Maternal glycaemia during pregnancy and child carotid intima media thickness, pulse wave velocity and augmentation index

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Abstract (247/250)

Background: in women without diabetes, little is known about the consequences of hyperglycaemia during pregnancy for the offspring cardiovascular structure and function.

Objective: To investigate the association of maternal glycaemia during pregnancy with cardiovascular risk markers in their children in GUSTO, a Singaporean birth cohort study.

Methods: Around 26 weeks' gestation, a 75g oral glucose tolerance test was performed and fasting (FPG) and 2-hr postprandial plasma glucose (2-hr PPPG) concentrations were measured. Gestational diabetes mellitus (GDM) was defined using WHO 1999 diagnostic criteria. At age 6 years, we measured the child's carotid intima-media thickness (cIMT), carotid-femoral pulse wave velocity (cfPWV), aortic augmentation index (AIx) and blood pressure (BP). Association of maternal glycaemia during pregnancy with cardiovascular risk markers in their children were analysed using multiple linear and logistic regressions.

Results: Analysis were performed on 479 mother-child dyads. Higher maternal FPG was associated with higher cIMT and in male, higher cfPWV in the offspring (adjusted β [CI 95%], cIMT: 0.08 per 10mm increase [0.02; 0.15], cfPWV: 0.36 m/s [0.01; 0.70]). Higher 2-hr PPPG was associated with higher cfPWV and AIx. GDM was associated with higher AIx. No association was found between maternal glycaemia and their offspring blood pressure.

Conclusions: among mothers without pre-existing diabetes, higher glycaemia during pregnancy was associated with mild structural and functional vascular changes in their children at age 6 years across a continuum. These results support the necessity to monitor maternal glycaemia during pregnancy even in absence of pre-existing diabetes or diagnosed gestational diabetes.

Précis

In the present study, using data from GUSTO, a Singaporean birth cohort study, we analyzed the link between maternal glycaemia measured around 26 weeks' gestation in non-diabetic mothers and cardiovascular risk markers measured in their offspring at age 6 years (carotid intima-media thickness, carotid-femoral pulse wave velocity, aortic augmentation index, blood pressure). Higher maternal fasting plasma glucose was associated with higher carotid intima media thickness in their offspring and in male, higher carotid femoral pulse wave velocity. Higher 2-hr postprandial plasma glucose was associated with higher carotid femoral pulse wave velocity and augmentation index. Higher maternal glycaemia during pregnancy was associated with changes in their offspring vascular structure and function in mid-childhood across a continuum.

Introduction

Gestational diabetes mellitus (GDM) is a state of glucose intolerance occurring for the first time during pregnancy and is associated with an increased risk of later type 2 diabetes mellitus (DM)[1]. There is scant evidence of the the adverse impact of GDM on the offspring's cardiovascular (CV) health[2] as most research has focused on type 1 and type 2 DM. Maternal hyperglycaemia during pregnancy is associated with an elevated risk of congenital cardiac (and non-cardiac) malformations and cardiac hypertrophy among pre-existing diabetic mothers[3,4]. Based on the developmental origin of health and disease paradigm, maternal hyperglycaemia during pregnancy may play a role in "fetal programming" and lead to detrimental later-life health outcomes[5].

During pregnancy, a hyperglycaemic intrauterine environment is associated with fetal hyperinsulinemia which itself can cause cardiovascular structural and functional changes[3,4]. Growing evidence suggests that even in the absence of manifest diabetes, increasing maternal glycaemia across a continuum during pregnancy is associated with adverse perinatal outcomes such as perinatal death, bone fracture, nerve palsy, large-for-gestational age or neonatal hypoglycaemia[6-8]. Yet little is known about the influence of maternal hyperglycaemia on child CV health at later ages. Markers of subclinical CV disease such as carotid intima media thickness (cIMT), carotid-femoral pulse wave velocity (cfPWV), and augmentation index (AIx) can now be measured non-invasively. These markers provide surrogate information on early CV structure and function and are important predictors of future CV disease events[9,10] but they have been rarely examined in children

Using data from a Singaporean multi-ethnic birth cohort study, we prospectively investigated the association of maternal glycaemia during pregnancy with cIMT, cfPWV, AIx and blood pressure (BP) in their children aged 6 years.

Methods

Study population

GUSTO (Growing Up in Singapore Towards healthy Outcomes) is a multi-ethnic mother-child cohort study. The detailed study description has been published[11]. Between 2009 and 2010, 1247 pregnant women aged ⩾18 years who attended their first-trimester ultrasound scan at Singapore's two major public maternity units: National University Hospital (NUH) or KK Women's and Children's hospital (KKH). Inclusion criteria included Singaporean citizenship or permanent resident status, Chinese, Malay or Indian ethnicity, intention to deliver either at NUH or KKH, intention to remain in Singapore for five years, and willingness to donate birth tissues at delivery. Mothers receiving chemotherapy or psychotropic drugs or who had a diagnosis of type 1 DM were excluded. Informed written consent was obtained from the women and their children when they reached age 6 years. The study was approved by both the National Healthcare Group Domain Specific Review Board (reference D/09/21) and SingHealth Centralized Institutional Review Board (reference 2009/280/D).

Maternal glycaemia

After an overnight fast during the clinic visit around 26 weeks' gestation, a 75g oral glucose tolerance test (OGTT) was performed only in mothers without pre-existing DM and not known GDM. Venous blood samples were collected in fluoride-containing tubes, with fasting (FPG) and 2-hr postprandial (2-hr PPPG) plasma glucose concentrations measured by colorimetry [Advia 2400 Chemistry system (Siemens Medical Solutions Diagnostics) or Beckman LX20 Pro analyzer (Beckman Coulter)]. GDM was defined as FPG ≥7.0 mmol/L and/or 2-h PPPG ≥7.8 mmol/L (WHO diagnostic criteria 1999). Following their OGTT in

GUSTO, mothers diagnosed as having GDM were either diet-treated only or diet- and insulin-treated.

Child cIMT, cfPWV, and BP

At age 6 years, all participants were invited for a non-invasive vascular assessment performed by trained sonographers using a standard protocol. CIMT was assessed by high resolution B-mode ultrasound using a high-frequency linear transducer and commercially available ultrasound systems (Philips CX-50 xMatrix at KKH and Aloka Prosound Alpha-10 at NUH) in accordance with the recommendations of the American Society of Echocardiography[12]. The right common carotid artery was scanned in the lateral plane and cIMT measured at the far wall, 1 cm proximal to the carotid bulb, in an area devoid of plaque. Triplicate cIMT measurements were averaged and used for analysis. Observer variability for cIMT assessed in 88 subjects suggested moderate rater reliability. For intra-observer variability (n=32), the intraclass correlation coefficient (ICC) based on a mean-rating (k = 2), absolute-agreement, 2-way mixed-effects model was 0.66 (95% CI, 0.30 – 0.84). The ICC for inter-observer variability (n=56) was 0.70 (95% CI, 0.54 – 0.81).

CfPWV was measured in the supine position using applanation tonometry (SphygmoCorVx, AtCor Medical, West Ryde, NSW, Australia). The carotid-femoral path length was obtained by subtracting right common carotid-suprasternal notch distance measured with a tape ruler from suprasternal notch-femoral distance. Carotid-femoral transit time was obtained by subtracting the time between onset of the electrocardiographic R-wave and the foot of the carotid pulse and the time between the R-wave and the femoral pulse, each averaged from 8 to 10 sequential waveforms. CfPWV was calculated as the carotid-femoral path length divided by the transit time. In a previous study of 96 subjects conducted at NUH, the intra-

observer ICC assessed as per cIMT above was 0.94 (95% CI, 0.92 – 0.97), indicating excellent reliability.

Pulse wave analysis: from about 10 seconds of sequential radial artery waveforms obtained by the high-fidelity tonometer, the aortic pressure waveform was reconstructed by the SphygmoCorPx System using a transfer function[13]. This waveform depends on left ventricular ejection, as well as the timing and amount of wave reflection from branch points or areas of impedance mismatch which are determined by aortic stiffness and arteriolar tone[14]. From the aortic pressure waveform, central systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure were derived. Central or aortic augmentation index (AIx) was calculated as the increment in pressure from the first systolic shoulder of the ascending aortic pressure wave to the peak of the second, late systolic shoulder, expressed as a percentage of the pulse pressure. Because AIx is influenced by heart rate, it was normalized to a heart rate of 75 beats/min (AIx@75) to facilitate comparison.

During the 6-year clinic visit, BP was measured using Dynamap CARESCAPE^{TM} V100 (GE Healthcare, Milwaukee, WI), with an appropriate child cuff, by trained research staff. The measurement was taken in a quiet room from the right upper arm in a seated position, with legs uncrossed and the arm resting at chest level. The child was asked to rest for 5 minutes before the measurement. Two measurements were recorded. If the second SBP or DBP differed from the first by >10 mm Hg, a third measurement was taken. The highest BP was discarded to account for child anxiety and the two lowest BP readings averaged.

Covariates

Maternal BP before 20 weeks' gestation, child's sex and gestational age were extracted from the maternity hospital records. Mothers were classified as normal BP (SBP<120 and DBP<80 mmHg), elevated BP (120≤SBP≤129 and DBP<80 mmHg), stage 1

hypertension (130≤SBP≤139 or 80≤DBP≤89 mmHg) and stage 2 hypertension (140≥SBP or 90≥DBP mmHg)[15]. Owing to the low percentage of stage 2 hypertensive mother (2.4 %), stage 1 and 2 hypertensive status were combined as one group. At the recruitment visit, educational attainment, ethnicity, and pre-pregnancy weight were collected through interviewer-administered questionnaires. Around 26 weeks' gestation, maternal plasma cotinine level (ng/mL) was analysed; her current and early pregnancy active smoking status and environmental tobacco exposure at home and work were ascertained through interviewer-administered questionnaire. We defined maternal active smoking exposure as having a cotinine level ≥3.0 ng/mL[16], or as having an active smoking status as reported by the subjects. At the 26-28-week clinic visit, maternal height was measured twice to the nearest 0.1 cm using a stadiometer (SECA 213, Hamburg, Germany). If the two measurements differed by >1.0 cm, a third measurement was taken. The two closest measurements were then averaged. Maternal pre-pregnancy weight status, derived from the self-reported pre-pregnancy weight and the height at 26-28 weeks' gestation, was defined using body mass index and the WHO classification for Asian population. At 24 or 36 months, paternal height was measured following the same protocol as for the maternal height.

At age 6 years, the child's weight and height were measured in duplicate. A third measurement was taken if the first two differed by >0.2 kg and >1.0 cm for weight and height, respectively. The two closest measurements were averaged. Age and sex-specific BMI z-scores were derived using WHO reference[17].Children were classified as overweight or obese when their BMI z-scores exceeded +2SD.

Analytic sample

A study flow chart is shown in **Figure 1**. Multiple pregnancies were excluded (n=10). From the 1237 singleton pregnancies, 1172 mother-child dyads were followed-up after

delivery. At age 6 years, 1026 children remained in follow-up, of whom 545 consented to cardiovascular assessment. Due to either parent or child refusal to be measured or technical problem on the day of measurement, 498 children had cIMT, cfPWV, AIx and AIx@75 measurements. Finally, only mother-child dyads with data on maternal glycaemia during pregnancy (n=479) were retained in the analysis.

Statistical analysis

Student's t-tests and chi-square tests were used to compare characteristics of included and non-included participants. Unadjusted associations of FPG, 2-hr PPPG at 26 weeks' gestation, GDM status with cIMT, cfPWV, AIx and AIx@75 were analysed using simple linear and logistic regression. Multiple linear and logistic regression models adjusted for study center, child's sex and the following maternal characteristics: age at delivery, ethnicity, educational attainment, pre-pregnancy BMI, smoking status weeks' gestation and environmental tobacco exposure during pregnancy, and BP category before 20 weeks' gestation were performed. Since few mothers reported consuming alcohol (1%), models were not adjusted for this covariate. In a subsample of children with BP measurements (n=452), we examined associations of FPG, 2-hr PPPG, GDM with SBP and DBP. Interactions with child's sex and maternal ethnicity were tested; when p-value significance was reached, an interaction term was added in the model and subgroups estimates were calculated. As a sensitivity analysis, models were run after excluding preterm infants (gestational age <37 weeks' gestation, n=30). Models were also run among non-GDM mothers to study the influence of lower levels of maternal glycaemia and of receiving no treatment on child functional CV markers. As it has been proposed that negative AIx values be disregarded when studying wave reflection magnitude, we omitted these (n=20) in a subanalysis[18]. To provide insight into potential causal pathways, we performed mediation analysis to explore if

241 the observed associations between maternal glycaemia and child cardiovascular risk markers
242 were explained by gestational age and sex-specific birthweight z-scores, child age- and sex-
243 specific BMI z-scores at age 5 and 6 years and child blood lipids (total cholesterol and
244 triglycerides)[19]. In a post hoc analysis, we analysed the association between GDM defined by
245 WHO 2013 criteria[20] and child CV outcomes. Missing values for confounders, which were
246 assumed to be missing at random, were handled using multiple imputations. Twenty
247 independent datasets were generated using the Markov Chain Monte Carlo method, and
248 pooled effect estimates were calculated. We imputed 20 datasets based on Graham et al
249 recommendations for 10 to 30% of missing information[21]. All analyses were performed with
250 SAS software (version 9.4; SAS Institute, Cary, NC, USA). Significance was set at $P<0.05$
251 except for interaction test ($P<0.10$) and hypothesis tests were 2-sided.
252

253 **Results**

254 Characteristics of the included and non-included participants are compared in **Table**
255 **1**. Of the mothers studied, 61 % were Chinese, 35 % had a university diploma, 23 % were
256 obese and 18 % had GDM. Among mothers diagnosed with GDM, 95% were treated either
257 by diet alone (90%) or with insulin as well (6%). Of the mothers, 95% had a FPG<5.1
258 mmol/L (normal range according to WHO 2013 GDM diagnosis criteria) and 82% had a 2-hr
259 PPPG<7.8 mmol/L at 26 weeks' gestation. Compared with non-included mothers, those
260 included were less likely to being classified as having hypertension (13% vs 20%, overall
261 *P*=.001 for BP category) and more likely to be Chinese (61% vs 53%, overall *P*=.03 for
262 maternal ethnicity). No other significant differences were observed.
263
264 *CIMT, cfPWV and AIx*
265 The associations of maternal FPG, 2-hr PPPG at 26 weeks' gestation, and GDM status
266 with their offspring cIMT, cfPWV, AIx, and AIx@75 at age 6 years are shown in **Table 2**.
267 FPG was not associated with AIx or AIx@75. Higher FPG was associated with higher cIMT
268 and in male only (adjusted β [CI 95%], cfPWV: 0.35 m/s [0.01; 0.71] in male vs -0.06 [-0.39;
269 0.26] in female), with higher cfPWV (interaction test *P*=.09). No further interactions were
270 observed with child's sex and maternal ethnicity. Higher 2-hr PPPG was associated with
271 higher AIx, AIx@75, with higher cfPWV even if borderline significant (*P*=.058) but not with
272 cIMT. GDM status was associated with higher AIx and AIx@75 but not with cIMT and
273 cfPWV. No interactions were found of child's sex and maternal ethnicity on the association
274 of 2-hr PPPG and GDM with any child CV risk markers. After omitting the preterm births
275 from the study sample, the findings were mostly similar and the trends remained (**Table 3**).
276 Associations with CV markers were non-significant when only non-GDM mothers were
277 analysed, yet similar but diminished trends were observed (Table 3). After excluding negative

AIx (Table 4), associations between AIx with 2-hr PPPG and GDM were reduced and not significant while the magnitude of the associations between AIx@75 with 2-hr PPPG and GDM were only reduced; all associations remained non-significant with FPG. In our mediation analysis, we found that the associations between FPG with cIMT was 24.2 % mediated by birthweight (indirect effect estimate, adjusted β [CI 95%]: 0.02 [-0.00; 0.02]. However, the association between FPG and cfPWV was not mediated by birthweight. Similarly, the associations between PPPG with cfPWV, AIx and AIx@75 and between GDM with AIx and AIx@75 were not mediated by birthweight. None of our studied associations were mediated by child BMI z-scores at age 5 and 6 years or child triglycerides and total cholesterol levels at age 6 years. In a post-hoc analysis, applying WHO 2013 criteria instead of WHO 1999 criteria to define GDM did not change the associations between GDM with cIMT and cfPWV. However, the magnitude of the association between GDM and AIx was attenuated (adjusted β [CI 95%]: 0.89 [-1.83; 3.62] for AIx and 0.95 [-1.79; 3.69] for AIx@75) and became non-significant.

Blood pressure

As shown in **Table 4**, maternal FPG, 2-hr PPPG and GDM were not associated with SBP or DBP in their offspring aged 6 years. After excluding the preterm births and GDM mothers, all associations remained unchanged (Table 4). Interactions of child's sex or maternal ethnicity were non-significant. Associations between GDM with child SBP and DBP remained similar when applying either WHO 2013 or 1999 criteria to define GDM.

Discussion

To our knowledge, this is the first study to investigate the link between maternal glycaemia during pregnancy, among women without pre-existing DM, and vascular structure and function in their offspring during mid-childhood. We found that among mothers without pre-existing diabetes, higher FPG at 26 weeks' gestation was associated with putative CV risk markers in their offspring aged 6 years, i.e. higher cIMT, and in male, higher cfPWV. Higher maternal 2-hr PPPG was associated with higher cfPWV, AIx and AIx@75 but not with cIMT. GDM status was associated with higher AIx but not with other CV risk markers. No associations were observed between maternal glycaemia and offspring SBP or DBP.

GDM can cause oxidant stress which itself can lead to altered placental function and hence, impacts fetal growth and epigenetic programming that are associated with higher cardiometabolic risk in later ages[22]. In both the ACHOIS trial and in the HAPO cohort study, maternal hyperglycaemia was associated with increasing risk of perinatal morbidity and mortality in a continuum, but to a lesser degree than manifest DM[6-8], and GDM treatment was beneficial in reducing the risk[6]. From previous studies in pre-existing diabetic mothers, macrosomic neonates are known to have an increased left ventricular mass and a larger aortic intima-media thickness compared with their normal-sized counterparts[3]. Similarly, children born macrosomic from mothers with either pre-existing type 1 DM or GDM had higher aortic intima media thickness[23].

In line with previous studies results, we found that higher FPG at 26 weeks' gestation among GUSTO mothers was related to higher cIMT in their offspring at age 6 years, and in male to greater conduit arterial stiffness (increase in cfPWV of 0.36 m/s). Higher maternal 2-hr PPPG was associated with higher cfPWV (increase of 0.08 m/s) and AIx. GDM status was only associated with offspring's AIx but not with cfPWV and cIMT. As a gauge of the clinical significance of our findings, an increase in cfPWV of 0.18 m/s and 0.11 m/s were

326 associated with obesity and higher HOMA-IR, respectively, in children aged 8 years[24]. We
327 acknowledge that the magnitude of our associations was small and further investigation are
328 needed to confirm our findings. In our study, only a small number of GDM mothers were
329 treated with insulin (6%) while the vast majority (90%) were controlled with diet alone. Since
330 almost all GDM mothers were treated following diagnosis and strict glycaemic control
331 enforced, this could have masked an association of GDM with cfPWV and cIMT. Evidence
332 from randomized trials has shown that treatment of GDM led to a reduced risk of fetal
333 overgrowth and other adverse perinatal outcomes[25]. Furthermore, the association between
334 maternal glycaemia and the risk of neonatal adverse outcomes has been shown to occur
335 across a continuum, inferring that any diagnostic threshold indicative for GDM is arbitrary[8].
336 Indeed, among non-GDM mothers, we were able to discern parallel albeit non-significant
337 trends between maternal glycaemia with child's vascular metrics. This lack of significance
338 might partly be explained by the low levels of maternal glycaemia in our study. Even if some
339 outcomes did not attain statistical significance, it is remarkable that the associations between
340 child vascular measures and glycaemic status measured at a single time-point in pregnancy
341 were directionally consistent. This consistency suggests common pathophysiologic
342 mechanisms linking hyperglycaemia to arterial disease, including inflammation, endothelial
343 dysfunction, and extracellular matrix alterations[26]. Besides expedient treatment of mothers
344 with higher levels of glycaemia, another explanation of the differences observed across the
345 different exposures could be that FPG and PPPG may influence outcomes through different
346 causal pathways. It has been suggested that PPPG levels could be more predictive than FPG
347 for macrosomia and hypoglycaemia in diabetic mothers or insulin treated GDM mothers[27-29].
348 While both FPG and PPPG have been associated with greater arterial stiffness, several studies
349 have now shown a unique ability of postprandial hyperglycaemia to acutely increase arterial
350 stiffness[30]. In one large Taiwanese study of nearly 5000 subjects, impaired glucose tolerance

but not impaired fasting glucose was associated with greater arterial stiffness, possibly because the more pronounced degree of insulin resistance in the former leads to higher serum and tissue levels of advanced glycation end-products[31].

Most previous studies of child CV outcomes have focused on BP. In a Chinese population, children aged 3-10 years of GDM mothers had higher sex- and height-specific BP z-scores for SBP and higher rates of hypertension than those of non-GDM mothers[32] while in Project Viva, higher SBP at age 3 years was associated with maternal GDM[33]. By contrast, we found no association between maternal glycaemia and SBP or DBP of their 6-year-old offspring. We used a single stage GDM screening while the two referenced studies[32,33] used a two stage GDM screening. As our results are consistent with studies that have used a single stage GDM screening[34-36], the discrepant findings could be explained by different degrees of hyperglycaemia or GDM across studies.

We found that higher maternal FPG at 26 weeks' gestation was associated with higher cfPWV only in males. Several mechanisms of sexual dimorphism in developmental programming of cardiovascular disease have been stated, but mainly from animal studies[37]. Human studies suggested that males may be more responsive to GDM treatment than females, as reflected in lower neonatal fat mass and lower birthweight centiles[38]. The risk of developing hypertension in the offspring, associated with maternal GDM, also appears more pronounced in male children[32].

We acknowledge that the generalized transfer function used by the SphygmoCor device to convolve radial to aortic pressure was derived in adults. However, even when the standard adult transfer function is applied to young children, underestimation of aortic systolic BP was small (mean, 5-6 mmHg) and >90% of them had values within 10% of "reference" aortic pressure[39]. Irrespective of the algorithm used, any over- or under-

estimation of central pressure appears to be systematic, without bias for any specific individual or subgroup[39].

Our study findings may not be generalizable to the Singapore population. In the GUSTO study, only 54% of women who initially volunteered met the eligibility criteria[11], and of these, half of the GUSTO families at age 6 years agreed to participate in CV assessment. We found differences in maternal ethnicity and hypertensive status between included and non-included participants. However, we believe that potential reasons of non-participation to the CV assessment should not have a strong influence on our findings although they may limit their generalizability. In GUSTO, WHO 1999 criteria was used to diagnose GDM. This limits comparisons with other studies which use WHO 2013 criteria. WHO 1999 criteria may over-diagnose GDM on the basis of 2hr-PPPG and underdiagnose GDM on the basis of FPG. However, our post hoc analysis applying WHO 2013 criteria to define GDM showed mostly similar association, except for AIx. This difference may be explained by the lower prevalence of GDM diagnosed using WHO 2013 criteria. Our results are strengthened by the prospective design of GUSTO study, the comprehensive information collected on the participants and the relatively large sample size compared to previous studies.

We found that higher maternal FPG and 2-hr PPPG at 26 weeks' gestation was associated with offspring mild structural and functional CV changes, but not BP, in children aged 6 years. Given the magnitude of the associations found in our study and the absence of longitudinal data linking subclinical CV changes in early childhood to CV events in adult life, we believe that it would be premature to recommend public health interventions. Further follow-up of our cohort will reveal whether these risk markers predict higher risk of CV events in adulthood. If these alterations do indeed persist into adulthood and portend adverse outcomes, the important implication of our study is that CV changes due to maternal

hyperglycemia can be detected as young as 6 years of age. The corollary is that at risk individuals should be more carefully monitored and targeted for health education or intervention.

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Figure 1. Study flow chart

Table 1. Characteristics of included and non-included participants[a]

[a]Values are mean ± SD or n (%). Values before multiple imputation.
[b]Using WHO 2006 definition.
[c]Using ACC/AHA 2017 definition[15].
Abbreviations: cIMT: carotid intima media thickness, cfPWV: carotid femoral pulse wave velocity, AIx: augmentation index, AIx@75: augmentation index normalized to a heart rate of 75 beats/min, SBP: systolic blood pressure, DBP: diastolic blood pressure, BP: blood pressure, BMI: body mass index, GDM: gestational diabetes mellitus, FPG: fasting plasma glucose, PPPG: postprandial plasma glucose.

Table 2. Associations of FPG, 2-hr PPPG, GDM status at 26 weeks' gestation with cIMT, cfPWV, AIx AIx@75 at age 6 years

[a]Multiple linear and logistic regressions on multiple imputed datasets (n=20) also adjusted for study center, child's sex, paternal height, maternal characteristics (ethnicity, age at delivery, height, pre-pregnancy BMI, educational attainment, BP category before 20 weeks' gestation, active smoking status at 26 weeks' gestation, exposure to tobacco at home and/or work in early pregnancy and at 26 weeks).
[b]*P*-value<0.05.
[c]GDM defined using WHO 1999 criteria.
[d]*P*-value<0.01.
[e]*P*-value<0.0001.
Abbreviations: cIMT: carotid intima media thickness, cfPWV: carotid femoral pulse wave velocity, AIx: augmentation index, AIx@75: augmentation index normalized to a heart rate of 75 beats/min, SBP: systolic blood pressure, DBP: diastolic blood pressure, FPG: fasting plasma glucose, PPPG: postprandial plasma glucose, GDM: gestational diabetes mellitus.

Table 3. Associations of FPG, 2-hr PPPG, GDM status at 26 weeks' gestation with SBP and DBP at age 6 years

[a]Multiple linear and logistic regressions on multiple imputed datasets (n=20) also adjusted for study center, child's sex, paternal height, maternal characteristics (ethnicity, age at delivery, height, pre-pregnancy BMI, educational attainment, BP category before 20 weeks' gestation, active smoking status at 26 weeks' gestation, exposure to tobacco at home and/or work in early pregnancy and at 26 weeks).
[b]*P*-value<0.05.
[c]GDM defined using WHO 1999 criteria.
Abbreviations: cIMT: carotid intima media thickness, cfPWV: carotid femoral pulse wave velocity, AIx: augmentation index, AIx@75: augmentation index normalized to a heart rate of 75 beats/min, SBP: systolic blood pressure, DBP: diastolic blood pressure, FPG: fasting plasma glucose, PPPG: postprandial plasma glucose, GDM: gestational diabetes mellitus.

Table 4. Sensitivity analysis: adjusted associations of FPG, 2-hr PPPG, GDM status (according to WHO 1999 definition) at 26 weeks' gestation with cIMT, cfPWV, AIx, AIx@75 SBP and DBP at age 6 years excluding preterm children or mothers with GDM or children with negative AIx[a]

[a]Multiple linear and logistic regressions on multiple imputed datasets (n=20) also adjusted for study center, child's sex, paternal height, maternal characteristics (ethnicity, age at delivery, height, pre-pregnancy BMI, educational attainment, BP category before 20 weeks of gestation, active smoking status at 26 weeks of gestation, exposure to tobacco at home and/or work in early pregnancy and at 26 weeks).
[b]*P*-value<0.05.
[c]GDM defined using WHO 1999 criteria.
Abbreviations: cIMT: carotid intima media thickness, cfPWV: carotid femoral pulse wave velocity, AIx: augmentation index, AIx@75: augmentation index normalized to a heart rate of 75 beats/min, SBP: systolic blood pressure, DBP: diastolic blood pressure, FPG: fasting plasma glucose, PPPG: postprandial plasma glucose, GDM: gestational diabetes mellitus.

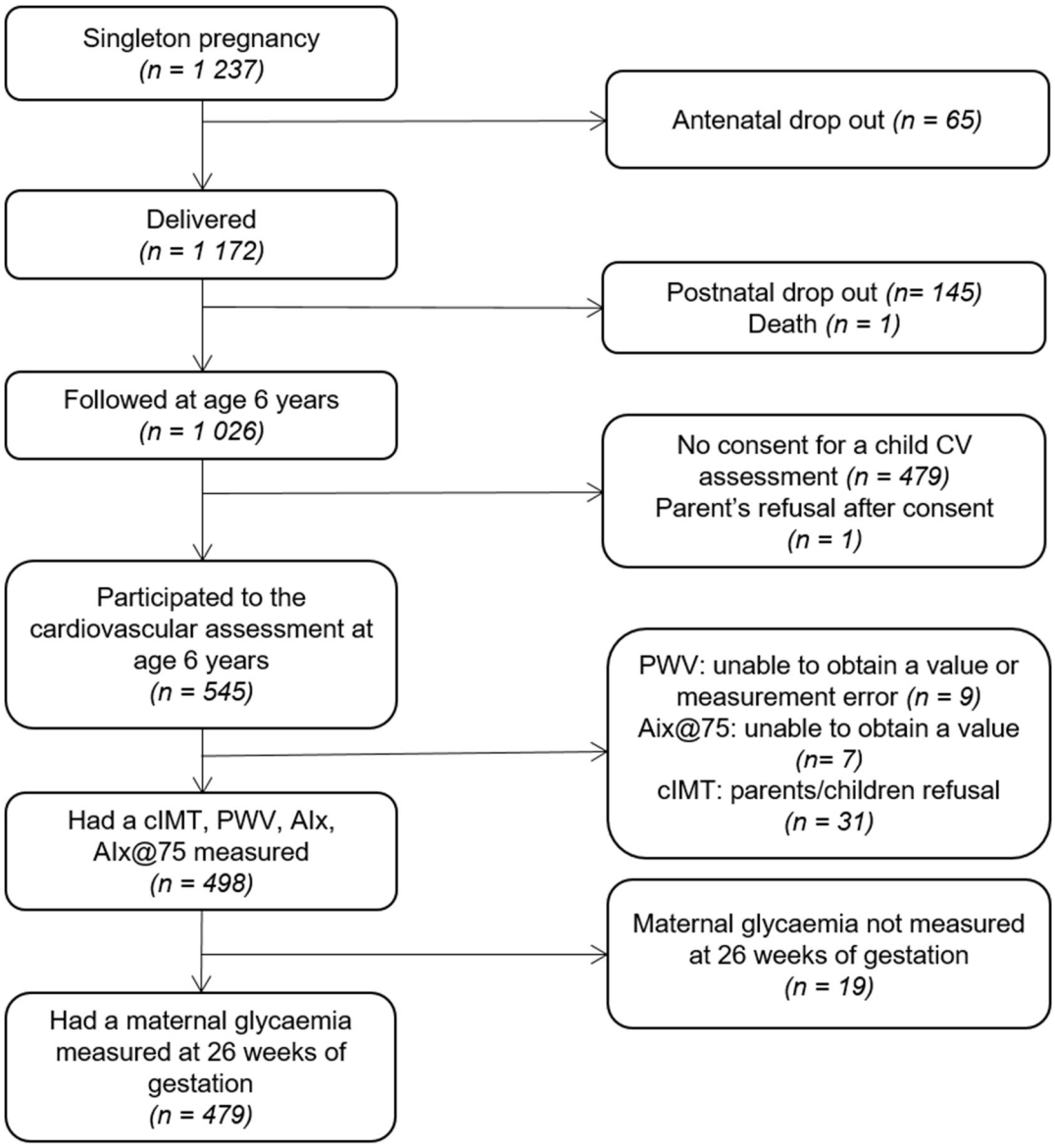

Figure 1. Study flow chart

Table 1. Characteristics of included and non-included participants[a]

	n	Included	n	Non-included	*P*-value
Child characteristics					
Male sex	479	251 (52)	758	368 (49)	0.19
Birth weight, kg	479	3.1 (0.4)	692	3.0 (0.5)	0.74
Gestational age, weeks	479	38.4 (1.5)	692	38.2 (1.6)	0.13
At year 6					
cIMT, mm	479	0.42 (0.29)	34	0.42 (0.24)	0.37
cfPWV, m/s	479	4.8 (1.2)	63	5.3 (5.2)	0.47
AIx, %	479	17.9 (10.1)	66	16.3 (11.5)	0.21
AIx@75, %	479	21.9 (10.1)	59	21.8 (9.3)	0.94
SBP, mmHg	452	98.8 (8.1)	241	99.1 (8.4)	0.61
DBP, mmHg	452	59.1 (6)	241	59.6 (6.3)	0.35
BMI, kg/m^2	478	15.5 (2.4)	358	15.5 (2.2)	0.92
BMI z-score, SD	478	-0.03 (1.40)	358	-0.02 (1.32)	0.81
Overweight (obesity included)[b]	478	89 (19)	358	65 (18)	0.96
Maternal characteristics					
Age at delivery, years	479	31.4 (5)	692	31 (5.2)	0.18
Primiparous, %	479	216 (45)	692	319 (46)	0.73
Ethnicity, %	479		758		0.03
Chinese		290 (61)		401 (53)	
Malay		111 (23)		211 (28)	
Indian		78 (16)		146 (19)	
Educational attainment, %	476		744		0.69
No formal education/primary/secondary		145 (30)		237 (32)	
Postsecondary		165 (35)		265 (36)	
University		166 (35)		242 (33)	
BMI before pregnancy, kg/m^2	437		639		0.27
<18.5		45 (10)		79 (12)	
18.5-22.9		230 (53)		311 (49)	
23.0-24.9		60 (14)		76 (12)	
≥25.0		102 (23)		173 (27)	
Glycaemia at 26 weeks' gestation					
FPG, mmol/L	479	4.3 (0.5)	649	4.4 (0.5)	0.19
2-hr PPPG, mmol/L	479	6.5 (1.4)	649	6.6 (1.5)	0.13
GDM using WHO 2013 criteria, %	479	62 (13)	649	80 (12)	0.76
GDM using WHO 1999 criteria, %	479	84 (18)	649	127 (20)	0.39
Diet-treated only		76 (90)		107 (84)	
Diet and insulin-treated		5 (6)		12 (9)	
Not treated		3 (4)		6 (5)	
Unknown		0 (0)		2 (2)	
Pre-pregnancy or early gestational (<20 weeks' gestation) BP category[c], %	464		676		0.001
Normotensive		314 (68)		399 (59)	
Elevated BP		92 (20)		139 (21)	
Hypertensive		58 (13)		138 (20)	
Active smoking at 26 weeks' gestation %	429	24 (6)	549	29 (5)	0.83
Exposure to tobacco at home and/or work in early pregnancy and at 26 weeks, %	458	173 (38)	671	255 (38)	0.94

Table 2. Associations of FPG, 2-hr PPPG, GDM status at 26 weeks' gestation with cIMT, cfPWV, AIx AIx@75 at age 6 years

	cIMT, per 10 mm n = 479		cfPWV, m/s n = 479		AIx, % n = 479		AIx@75, % n = 479	
	β [CI 95%]		β [CI 95%]		β [CI 95%]		β [CI 95%]	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
FPG, mmol/L	0.06 [0.00; 0.12]	0.08 [0.02; 0.15][b]	0.29 [0.06; 0.52][b]	0.16 [-0.09; 0.40]	-0.15 [-2.14; 1.84]	-0.04 [-2.19; 2.11]	0.25 [-1.74; 2.24]	0.09 [-2.07; 2.25]
2-hr PPPG, mmol/L	0.01 [-0.01; 0.03]	0.01 [-0.01; 0.03]	0.05 [-0.02; 0.13]	0.08 [0.00; 0.15]	0.51 [-0.12; 1.13]	0.77 [0.10; 1.45][b]	0.75 [0.13; 1.38][b]	0.89 [0.22; 1.57][b]
GDM[e]	0.02 [0.01; 0.04][d]	0.01 [-0.07; 0.08]	0.22 [0.16; 0.28][e]	0.23 [-0.05; 0.51]	2.17 [1.64; 2.70][e]	2.69 [0.28; 5.10][b]	2.84 [2.31; 3.36][e]	3.07 [0.65; 5.50][b]

Table 3. Sensitivity analysis: adjusted associations of FPG, 2-hr PPPG, GDM status at 26 weeks' gestation with cIMT, cfPWV, AIx, AIx@75 SBP and DBP at age 6 years excluding preterm children or mothers with GDM or children with negative AIx[a]

	cIMT, per 10 mm	cfPWV, m/s	AIx, %	AIx@75, %	SBP, mmHg	DBP, mmHg
	β [CI 95%]	β [CI 95%]	β [CI 95%]	β [CI 95%]	β [CI 95%]	β [CI 95%]
Excluding preterm children	*n = 447*	*n = 447*	*n = 447*	*n = 447*	*n = 421*	*n = 421*
FPG, mmol/L	0.09 [0.02; 0.15][b]	0.10 [-0.15; 0.36]	0.04 [-2.09; 2.17]	0.00 [-2.13; 2.14]	1.45 [-0.34; 3.24]	0.07 [-1.30; 1.44]
2-hr PPPG, mmol/L	0.01 [-0.01; 0.03]	0.09 [0.00; 0.17][b]	0.75 [0.06; 1.43][b]	0.94 [0.26; 1.63][b]	0.03 [-0.56; 0.62]	-0.31 [-0.76; 0.13]
GDM[c]	0.02 [-0.06; 0.10]	0.20 [-0.09; 0.49]	2.37 [-0.06; 4.81]	2.39 [0.49; 5.36][b]	0.89 [-1.22; 3.01]	0.16 [-1.45; 1.77]
Excluding mothers with GDM	*n = 395*	*n = 395*	*n = 395*	*n = 395*	*n = 373*	*n = 373*
FPG, mmol/L	0.09 [0.01; 0.17]	0.12 [-0.18; 0.42]	-0.41 [-3.18; 2.36]	-0.57 [-3.37; 2.22]	1.75 [-0.45; 3.95]	0.49 [-1.18; 2.16]
2-hr PPPG, mmol/L	0.02 [-0.01; 0.05]	0.04 [-0.07; 0.16]	0.71 [-0.34; 1.76]	0.79 [-0.27; 1.84]	0.07 [-0.76; 0.90]	-0.41 [-1.04; 0.22]
Excluding negative AIx	*n = 459*	*n = 459*	*n = 459*	*n = 459*	*n = 434*	*n = 434*
FPG, mmol/L	0.07 [0.00; 0.14] [b]	0.12 [-0.14; 0.38]	-0.95 [-2.84; 0.95]	-0.76 [-2.67; 1.15]	1.55 [-0.26; 3.37]	0.22 [-1.16; 1.60]
2-hr PPPG, mmol/L	0.01 [-0.01; 0.03]	0.06 [-0.02; 0.14]	0.49 [-0.10; 1.08]	0.62 [0.03; 1.21][b]	-0.05 [-0.62; 0.53]	-0.40 [-0.83; 0.03]
GDM[c]	0.02 [-0.06; 0.09]	0.20 [-0.08; 0.49]	1.63 [-0.46; 3.72]	2.09 [-0.02; 4.19][b]	0.53 [-1.53; 2.59]	0.08 [-1.49; 1.64]

Table 4. Associations of FPG, 2-hr PPPG, GDM status at 26 weeks' gestation with SBP and DBP at age 6 years

	SBP, mmHg n = 452		DBP, mmHg n = 452	
	β [CI 95%]		β [CI 95%]	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
FPG, mmol/L	1.75 [0.13; 3.37][b]	1.52 [-0.23; 3.26]	0.09 [-1.12; 1.30]	0.17 [-1.16; 1.50]
2-hr PPPG, mmol/L	-0.15 [-0.66; 0.37]	-0.02 [-0.58; 0.54]	-0.41[-0.79; -0.03][b]	-0.36 [-0.78; 0.07]
GDM[c]	0.48 [0.04 ; 0.92][b]	0.66 [-1.36; 2.69]	-0.10 [-0.42 ; 0.23]	0.20 [-1.33; 1.73]