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Clinical Research Article

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Dichotomy in the Impact of Elevated Maternal Glucose Levels on Neonatal Epigenome

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Abbreviations: BMI, body mass index; EWAS, epigenome-wide association study; FDR, false discovery rate; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; GO, gene ontology; GUSTO, Growing Up in Singapore Towards Healthy Outcomes; IVF, in vitro fertilization; KKH, Kandang Kerbau Women's and Children's Hospital; NUH, National University Hospital; OGTT, oral glucose tolerance test; SV, surrogate variable; T2DM, type 2 diabetes mellitus.

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Abstract

Context: Antenatal hyperglycemia is associated with increased risk of future adverse health outcomes in both mother and child. Variations in offspring's epigenome can reflect the impact and response to in utero glycemic exposure, and may have different consequences for the child.

Objective: We examined possible differences in associations of basal glucose status and glucose handling during pregnancy with both clinical covariates and offspring cord tissue DNA methylation.

Research Design and Methods: This study included 830 mother-offspring dyads from the Growing Up in Singapore Towards Healthy Outcomes cohort. The fetal epigenome of umbilical cord tissue was profiled using Illumina HumanMethylation450 arrays. Associations of maternal mid-pregnancy fasting (fasting plasma glucose [FPG]) and 2-hour plasma glucose (2hPG) after a 75-g oral glucose challenge with both maternal clinical phenotypes and offspring epigenome at delivery were investigated separately.

Results: Maternal age, prepregnancy body mass index, and blood pressure measures were associated with both FPG and 2hPG, whereas Chinese ethnicity ($P = 1.9 \times 10^{-4}$), maternal height ($P = 1.1 \times 10^{-4}$), pregnancy weight gain ($P = 2.2 \times 10^{-3}$), prepregnancy alcohol consumption ($P = 4.6 \times 10^{-4}$), and tobacco exposure ($P = 1.9 \times 10^{-3}$) showed significantly opposite associations between the 2 glucose measures. Most importantly, we observed a dichotomy in the effects of these glycemic indices on the offspring epigenome. Offspring born to mothers with elevated 2hPG showed global hypomethylation. CpGs most associated with the 2 measures also reflected differences in gene ontologies and had different associations with offspring birthweight.

Conclusions: Our findings suggest that 2 traditionally used glycemic indices for diagnosing gestational diabetes may reflect distinctive pathophysiologies in pregnancy, and have differential impacts on the offspring's DNA methylome.

Key Words: epigenome wide association study, gestational diabetes, fasting plasma glucose, 2h oral glucose tolerance test, DNA methylation

Maternal hyperglycemia is associated with increased risk of adverse perinatal outcomes, including fetal macrosomia, intrauterine growth restriction, neonatal hypoglycemia, and neonatal intensive care unit admission (1). Furthermore, children born to mothers with antenatal hyperglycemia are at increased risk of developing childhood obesity (2), adult obesity, and diabetes (3), thus exacerbating a vicious health cycle. Gestational diabetes mellitus (GDM), defined by maternal hyperglycemia during pregnancy (4), is clinically diagnosed by oral glucose tolerance tests (OGTTs), with several definitions in use worldwide. A positive clinical diagnosis typically entails an abnormality in at least 1 of several glucose measures: fasting plasma glucose (FPG) collected after an overnight (8-10 hours) fast, and plasma glucose collected 1, 2, and/or 3 hours (x-hPG, where x = 1, 2, or 3 and hPG is hour plasma glucose) after administration of a fixed OGTT (5). Globally, American and European cohorts report lower prevalences of GDM (generally below 10%), whereas Asian cohorts typically reflect higher prevalences (up to 28%) (6). Outside of pregnancy, fasting and 2hPG measures are also used in the diagnosis of type 2 diabetes mellitus (T2DM). Closely related to T2DM are 2 prediabetic metabolic states (7), impaired fasting glucose (8) and impaired glucose tolerance (9), conditions associated with an abnormal FPG and an abnormal 2hPG, respectively. Different but overlapping physiological mechanisms are involved in maintaining fasting glucose levels and in determining the glycemic response to an oral glucose load. The differential effects of an elevated gestational FPG and x-hPG on maternal (10), birth (11), and childhood outcomes (12) further suggest that these 2 parameters have different underlying etiologies and pathophysiologies, even in pregnancy.

Maternal hyperglycemia may affect an offspring's future health outcomes through perturbations in the fetal methylome (13). Previous epigenome-wide association studies (EWAS) investigating effects of maternal gestational hyperglycemia on offspring methylome at delivery were primarily conducted on Caucasian cohorts (14-16), did not compare differences between elevated FPG and x-hPG

on the offspring methylome (16-18), or had small sample sizes (< 100) (17-20). Because the incidence of GDM is high in Asian populations, our study focuses on developing insights into the preconception and antenatal factors associated with FPG and 2hPG status mid-pregnancy (such as maternal anthropometry, pregnancy plasma biomarkers, environmental and mental health measures), as well as investigating the associated impact of antenatal glycemic levels on the offspring's DNA methylome.

Materials and Methods

Study population

Expecting mothers were enrolled in the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) birth cohort as previously described (21). Briefly, women were recruited from 2 public hospitals (National University Hospital [NUH] and Kandang Kerbau Women's and Children's Hospital [KKH]) in Singapore during their first trimester visit. Women ≥ 18 years of age, homogenous ethnic background (up to grandparents), and of Chinese/Malay/Indian ethnic origin were recruited. Subjects on chemotherapy or psychotropic medication, or with diabetes before recruitment, or had second trimester OGTTs fulfilling the clinical threshold for possible undiagnosed preexisting diabetes (FPG ≥ 7.0 mmol/L, 2hPG ≥ 11.1 mmol/L) were excluded from this study. To prevent outlier-driven results, 2 subjects whose OGTT values below the 0.001th centile were also removed (1 with FPG = 2.9 mmol/L, another with 2hPG = 2.9 mmol/L). Written informed consent was obtained from the participants. The GUSTO study was approved by the Centralized Institutional Review Board of KKH and the Domain-Specific Review Board of NUH. In this study, only live, singleton, full-term births with no congenital anomalies, with complete information of maternal OGTT results, and infant umbilical cord DNA methylome were included (n = 830, Tables 1 and 2).

Maternal anthropometry and pregnancy environmental exposures

Midpregnancy (approximately 26 weeks gestation), after overnight fasting of at least 8 hours, fasting, and 2 hours after 75-g OGTT (as recommended by World Health Organization at the time of collection (5)) plasma was collected from pregnant mothers and measured on a colorimeter (Advia 2400 Chemistry system [Siemen Medical Solutions Diagnostics] in NUH; Beckman LX20 Pro analyser [Beckman Coulter] in KKH) within 4 hours of sampling. Additional plasma was collected to determine fatty acid (n-6 polyunsaturated fatty acids, n-3 polyunsaturated fatty acids, monounsaturated fatty acids, saturated fatty acids), and micronutrient concentrations (folate; vitamins B6, B12, D; and minerals Ca and Zn) on the same visit. Micronutrient extraction and quantification, as well as acquisition of other risk factors/predictors are elaborated in Lim et al (22).

Cord tissue DNA methylation data

Methylation profiling of DNA from infant umbilical cords was performed using the Illumina HumanMethylation450 BeadChip arrays per the manufacturer's instructions (23) and processed as described previously (24), including the removal of cross-hybridizing probes (25, 26). Randomization of samples was performed across chip and chip position with respect to important clinical covariates such as ethnicity, infant sex, and gestational age. Although both glucose measures did not associate with chip or chip position, the first principal component of a principal component analysis on raw infant cord tissue DNA methylation data revealed a significant association with chip. COMBAT (27) was used to adjust cord tissue DNA methylation data for chip effects, removing CpGs with missing values across all 12 positions on any chip. DNA extraction batch, bisulfite conversion batch, and hospital remained significantly associated with the top principal components of the chip-adjusted cord tissue DNA methylation data, as well as both glucose measures, and thus were included in regression models involving cord tissue DNA methylation. Cellular composition of neonatal umbilical cords was estimated using a reference panel of stromal, endothelial, epithelial, and granulocytes as a surrogate for potential residual cord blood contamination within the cord tissue samples (28). Their principal components were adjusted as covariates in all cord tissue DNA methylation regression models. To ensure statistical robustness, probes with low cord tissue DNA methylation variation (2.5th-97.5th centile range below 10%), absent in > 10% of samples, containing single nucleotide polymorphisms on the CpG dinucleotide, and showing multimodal distributions (ENmix (29), minimum distance between nodes = 0.2) were removed, resulting in a total of 102 498 autosomal CpGs for analysis. The majority of the removed probes had low interindividual variability (234 186 probes, 61% of all removed probes). Random technical variations make probes with such low variability prone to false-positive results and thus these were removed as recommended (30). Homer *annotatePeaks* function (hg19) was used to annotate CpGs with respect to gene features (promoter, 5′-UTR, exon, intron, 3′-UTR, TTS, and intergenic regions) and CpG islands (island, shore, shelf, open sea). With respect to enhancer regions, CpGs were annotated using the combination of the Infinium HumanMethylation450 manifest file, predicted enhancers

Table 1. Study cohort characteristics (categorical)

Covariate (N)	Category	No.	Proportion
Hospital (N = 830)	KKH	619	74.6%
	NUH	211	25.4%
Ethnicity (N = 830)	Chinese	486	58.6%
	Malay	207	24.9%
	Indian	137	16.5%
Maternal education (N = 830)	Secondary and below	443	53.4%
	Tertiary and above	387	46.6%
Maternal occupation (N = 830)	Others	355	42.8%
	White collar	475	57.2%
Monthly household income (N = 782)	<S$4,000	331	42.3%
	≥S$4,000	451	57.7%
Familial T2DM (N = 830)	No history	586	70.6%
	Yes	244	29.4%
Previous GDM (N = 830)	No	799	96.3%
	Yes	31	3.7%
Previous pregnancy (N = 641)	Non-first pregnancy	373	58.2%
	First pregnancy	268	41.8%
Previous delivery (N = 641)	Non-firstborn	321	50.1%
	Firstborn	320	49.9%
IVF status (N = 818)	No	761	93%
	Yes	57	7%
Prepregnancy alcohol consumption (N = 822)	No	534	65%
	Yes	288	35%
Pregnancy alcohol consumption (N = 808)	No	794	98.3%
	Yes	14	1.7%
Prepregnancy tobacco exposure (N = 825)	No	470	57%
	Yes	355	43%
Pregnancy tobacco exposure (N = 824)	No	519	63%
	Yes	305	37%
Overall tobacco exposure (N = 825)	No	456	55.3%
	Yes	369	44.7%
Depression (BDI) (N = 784)	< 14	655	83.5%
	≥ 14	129	16.5%
Depression (EPDS) (N = 805)	< 14	740	91.9%
	≥ 14	65	8.1%
Depression (BDI/EPDS/STAI) (N = 791)	Not depressed	640	80.9%
	Depressed	151	19.1%
Plasma folate (N = 731)	≤ 6 ng/mL	68	9.3%
	> 6 ng/mL	663	90.7%
Plasma vitamin B6 (PLP) (N = 730)	≤ 20 nmol/L	115	15.8%
	> 20 nmol/L	615	84.2%
Plasma vitamin B12 (N = 731)	≤ 300 pg/mL	415	56.8%
	> 300pg/mL	316	43.2%
Plasma vitamin D (N = 695)	≤ 50 nmol/L	88	12.7%
	> 50nmol/L	607	87.3%
Plasma calcium (N = 730)	≤ 81.8 mg/L	64	8.8%
	> 81.8mg/L	666	91.2%
Plasma zinc (N = 730)	≤ 700 µg/L	120	16.4%
	> 700 µg/L	610	83.6%

Abbreviations: BDI, Beck Depression Inventory; EPDS, Edinburgh Postnatal Depression Scale; GDM, gestational diabetes mellitus; IVF, in vitro fertilization; KKH, Kandang Kerbau Women's and Children's Hospital; NUH, National University Hospital; PLP, pyridoxal 5′ phosphate; STAI, State-Trait Anxiety Inventory; T2DM, type 2 diabetes mellitus.

Table 2. Study cohort characteristics (numerical)

Covariate	Category	No.	Mean	SD
OGTT (mmol/L)	FPG	830	4.3	0.4
	2hPG	830	6.5	1.4
Maternal age (y)		830	30.9	5.1
Maternal height (cm)		819	158.3	5.6
Adiposity	Prepregnancy BMI (kg/m^2)	781	22.7	4.4
	Pregnancy weight gain (kg)	777	8.6	4.5
Midpregnancy blood pressure (mmHg)	Systolic	830	114.5	13.0
	Diastolic	830	66.2	9.2
Midpregnancy caloric intake (kCal)		807	1886.4	571.5
Midpregnancy plasma fatty acids (mcg/mL)	Total saturated	730	1116.2	372.7
	Total omega-3	730	159.2	80.4
	Total omega-6	730	828.6	274.1
	Total monounsaturated	730	328.3	109.2
	Total polyunsaturated	730	987.9	337.4
	Total	730	2432.4	788.8

Abbreviations: 2hPG, 2-hour plasma glucose; BMI, body mass index; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test.

from the Encyclopedia of DNA Elements consortium (31), as well as the Functional Annotation of the Mammalian Genome consortium's FANTOM5 phase 1 and 2 data (32).

Analysis of association between midpregnancy FPG/2hPG and clinically relevant variables

Linear regression models were first used to investigate the relationship between maternal midpregnancy plasma glucose concentrations and various prenatal/pregnancy covariates, adjusting for hospital and ethnicity. We note that the different clinical variables were examined individually with the glucose measures. For clinical covariates that were associated with at least 1 of the OGTT measures ($P < 0.05$), general linear models with unstructured covariance structure were used to examine whether the associations of the covariates with the OGTT measures were different.

Analysis of association between infant cord tissue DNA methylation and midpregnancy FPG/2hPG

Linear regression models were then used to examine the association of neonatal cord tissue DNA methylation values with maternal plasma glucose concentrations, with adjustment for hospital, infant sex, ethnicity, gestational age at delivery, principal components of cellular composition, DNA extraction batch, and bisulfite conversion batch effects. To allow for the associations between cord tissue DNA methylation and cellular proportions to be ethnicity dependent, models also included the interaction between ethnicity and principal components of cellular proportions. Because both maternal glucose measures possessed different ranges, levels were normalized with mean = 0, SD = 0.5 before analysis to ensure effect sizes were comparable. Because of this normalization, regression effect sizes are reported as increase in cord tissue DNA methylation Z-score for every 2 SD increase in maternal glucose levels. Additionally, to ensure results are not driven by outliers, outlier values in cord tissue DNA methylation and continuous variables were truncated to the next possible boundary values.

As a technical sensitivity analysis, also reported are results additionally adjusted for chip row, as well as adjusted for unaccounted technical covariates with the use of surrogate variable analysis (33). Five surrogate variables (SVs) were generated from scaled infant umbilical cord tissue DNA methylation values, with covariates protected in generating the SVs (FPG or 2hPG, ethnicity, gestational age, infant sex, DNA extraction batch, bisulfite conversion batch, chip position, and cell type proportion Principal Components). These SVs were included in linear regression models in addition to the previously mentioned covariates.

For the top 50 CpGs most associated with either OGTT measure, general linear models with unstructured covariance structure were used to examine whether the associations of the cord tissue DNA methylation with the respective OGTT measure were significantly different, reporting CpGs passing Bonferroni as well as nominal $P = 0.05$ thresholds.

Effect sizes between the glucose measures are denoted as β_f for FPG and β_{2h} for 2hPG associations, respectively. Similarly, P values between the glucose measures are denoted as p_f and p_{2h} for FPG and 2hPG associations, respectively.

Gene ontology enrichment

To identify biological pathways (GO and Kyoto Encyclopedia of Genes and Genomes pathways) potentially affected by the differential associations maternal antenatal glycemic measures have on infant cord tissue DNA methylation, the top 500 most associated CpGs per glucose measure were input into missMethyl (34). missMethyl adjusts GO enrichments for both the number of CpGs per gene, as well as the length of the gene, mitigating the nonuniform coverage of genes on the Infinium HumanMethylation450 array. Because CpGs may map onto more than 1 gene, a single CpG with multiple gene annotations may skew GO results; hence, CpGs with more than 3 gene annotations were removed before GO analysis. Subsequently, missMethyl results provide a comprehensive list of GO terms, including GOs with very large parent gene lists, as well as GOs with very similar gene lists between them. Because GOs with large parent gene lists may not inform gene functionalities, GOs with more than 400 genes were removed. GOs with very few genes present in the enriched CpG list may also be prone to false positives; hence, GOs with < 3 genes present within the enriched CpG list were also removed. To address highly similar GOs, REVIGO (35) was used to condense the GO terms obtained. REVIGO removes GO terms with > 70% semantic similarity measure between gene lists, retaining the GO term with a more unique gene list.

Comparison against existing EWAS

We compiled a list of known differentially methylated CpGs associated with gestational diabetes across various EWASs to find CpG candidates that may have biomarker potential for diabetes-related pathophysiologies. Because this is the first study investigating offspring cord tissue DNA methylation with antenatal glycemic levels, we expanded the scope of comparable EWAS to include fetal-origin biosamples (such as cord blood) and different GDM diagnostic criteria. However, given known reproducibility issues among DNA methylation studies (36, 37), we limited our comparison to genome-wide (as opposed to candidate-based) studies investigating offspring DNA methylation with study cohorts greater than 100 participants.

Analysis of association between infant cord tissue DNA methylation and birthweight

To assess whether these differences in cord tissue DNA methylation corresponded to differences in infant outcomes, we performed additional linear regression models to examine the association of neonatal cord tissue DNA methylation values with infant birthweight, with adjustment for hospital, infant sex, ethnicity, gestational age at delivery, principal components of cellular composition, DNA extraction batch, bisulfite conversion batch effects, and interactions between ethnicity and principal components of cellular proportions.

Results

Study demographics

This analysis used 830 mother-offspring dyads that had complete maternal glucose, gestational age, infant sex, ethnicity, and umbilical cord tissue DNA methylation data (Tables 1 and 2). This study comprised 59% Chinese, 25% Malay, and 17% Indian participants. Average prepregnancy body mass index (BMI) was 22.7 kg/m^2 and average weight gain was 8.6 kg. The mean midpregnancy glucose concentrations were 4.3 mmol/L and 6.5 mmol/L for FPG and 2hPG, respectively. Fifty-three percent of infants were born male; 47% of infants were born female. The average gestation was 39 weeks (SD 1 week) and the average birthweight was 3.2 kg (SD 0.4 kg). Because maternal glycemia is known to associate with adverse maternal and infant outcomes on a continuous basis (38), we investigated these glucose measures as continuous variables as opposed to a clinically defined GDM status. Nevertheless, as a benchmark reference, 18% of participants were diagnosed with GDM given the prevailing GDM criteria during recruitment (1999 World Health Organization GDM criteria: FPG ≥ 7.0 mmol/L, 2hPG ≥ 7.8 mmol/L) (39). Proportionally, this affected Indian women the most (24%), followed by Chinese (19%), and then Malay women (9.0%).

Common and distinct prenatal variables contributing to midpregnancy FPG and 2hPG status

Looking at the relationship between both pregnancy glucose measures with various clinical covariates independently (ie, FPG vs clinical covariates; 2hPG vs clinical covariates), 4 covariates were significant associated (at a Bonferroni adjusted P value of $P < 0.05/38 = 1.3 \times 10^{-3}$) with both glucose measures: maternal age ($\beta_f = 0.11$, $p_f = 1.1 \times 10^{-3}$; $\beta_{2h} = 0.23$, $p_{2h} = 3.0 \times 10^{-11}$), prepregnancy BMI ($\beta_f = 0.24$, $p_f = 3.6 \times 10^{-11}$; $\beta_{2h} = 0.20$, $p_{2h} = 5.8 \times 10^{-8}$), and blood pressure (systolic [$\beta_f = 0.17$, $p_f = 1.3 \times 10^{-6}$; $\beta_{2h} = 0.13$, $p_{2h} = 1.9 \times 10^{-4}$] and diastolic [$\beta_f = 0.13$, $p_f = 2.0 \times 10^{-4}$; $\beta_{2h} = 0.14$, $p_{2h} = 1.0 \times 10^{-4}$]) (Table 3). Several other covariates, however, were only significantly associated with 1 pregnancy glucose measure and not the other. Indian ethnicity ($\beta_f = 0.18$, $p_f = 5.8 \times 10^{-5}$; $\beta_{2h} = 0.04$, $p_{2h} = 0.40$)

Table 3. Relationship of maternal midpregnancy OGTT with clinical covariates adjusted by hospital and ethnicity

	Covariate	FPG[a,c]			2hPG[b,d]		
		Effect size	95% CI	*P* value	Effect size	95% CI	*P* value
Demographics	±Chinese ethnicity[b]	-6.46e-02	-0.13 to 0	6.42e-02	8.76e-02	0.02 to 0.16	**1.30e-02**
	Malay ethnicity[d]	-5.37e-02	-0.13 to 0.02	1.78e-01	-1.44e-01	-0.22 to -0.07	**3.50e-04**
	Indian ethnicity[c]	1.85e-01	0.1 to 0.27	**5.78e-05**	3.96e-02	-0.05 to 0.13	3.97e-01
	Education	-1.89e-02	-0.09 to 0.05	5.89e-01	-1.65e-03	-0.07 to 0.07	9.63e-01
	± Occupation	-7.53e-04	-0.07 to 0.07	9.83e-01	2.62e-02	-0.04 to 0.1	4.60e-01
	±Household income[b]	-5.02e-03	-0.08 to 0.07	8.97e-01	8.65e-02	0.01 to 0.16	**2.64e-02**
	Age[c,d]	1.14e-01	0.05 to 0.18	**1.11e-03**	2.32e-01	0.16 to 0.3	**3.03e-11**
	Familial T2DM	6.10e-02	-0.01 to 0.14	1.07e-01	3.94e-02	-0.04 to 0.11	3.05e-01
	Previous GDM[a,d]	2.27e-01	0.05 to 0.4	**1.15e-02**	4.85e-01	0.31 to 0.66	**8.13e-08**
	Previous pregnancy[a]	-1.15e-01	-0.19 to -0.04	**3.52e-03**	-7.30e-02	-0.15 to 0.01	6.97e-02
	Previous delivery[a]	-1.09e-01	-0.18 to -0.03	**5.06e-03**	-7.44e-02	-0.15 to 0	6.12e-02
	IVF status[d]	8.09e-02	-0.05 to 0.22	2.37e-01	2.55e-01	0.12 to 0.39	**2.21e-04**
Adiposity/anthropometry	± Height[d]	3.52e-02	-0.03 to 0.1	3.12e-01	-1.22e-01	-0.19 to -0.05	**5.40e-04**
	Prepregnancy BMI[c,d]	2.42e-01	0.17 to 0.31	**3.57e-11**	2.03e-01	0.13 to 0.28	**5.80e-08**
	± Pregnancy weight gain[a]	7.88e-02	0.01 to 0.15	**2.53e-02**	-4.80e-02	-0.12 to 0.02	1.82e-01
	Systolic BP[c,d]	1.65e-01	0.1 to 0.23	**1.28e-06**	1.30e-01	0.06 to 0.2	**1.87e-04**
	Diastolic BP[c,d]	1.33e-01	0.06 to 0.2	**1.95e-04**	1.40e-01	0.07 to 0.21	**1.03e-04**
Environment/mental health	± Prepregnancy alcohol consumption[b]	6.99e-02	0 to 0.14	6.65e-02	-8.56e-02	-0.16 to -0.01	**2.63e-02**
	± Pregnancy alcohol consumption	1.40e-01	-0.12 to 0.4	2.87e-01	-5.69e-02	-0.32 to 0.21	6.72e-01
	± Prepregnancy tobacco exposure[b]	4.99e-02	-0.02 to 0.12	1.79e-01	-9.41e-02	-0.17 to -0.02	**1.27e-02**
	± Pregnancy tobacco exposure[b]	6.81e-02	-0.01 to 0.14	7.31e-02	-9.67e-02	-0.17 to -0.02	**1.23e-02**
	± Overall tobacco exposure[b]	3.36e-02	-0.04 to 0.11	3.60e-01	-1.00e-01	-0.17 to -0.03	**7.28e-03**
	Depression (BDI)	2.89e-02	-0.06 to 0.12	5.45e-01	2.78e-02	-0.07 to 0.12	5.70e-01
	± Depression (EPDS)	1.20e-02	-0.11 to 0.14	8.49e-01	-8.68e-02	-0.21 to 0.04	1.81e-01
	Depression (BDI/EPDS/STAI)	2.39e-02	-0.06 to 0.11	5.93e-01	9.65e-03	-0.08 to 0.1	8.33e-01
Pregnancy plasma biomarkers	± Caloric intake	1.45e-02	-0.05 to 0.08	6.78e-01	-4.54e-02	-0.12 to 0.02	2.02e-01
	± Plasma folate[b]	-3.95e-02	-0.17 to 0.09	5.39e-01	1.61e-01	0.04 to 0.29	**1.14e-02**
	Plasma vitamin B6	1.04e-02	-0.09 to 0.11	8.39e-01	5.45e-02	-0.05 to 0.15	2.83e-01
	Plasma vitamin B12[c]	-1.36e-01	-0.21 to -0.06	**2.66e-04**	-1.71e-02	-0.09 to 0.06	6.46e-01
	± Plasma vitamin D	-6.46e-03	-0.12 to 0.11	9.12e-01	9.49e-02	-0.02 to 0.21	1.01e-01
	Plasma calcium[b]	9.16e-02	-0.04 to 0.22	1.63e-01	1.58e-01	0.03 to 0.28	**1.52e-02**
	Plasma zinc[b]	2.37e-02	-0.07 to 0.12	6.34e-01	1.19e-01	0.02 to 0.22	**1.57e-02**
	Plasma ∑ saturated FA	7.90e-03	-0.06 to 0.08	8.30e-01	1.08e-02	-0.06 to 0.08	7.68e-01
	Plasma ∑ omega-3 FA	2.08e-03	-0.07 to 0.07	9.55e-01	2.97e-02	-0.04 to 0.1	4.21e-01
	Plasma ∑ omega-6 FA	2.49e-02	-0.05 to 0.1	4.97e-01	7.16e-03	-0.06 to 0.08	8.44e-01
	Plasma ∑ monounsaturated FA	-8.41e-03	-0.08 to 0.07	8.23e-01	-2.13e-02	-0.09 to 0.05	5.68e-01
	Plasma ∑ polyunsaturated FA	1.96e-02	-0.05 to 0.09	5.94e-01	1.20e-02	-0.06 to 0.08	7.42e-01
	Plasma ∑ FA	1.28e-02	-0.06 to 0.08	7.29e-01	7.00e-03	-0.06 to 0.08	8.48e-01

Clinical covariates were analyzed using linear regression, adjusted for hospital and ethnicity except in the case of individual ethnicities, where specific ethnicities were analyzed as a binary variable (yes/no) where only hospital was adjusted in the model. Clinical covariates were generally classified into 4 major categories labelled on the left side of the table. Standardized effect size of the regression is presented alongside the 95% CIs and *P* values.

Clinical covariates found to have $P < 0.05$ with respect to [a]fasting plasma glucose (FPG) or [b]2-hour plasma glucose (2hPG) have *P* values indicated in bold type, represented in Fig. 1. Those found to have *P* values below the Bonferroni threshold ($0.05/38 = 1.3 \times 10^{-3}$) for [c]FPG or [d]2hPG have a different superscript suffix. Antagonistic effect sizes with relation to the same clinical covariate is prefixed with a ±.

Abbreviations: BDI, Beck Depression Inventory; BP, blood pressure; CI, Confidence Interval; EPDS, Edinburgh Postnatal Depression Scale; FA, fatty acid; STAI, State-Trait Anxiety Inventory.

and midpregnancy maternal vitamin B12 status (β_f = -0.14, $p_f = 2.7 \times 10^{-4}$; β_{2h} = -0.02, p_{2h} = 0.65) were significantly associated with FPG but not 2hPG, whereas Malay ethnicity (β_f = -0.05, p_f = 0.18; β_{2h} = -0.14, $p_{2h} = 3.5 \times 10^{-4}$), history of previous GDM (β_f = 0.23, $p_f = 1.1 \times 10^{-2}$; β_{2h} = 0.48, $p_{2h} = 8.1 \times 10^{-8}$), current pregnancy IVF status (β_f = 0.08, p_f = 0.24; β_{2h} = 0.26, $p_{2h} = 2.2 \times 10^{-4}$), and maternal height ($\beta_f$ = 0.04, p_f = 0.31; β_{2h} = -0.12, $p_{2h} = 5.4 \times 10^{-4}$) were significantly associated with 2hPG but not FPG.

We examined whether the association of a covariate with 1 glucose measure was significantly different from the corresponding association with other glucose measure (ie, [FPG vs clinical covariate] vs [2hPG vs the same clinical covariate]). As such, we selected 22 covariates nominally associated ($P < 0.05$) with at least 1 OGTT measure (Fig. 1, Table 4). Maternal midpregnancy (approximately 26 weeks gestation) FPG and 2hPG measurements were significantly different (Bonferroni threshold = 0.05/22 = 2.3×10^{-3}) with respect to 7 of these 22 clinical covariates, including Chinese ethnicity ($P = 1.9 \times 10^{-4}$), maternal height ($P = 1.1 \times 10^{-4}$), pregnancy weight gain ($P = 2.2 \times 10^{-3}$), prepregnancy alcohol consumption ($P = 4.6 \times 10^{-4}$), and tobacco exposure (prepregnancy $P = 9.2 \times 10^{-4}$; pregnancy $P = 2.1 \times 10^{-4}$; overall $P = 1.9 \times 10^{-3}$). Another 7 covariates were significant at $P < 0.05$ but not passing Bonferroni cutoff, including Indian ethnicity ($P = 7.1 \times 10^{-3}$), household

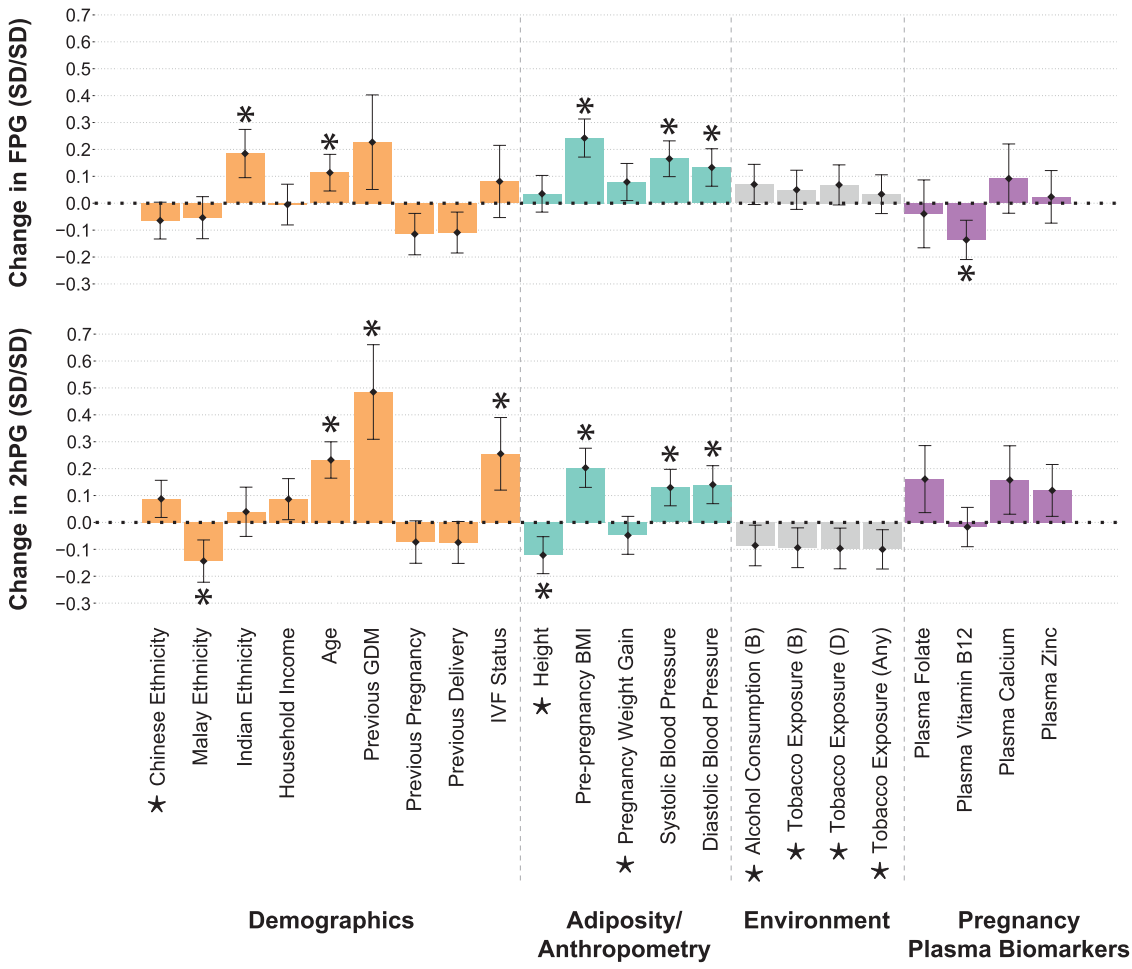

Figure 1. Clinical covariates significantly associated with either glucose measure, adjusted for hospital and ethnicity. Linear regression analyses of maternal mid-pregnancy FPG and 2hPG was performed on 38 different risk factors/predictors, adjusting for hospital and ethnicity. Individual ethnicities were analyzed as a binary variable (yes/no) adjusted for hospital. Risk factors/predictors which were found to have $P < 0.05$ are grouped into 4 categories here on the horizontal axis: demographics (orange), adiposity/anthropometry (green), environment (gray), and pregnancy plasma biomarkers (purple). On the vertical axis, point estimates (height of bars) and 95% confidence intervals (top and bottom whiskers) reflect standardized effect sizes. For reporting of standardized effect sizes, OGTT and continuous variables were scaled to have an SD of 0.5, and binary variables retained their original coding (0 vs 1). Risk factors/predictors that significantly associate (passing Bonferroni, $P < 0.05/38 = 1.3 \times 10^{-3}$) with either glucose measure are indicated with an asterisk on that respective glucose measure. Of these 22 covariates plotted, general linear models with unstructured covariance structure were used to examine whether the associations of the covariates with the OGTT measures were different. Covariates found Bonferroni significantly different in the associations between FPG and 2hPG are indicated by a star prefix on the x-axis label. B, Before pregnancy; D, during pregnancy; GDM, gestational diabetes; IVF, in vitro fertilization.

Table 4. *P* values for testing whether there are significant differences between the associations of the 2 Maternal midpregnancy OGTT measures with clinical covariates

	Covariate	*P* value
Demographics	Chinese ethnicity[a]	**1.85e-04**
	Malay ethnicity	5.35e-02
	Indian ethnicity[b]	7.07e-03
	Household income[b]	4.32e-02
	Age[b]	3.61e-03
	Previous GDM[b]	1.40e-02
	Previous pregnancy	3.62e-01
	Previous delivery	4.46e-01
	IVF status[b]	2.95e-02
Adiposity/anthropometry	Height[a]	**1.09e-04**
	Prepregnancy BMI	3.69e-01
	Pregnancy weight gain[a]	**2.25e-03**
	Systolic blood pressure	3.75e-01
	Diastolic blood pressure	8.58e-01
Environment	Prepregnancy alcohol consumption[a]	**4.61e-04**
	Prepregnancy tobacco exposure[a]	**9.18e-04**
	Pregnancy tobacco exposure[a]	**2.05e-04**
	Overall tobacco exposure[a]	**1.85e-03**
Pregnancy plasma biomarkers	Plasma folate[b]	7.44e-03
	Plasma vitamin B12[b]	6.46e-03
	Plasma calcium	3.89e-01
	Plasma zinc	1.01e-01

Twenty-two clinical covariates were found to associate with at least 1 of the OGTT measures shown in Table 3 ($P < 0.05$). These were further evaluated, comparing whether the associations of the clinical covariates with the OGTT measures were different using general linear models with unstructured covariance structure. Specific ethnicities were analyzed as a binary variable (yes/no). Clinical covariates found to have differential association with fasting plasma glucose (FPG) as opposed to 2-hour post OGTT plasma glucose at [a]Bonferroni threshold ($0.05/22 = 2.3 \times 10^{-3}$) are indicated in bold type. Those at [b]$P < 0.05$ have a different suffix. Abbreviations: BMI, body mass index; GDM, gestational diabetes mellitus; IVF, in vitro fertilization; OGTT, oral glucose tolerance test.

income ($P = 4.3 \times 10^{-2}$), maternal age ($P = 3.6 \times 10^{-3}$), history of previous GDM ($P = 0.014$), in vitro fertilization (IVF) status ($P = 0.030$), midpregnancy plasma folate status ($P = 7.4 \times 10^{-3}$), and midpregnancy plasma vitamin B12 status ($P = 6.5 \times 10^{-3}$). If ethnicity was modelled jointly as 2 binary variables, the associations remain different for the 2 glucose measures (22). Supplementary analyses such as this, generated during this study, are included in the data repository listed in Lim et al (22).

Maternal FPG and 2hPG influences umbilical cord tissue DNA methylation

In total, 336 684 CpGs from the infant umbilical cord tissue DNA methylome passed quality control, of which 102 498 probes had DNA methylation 2.5th to 97.5th centile range > 10%, and were selected for subsequent analysis. The correlation between the 2 glucose measures was low (Fig. 2A, Pearson correlation = 0.31), and there were differences in association of cord tissue DNA methylation with respect to maternal FPG and 2hPG measures. The association of FPG with the infant umbilical cord tissue DNA methylome appeared to be equally balanced between hypo- and hypermethylation (Fig. 2B), whereas the association of 2hPG appeared to skew toward hypomethylation, especially among the more statistically associated CpGs (Fig. 2C). Although the directionality of change in cord tissue DNA methylation appeared to be mostly preserved among differentially methylated CpGs from 1 glucose measure to the other (Fig. 2D), statistical association was typically lost (Fig. 2E). Notably, the top 50 most associated CpGs with respect to FPG (Fig. 2F, (22)) were mostly different from the corresponding CpGs with respect to 2hPG (Fig. 2G, (22)). Genes in proximity to the most strongly associated CpGs also differed markedly between the 2 glucose measures (22). Although none of CpGs attained genome-wide significance on either glucose measure, among the top 500 most associated CpGs, all 500 retained effect size directionality with nominal P value < 0.005 in sensitivity analysis 1 in both glucose measures, and 497 (99.4%) and 495 (99.0%) CpGs retained directionality with nominal P value < 0.005 in sensitivity analysis 2 for FPG and 2hPG, respectively.

For the top 50 CpGs most associated with each glucose measure, we examined whether the association of that CpG

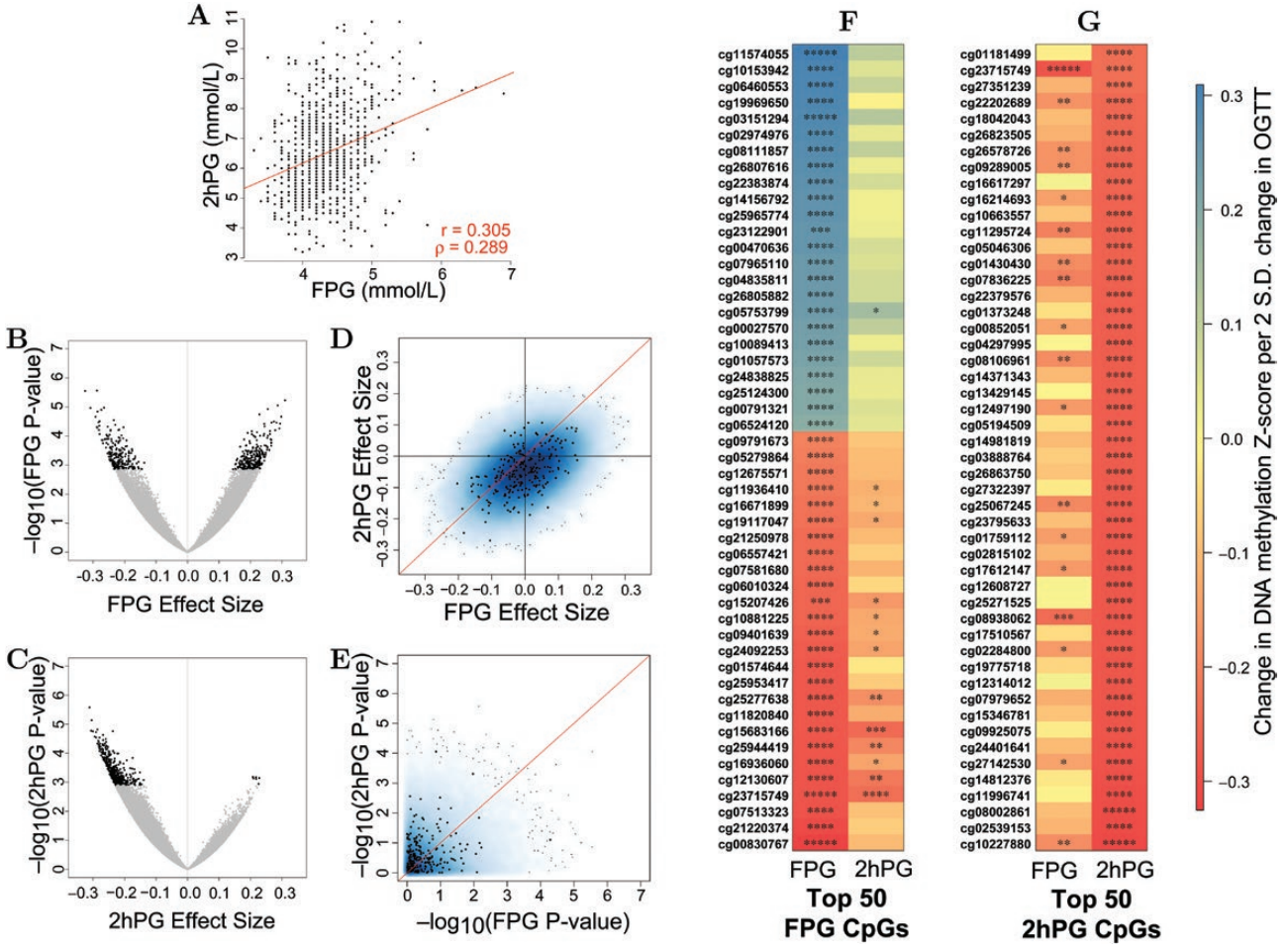

Figure 2. Comparing midpregnancy fasting plasma glucose (FPG) and 2h plasma glucose (2hPG). (A) Scatterplot of raw OGTT. Vertical axis represents raw 2hPG values, whereas the horizontal axis represents corresponding FPG values. The red line in this scatterplot is the regression line between both glucose measures. Pearson correlation between both glucose measures given as *r*, whereas the Spearman correlation coefficient provided as ρ. (B, C) Volcano plot of FPG/2hPG on CpG. Horizontal axis represents the change in cord tissue DNA methylation Z-score per 2 SD change in glucose measure (standardized effect size; FPG in panel B, 2hPG in panel C), whereas the vertical axis represents the corresponding negative logarithmic significance of corresponding regression betas. Black dots represent 500 CpGs most associated ranked by *P* value, whereas the rest of the CpGs are represented in gray. (D) Cloud scatter of OGTT on CpG effect size. Vertical axis represents the change in cord tissue DNA methylation Z-score per 2 SD change in 2hPG (standardized effect size), whereas the horizontal axis represents the change in cord tissue DNA methylation Z-score per 2 SD change in FPG (standardized effect size). (E) Cloud scatter of OGTT on CpG significance. Horizontal axis represents the negative logarithmic significance of corresponding regression betas previously indicated in panel B (FPG), whereas the vertical axis represents the negative logarithmic significance of corresponding regression betas previously indicated in panel C (2hPG). The red line on both cloud scatters represents y = x. Associations between CpGs and midpregnancy PGs in panel B-E were adjusted for hospital, DNA extraction batch, bisulfite conversion batch, infant sex, gestational age, ethnicity, principal components of cellular proportions, and interactions between ethnicity and principal components of cellular proportions. (F, G) Heatmap of the top 50 CpGs most associated with respect to FPG/2hPG ranked by *P* value. These CpGs with respect to FPG (F) and 2hPG (G) are represented in a heatmap, ordered by standardized effect sizes, with increasing magnitudes reflected by a color change from yellow to blue (for positive beta coefficients) or yellow to red (for negative coefficients). Significances of each CpG to corresponding glucose measure: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, ... , $*****P < 10^{-5}$. In each heatmap, the effect size and significance of the same CpG to the corresponding glucose measure is provided for comparison.

with 1 glucose measure was significantly different from the corresponding association with other glucose measure. More than one-half of the top 50 most associated CpGs with each glucose measure showed evidence that the association between cord tissue DNA methylation and glucose was different in the 2 measures. Specifically, of the top 50 CpGs most associated with FPG, 1 CpG (cg19969650) was significant at Bonferroni cutoff ($P < 0.05/50 = 1.0 \times 10^{-3}$), and 29 were significant at $P < 0.05$. Of the top 50 CpGs most associated with 2hPG, 8 CpGs (cg12314012, cg16617297, cg04297995, cg11996741, cg25271525, cg126098727, cg01181499, and cg13429145) were significant at Bonferroni cutoff, and 30 were significant at $P < 0.05$.

Gene ontology (GO) enrichment using the top 500 most associated CpGs with respect to either glucose measures

suggested CpGs differentially methylated with respect to FPG were associated with processes relating to regulation of reactive oxygen species metabolism, nerve development, and regulation of membrane potential; those differentially methylated with respect to 2hPG were enriched in processes relating to sense of taste and antigen processing and presentation (22). GO enrichment using CpGs from sensitivity analysis 2 (surrogate variable analysis) yielded similar observations (22). Notably, although both glucose measures had almost completely different enriched GOs, they shared some REVIGO-defined general GO clusters (22).

In comparing our findings against 142 CpGs reported to be differentially methylated with respect to maternal antenatal hyperglycemia (from offspring cord and peripheral blood, summarized in Lim et al (22)), approximately two-thirds (96 of 142) of these CpGs either did not show sufficient variation in DNA methylation in cord tissue or pass the required quality control criteria. Specific breakdown included: 28 cross-hybridization probes, presence of single nucleotide polymorphisms on or a single-base pair away from the probe, or located on a sex chromosome, or probes significantly associated with batch variables; 13 with detection $P < 0.01$; and 55 with cord tissue DNA methylation of the 2.5th to 97.5th centile was below 10%.) Of the remaining 46 of these 142 CpGs found in our GUSTO infant cord tissue dataset that passed quality control filtering (22), cg12140144 (LINC00982 promoter [PRDM16 divergent transcript]) was suggestively associated to FPG ($\beta_{FPG} = 0.17$, $p_{FPG} = 9.5 \times 10^{-4}$; $\beta_{2hPG} = 0.05$, $p_{2hPG} = 0.28$) within this pool of candidate CpGs.

We also examined the associations of cord tissue DNA methylation with birthweight as a proxy for infant outcomes within the top 50 CpGs most associated with each glucose measure. Eighteen (associated with 13 unique genes) of the 50 CpGs most associated with FPG were associated with birthweight (passing Bonferroni, $0.05/50 = 1.0 \times 10^{-3}$). Thirteen of these 18 CpGs showed positive association, whereas the remaining 5 CpGs showed negative association with birthweight (22). In contrast, only 7 (associated with 4 unique genes) of the 50 CpGs most associated with 2hPG were significantly associated with birthweight, and all showed a negative association (22).

Discussion

This is the first study differentiating the relationship between midpregnancy FPG and 2hPG and their differential impact on offspring cord tissue DNA methylation at birth. We found that although general risk factors such as maternal age, prepregnancy BMI, and pregnancy blood pressure correlated strongly with both FPG and 2hPG, other clinical covariates associated independently with only 1 glucose measure. Interestingly, although most covariates share similar correlative directionalities between either glucose measures, albeit with differing magnitudes, a small group of covariates also reflected significant antagonistic relationships between FPG and 2hPG, such as Chinese ethnicity, maternal height, pregnancy weight gain, prepregnancy alcohol consumption, and cigarette smoke exposure. Importantly, although others have highlighted differences between FPG and *x*-hPG in the context of maternal (38) and childhood outcomes (12), we found that antenatal FPG and 2hPG also have differential influences on the offspring cord DNA methylome. Comparing associations between individual CpGs with each glucose measure, the association was nominally different ($P < 0.05$) from the corresponding glucose measure in more than one-half of the top 50 most associated CpGs. Additionally, we found associations in cg12140144 in agreement with another GDM-related EWAS. Collectively, our findings suggest the need to decouple fasting and *x*-hPG measures in the diagnosis of GDM to better appreciate the different pathophysiological contributions each glucose measure has, in context of the metabolic status of both mother and offspring.

Although maternal hyperglycemia has been characterized by either an elevated FPG or 2hPG, our data demonstrate that these 2 glucose measures associate with various conditions with differing magnitudes, just as impaired fasting glucose and impaired glucose intolerance manifest differently in nonpregnant populations (10). In prediabetes pathophysiology, these 2 groups of participants were demonstrated to belong to different populations with altered glucose metabolism (40), with considerable variation between ethnic groups and sex (41-44). Interestingly, although many of these associations (vitamin B12 (45–47), IVF (48), maternal height (49)) have been previously reported in context of GDM or poor insulin sensitivity and obesity, studies examining whether associations of clinical phenotypes with individual glucose measures differ between the glucose measures themselves are far fewer in number (50). In our study, these 2 glucose measures not only differ in the associations with ethnicity, but also with maternal height, pregnancy weight gain, alcohol, and tobacco exposure. The lack of resolution between associations of differential glucose measures and comparative associations between glucose measures is unfortunate, as our observations suggest, for one, ethnic differences in glucose handling may require different treatment methods (lower basal glucose levels as opposed to improving glucose tolerance), necessitating differential therapeutic options for a person with diabetes diagnosed based on how each glucose measurement presents as opposed to a generic treatment plan based on some combination of FPG or *x*-hPG levels. Second, our observations also suggest some environments reflect antagonistic

associations with either glucose measure. In fact, although certain covariates share similar directionalities, most appear to possess differential effect sizes between either glucose measures. This phenomenon does not appear to be unique to pregnancy (50). Different correlations observed between FPG and 2hPG, varying from poor ($r < 0.3$) (51) to strong ($r > 0.8$) (52), could lead to different proportional diagnosis of diabetes depending on the glucose measure used (53, 54). Hence, resolving between glucose measures within a single patient would perceivably provide more medically useful information.

The dissemblance between fasting and 2hPG is reinforced by the differential associations observed on the infant cord tissue DNA methylome. Both glucose measures are associated with unique epigenome-wide effect size profiles despite no individual CpG attaining epigenome-wide significance. In particular, elevated mid-pregnancy 2hPG appears to associate with global hypomethylation, a phenomenon also observed among significantly methylated CpGs in the livers of obese subjects with T2D (55). Moreover, ranking CpGs by statistical significance with respect to either glucose measure, both glucose measures share very few commonalities at the CpG, gene, or GO level. Among the top 50 CpGs most associated with each glucose measure, only 1 CpG overlaps between both glucose measures. A further examination of whether the association of CpGs with 1 glucose measure was significantly different from the corresponding association with other glucose measure also revealed more than one-half of the top 50 most associated CpGs with each glucose measure was nominally different ($P < 0.05$). Most of the CpGs that were highly associated with FPG also have a positive association with birthweight, whereas those highly associated with 2hPG were almost exclusively negatively associated with birthweight. Differences were also observed between gene ontological associations of the top 500 CpGs per glucose measure. That said, grouping GOs into more general clusters expectedly revealed some shared ontological processes that may have contributed to the amalgamation of the 2 measures for diagnostic purposes. Nevertheless, taken together, the distinct global cord tissue DNA methylation associations between both glucose measures lend credence to the notion that FPG and 2hPG may be reflective of 2 dissimilar pathologies under 1 disease condition (10), and that the differences between cord tissue DNA methylation associations could potentially relate to differential infant outcomes.

In our consolidation of existing known offspring differentially methylated CpGs associated with antenatal maternal glycemia, only 5 separate studies fit our selection criteria: 4 being individual studies and 1 a meta-analysis of 7 independent study cohorts, with all 5 investigating the exposure of GDM cases in association with the DNA methylation profile of offspring blood (cord (16, 56) and peripheral (36, 57, 58), summarized in Lim et al (22)). Notably, the meta-analysis study did not identify any CpG to be differentially methylated by maternal GDM status at a false discover rate (FDR)-adjusted P value threshold of 0.05, but did find 6 CpGs passing a less stringent FDR-adjusted P value of 0.10. This meta-analysis study of 7 cohorts predominantly comprised participants (n = 3677) of Caucasian ethnicity from the United Kingdom, Denmark, United States, Spain, Greece, Belgium, and Italy, with each cohort having distinct GDM diagnostic criteria (16). This meta-analysis also did not include cohorts from the other 4 individual studies we previously selected. Given the importance of such a large DNA methylation meta-analysis across 7 cohorts, we added these 6 CpGs to our list compiled from the studies of CpGs reported to be differentially methylated in association with maternal GDM, resulting in a total of 142 CpGs.

The reproducibility issues among DNA methylation studies have been pointed out by many authors (36, 37). This lack of replication with GDM EWAS cohorts can probably be attributed to small sample sizes used in currently published GDM EWAS cohorts, differences in the types of samples used (placenta, cord tissue, cord blood), as well as differences in GDM criteria between individual studies. Given GDM is diagnosed primarily through clinical thresholds derived from a combination of FPG and 2hPG, this incongruency between GDM studies with differing GDM diagnostic guidelines is concordant with our findings that FPG and 2hPG have differing associations with offspring cord tissue DNA methylation. It has important implications both for defining GDM and for studying its transgenerational influences.

Interestingly, in spite of tissue differences, 1 CpG (cg12140144) found on the LINC00982 promoter was hypermethylated in the peripheral blood of nondiabetic offspring born to mothers with type 2 diabetes at least 1 year before pregnancy (58) as well as with respect to GUSTO fasting glucose levels. This long intergenic nonprotein coding RNA is also known to be a PRDM16 divergent transcript (59), hypermethylation of which was associated with increased future diabetes risk (58). Although this exact CpG was not investigated/identified, CpGs in PRDM16 were associated with differential methylation with respect to maternal fasting glucose in the second and third trimesters of human placenta in another candidate-based study design (60), as well as in pancreatic islets of patients with T2DM (61). PRDM16 is well known for its crucial role in brown adipose tissue development (62), energy expenditure (63), as well as increasing importance in pancreatic development (64). The presence of differentially

methylated CpGs across tissues highlights its potential as a biomarker for diabetes-related screenings and its potential as a key player in multiorgan regulatory mechanisms influenced by antenatal maternal glycemia.

Our observation of differential DNA methylation patterns in cord tissue between fasting and 2hPG measures may be more clinically relevant than studies investigating cord blood samples. Previous analyses comparing DNA methylation profiles between cord tissue and cord blood samples demonstrated DNA methylation profiles obtained from cord tissue samples have more variability for subsequent phenotype association, are less likely to be associated with genotype, are equally likely to be associated with prenatal factors, and are likely to be better surrogates for target tissues of mesenchymal stem cell origin (65). Mesenchymal stem cells have the potential to differentiate into numerous clinically important tissues for metabolic disease, including but not limited to cardiac muscle cells, skeletal muscle cells, kidney tubule cells, fat, and bone (66-68). Because this is a clinical study, participants were given appropriate diet recommendations/treatment upon diagnosis of GDM, which may explain the lack of individual CpGs passing genome-wide significance in cord tissue despite a relatively large sample size of 830 mother-offspring dyads. Notably, a recent meta-analysis of 7 pregnancy cohorts with a total sample size of 3677 using cord blood samples (16) also did not identify any differentially methylated CpGs at an FDR < 0.05. The lack of significant changes at any single genomic loci (in cord blood or cord tissue) does not necessarily reflect an unperturbed methylome but perhaps an ameliorated response as a result of diet/therapeutic interventions (69). Additionally, differential DNA methylation patterns may thus be the reticent indications of maternal antenatal hyperglycemia, similar to indications of "metabolic memory" (70).

Within the Developmental Origins of Health and Disease paradigm, maternal pregnancy environments play an important role in influencing offspring future health and growth outcomes (71, 72). Such influences may be inherited by subsequent generations through persistent epigenetic markers (73). Specifically in maternal hyperglycemia, recent studies investigated associations of offspring DNA methylome in the placenta and cord blood through clinically defined GDM diagnoses (17-20), potentially missing differential changes associated with glycemic status of fasting vs 2 hours after OGTT. This is concordant with existing literature highlighting key differences in the factors contributing to glucose metabolism for the maintenance of basal glucose levels (potentially reflected by FPG levels) compared with response toward a glucose challenge (potentially reflected by 2hPG levels) (74).

Our study has several strengths and weaknesses. Strengths include the relatively large and diverse study population based in Singapore where ethnically diverse participants dwell in a relatively homogenous environment, with a thorough in-depth antenatal phenotyping of environmental exposures accounting for the multifactorial nature of diabetes pathophysiology. The main limitation was not obtaining robust CpGs with genome-wide significance, potentially because of insufficient sample size numbers. Despite such limitations, not only we observed significant differences in global cord tissue DNA methylation association between the 2 glucose measures analyzed, but also effect size directionalities and nominal significances were preserved among the top 500 most associated CpGs within our sensitivity analyses. Such findings are concordant with the differential attributes that impaired fasting glucose and impaired glucose tolerance conditions possess in their contributions to the progression toward T2DM. In summary, our results suggest the need to address both FPG and *x*-hPG separately in the analysis of GDM-related pathophysiological factors and outcomes, just as in nonpregnant diabetes-related studies.

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Clinical Trial Information: This study is registered under the Clinical Trials identifier NCT01174875; http://www.clinicaltrials.gov/ct2/show/NCT01174875?term=GUSTO&rank=2 (registered August 4, 2010).

Additional Information

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Data Availability: Data related to prenatal factors are not publicly available because of ethical restrictions but can be obtained from the authors upon reasonable request and subject to appropriate approvals, including from the GUSTO cohort's Executive Committee. Supplementary analyses generated during this study are included in the data repository listed in Lim et al (22).

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