs was clearly lower than reported in HAPO. These data are

Table 2
Cumulative contribution of each OGTT sample to GDM* diagnosis and sensitivity and specificity for LGA (*N* = 56), before and after correction for glycolysis, in ORCHID participants.

OGTT samples and correction	GDM n (%)	LGA infants of GDM mothers n (%)	Sensitivity for LGA (95% CI)	Specificity for LGA (95% CI)
FPG:				
Unadjusted (IADPSG criteria)	17 (3.4%)	3 (17.6%)	5.4% (1.1–14.9)	96.8% (94.7–98.2)
HAPO preanalytical protocol correction† (IADPSG criteria)	111 (22.4%)	18 (16.2%)	32.1% (20.3–46.0)	78.8% (74.7–82.6)
FC tube correction‡ (modified IADPSG criteria)	108 (21.8%)	18 (16.7%)	32.1% (20.3–46.0)	79.5% (75.4–83.2)
FPG & 1-h PG:				
Unadjusted (IADPSG criteria)	35 (7.1%)	4 (11.4%)	7.1% (2.0–17.3)	92.9% (90.1– 95.2)
HAPO preanalytical protocol correction† (IADPSG criteria)	124 (25.0%)	20 (16.1%)	35.7% (23.4–49.6)	76.3% (72.0–80.2)
FC tube correction‡ (modified IADPSG criteria)	128 (25.9%)	20 (15.6%)	35.7% (23.4–49.6)	75.4% (71.1–79.4)
FPG & 1-h PG & 2-h PG:				
Unadjusted (IADPSG criteria)	48 (9.7%)	6 (12.5%)	10.7% (4.0–21.9)	90.4% (87.3–93.0)
HAPO preanalytical protocol correction† (IADPSG criteria)	137 (27.7%)	23 (16.8%)	41.1% (28.1–55.0)	74.0% (69.7–78.1)
FC tube correction‡ (modified IADPSG criteria)	145 (29.3%)	24 (16.6%)	42.9% (29.7–56.8)	72.4% (68.0–76.6)

Data are cumulative number and proportion (%) of participants with GDM or number and proportion (%) of GDM with LGA newborn. Calculated sensitivity and specificity of GDM for LGA.
OGTT = 75 g oral glucose tolerance test; LGA = large-for-gestational-age (>90th centile for newborn birthweight); *n* = number; CI = confidence interval; FPG = fasting plasma glucose; IADPSG = International Association of the Diabetes in Pregnancy Study Groups; HAPO = Hyperglycaemia and Adverse Pregnancy Outcomes study; FC = fluoride-citrate; PG = plasma glucose.
Linear regression β-coefficients for FPG + delay-to-analysis + delay-to-analysis^2 + constant for FLOX^{ICE} and FC Mix algorithm are reported in Supplementary Table 1.
* Gestational diabetes mellitus (GDM) was defined IADPSG criteria; one of more PG equal to or above the following thresholds: FPG, 5.1 mmol/L; 1-h PG, 10.0 mmol/L; 2-h PG, 8.5 mmol/L. IADPSG thresholds were modified + 0.2 mmol/L for OGTT with FC tube correction.
† OGTT corrected for glycolysis compared to HAPO preanalytical protocol; fluoride-oxalate (FLOX) tube immediately stored on crushed ice and processed within 1 h; correction by FLOX^{ICE} algorithm.
‡ OGTT corrected for glycolysis compared to FC tubes stored at room temperature; correction by FC Mix algorithm.

different to the reported PG distributions of a retrospective study of routine OGTT samples from a low-risk regional and rural Australian population from Victoria (91% Caucasian; BMI: 27.7 ± 7.0 kg/m^2), where LGA frequencies relative to the three PG samples were similar to HAPO [28]. Their LGA incidence was not reported for women treated for GDM and was only 5.1% in women without GDM.

In our study the largest estimated shift in LGA frequency was observed in FPG after correction for time-dependant glycolysis, compared to 1- and 2-h PG. This is not surprising, as the standard deviation for FPG is very small (±0.4 mmol/L) [20] and highly susceptible to measurement inaccuracy. In HAPO, odds for LGA newborn increased 38% with every 0.4 mmol/L increment in FPG whilst larger increments in 1- and 2-h PG were required for similar odds increases (1-h PG: 1.7 mmol/L, 2-h PG: 1.3 mmol/L) [29]. In the Victorian cohort, non-time-dependant correction for glycolysis did not affect LGA frequency

relative to PG. In contrast to the standard WA sample handling approach, plasma was separated soon after the final sample, likely reducing the amount of glycolysis [28].

Concerningly, in our analysis almost one in five missed GDM cases had an LGA newborn. Our data suggest OGTT is only accurate for LGA risk assessment in these settings if glycolysis is minimised. The FC protocol corrected relative risk for LGA in women with GDM was similar to that reported for the HAPO cohort with GDM (1.81 ORCHID v 1.95 HAPO) [30]. Women with GDM in our study were also more likely to be overweight or obese. This was consistent with urban WA data showing high BMI in women with GDM by IADPSG criteria [31]. However, a significant risk between corrected GDM and LGA in ORCHID remained after adjusting for BMI.

Correction for estimated glycolysis did not significantly improve the low performance of individual OGTT samples for LGA as assessed by ROC curve. Similar low performance (AUC < 0.62) has been reported by other groups [32–35]. One case-control study (GDM $N = 78$; controls $N = 40$) reported moderately accurate AUC for FPG (0.782) and 1-h PG (0.719) but not 2-h PG (0.510) [36]. Samples from this case-control study were processed according to the HAPO preanalytical protocol and the comparatively higher AUC observed may be explained by the significantly higher LGA incidence (30.7%) and mean PG (FPG, 5.8 mmol/L; 1-h PG 11.3 mmol/L) in GDM cases compared to those from the prospective ORCHID cohort. Furthermore, as GDM diagnosis is based on prediction of risk for LGA, threshold selection is best approached by modelling methods (such as logistic regression as used by the IADPSG) rather than ROC curve analysis [37].

Despite only a small increase in cost ($0.24 AUD per OGTT), Australian laboratories are yet to follow European and ADA recommendations for FC tubes [16,17]. HAPO study clinical investigators also support the shift to FC tubes using current thresholds [15], however concerns of increasing GDM burden have been raised [19]. Our data demonstrate that modification of IADPSG criteria by + 0.2 mmol/L to compensate for higher PG with FC tubes is warranted for this population. This modification gave a similar positive predictive value for LGA as for GDM by HAPO preanalytical protocol correction [29]. To avoid clinician confusion it may be preferable for clinical laboratories to apply a systematic postanalytical correction factor to assay results from FC mix tubes prior to reporting results.

Minimisation of glycolysis in FLOX tubes using immediate centrifugation to separate plasma has been adopted in the Australian Capital Territory at the pathology collection centre level (urban and regional) [13]. However, many rural and remote ORCHID sites conducted OGTT in the antenatal clinic due to either a lack of local collection centre or to optimise OGTT completion. Aside from lack of access to equipment, immediate centrifugation cannot be guaranteed in the clinic setting and places additional time constraints on antenatal care staff. Following ORCHID study completion only one study location with an onsite pathology collection centre has implemented immediate centrifugation of OGTT samples, whereas nine participating clinics have implemented FC tubes.

The main limitation of this study was reliance on algorithmic correction for estimated glycolysis. It was not feasible to reproduce the HAPO preanalytical protocol in ORCHID. However paired sample validation of the FC algorithm in a small remote cohort demonstrated predictability of time-dependant glycolysis, particularly for FPG estimates on which most new GDM cases were identified [12]. Another limitation when assessing IADPSG criteria using real-world data is outcome modification due to treatment. We likely underestimated risk for LGA if treatment of 43 participants was effective. However, LGA incidence in participants with unmanaged FC protocol corrected GDM was comparable to blinded HAPO participants with GDM (19.6% ORCHID v 16.2% HAPO).

The universal applicability of IADPSG criteria has been questioned [21]. Unlike the ORCHID cohort, representation of Aboriginal participants in the HAPO cohort was likely low (<2.6% other ethnicity) [20]. Ethnic differences coupled with higher rates of obesity and multiparity potentially modified the incidence of LGA in ORCHID newborns. Furthermore, different β-coefficients were used to calculate birthweight centiles in ORCHID compared to HAPO which may also have altered LGA incidence. Although we cannot assert that our sample is representative of the rural and remote population Australia wide, correction for glycolysis did improve risk assessment for LGA even after accounting for differences in ethnicity, BMI and parity. This suggests an effect of glycaemia on LGA above that of confounding factors, that is better identified if glycolysis is minimised in these settings.

This study did not address other factors that can also introduce error into OGTT, including physiological factors, patient preparation and laboratory error [38]. Analytical error was not available for all sites, however all laboratories were National Association of Testing Authorities, Australia (NATA) accredited. Although the total allowable error of up to 6.9% for glucose measurement can significantly impact GDM incidence, this remains lower than the maximum error introduced by glycolysis (13.6%) [12].

In rural and remote Australia the potential to reduce excess fetal growth is likely to be missed due to preanalytical glycolysis. Adopting ADA and European recommendations to use FC tubes would address this. Changing the IADPSG thresholds or applying a postanalytical correction to assay results when using FC tubes will result in GDM diagnosis more consistent with the HAPO study and reduce the burden of unnecessary diagnoses while improving risk assessment for LGA newborn.

CRediT authorship contribution statement

Emma L. Jamieson: Conceptualization, Methodology, Data curation, Formal analysis, Software, Funding acquisition, Investigation, Validation, Visualization, Writing - original draft, Project administration, Resources. **Erica P. Spry:** Data curation, Funding acquisition, Investigation, Visualization, Writing - review & editing, Project administration, Resources. **Andrew B. Kirke:** Conceptualization, Methodology, Funding acquisition, Investigation, Visualization, Writing - review & editing, Resources. **Carly Roxburgh:** Conceptualization, Methodology, Investigation, Visualization, Writing - review & editing, Resources. **David N. Atkinson:** Conceptualization, Methodology, Funding acquisition, Investigation, Supervision, Visualization, Writing - review & editing, Resources. **Julia V. Marley:** Conceptualization, Methodology, Formal analysis, Supervision, Investigation, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcte.2020.100247.

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