Maternal glycemia during pregnancy and offspring abdominal adiposity measured by magnetic resonance imaging in the neonatal period and preschool years: The GUSTO prospective mother-offspring birth cohort study

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Abbreviations:

2hPG	2-hour plasma glucose concentration
AA	Abdominal adiposity
AAT	Abdominal adipose tissue compartment
BW	Birth weight
dSAT	Abdominal deep subcutaneous adipose tissue
FPG	Fasting plasma glucose concentration
GA	Gestational age
GDM	Gestational diabetes mellitus
IAT	Abdominal intra-abdominal adipose tissue
MRI	Magnetic resonance imaging
OGTT	Oral glucose tolerance test
SAT	Abdominal subcutaneous adipose tissue
SFT	Skinfold thickness
sSAT	Abdominal superficial subcutaneous adipose tissue
VAT	Visceral adipose tissue

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Abstract

Background

Gestational diabetes is associated with unfavorable body fat distribution in the offspring. However, less is known about the effects across the range of maternal gestational glycemia on offspring's abdominal adiposity in infancy and early childhood.

Objective

This study determined the association between gestational glycemia and offspring's abdominal adiposity measured by magnetic resonance imaging (MRI) in the neonatal period and at preschool years.

Design

Participants were mother-offspring pairs from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) prospective cohort study. Children who underwent MRI within 2weeks post-delivery (N=305) and/or at preschool age, 4.5 years (N=273), and whose mothers had a 2-hour 75g oral glucose tolerance test (OGTT) at 26-28 gestational weeks were included. Abdominal adiposity measured by adipose tissue compartment volumes; superficial (sSAT), deep subcutaneous (dSAT), and internal (IAT) abdominal adipose tissue were quantified from MRI images.

Results:

Adjusting for potential confounders including maternal pre-pregnancy BMI, each 1 mmol/L increase in maternal fasting glucose was associated with higher sSAT, differences in standard deviations scores (SD) (95%CI); 0.66 (0.45, 0.86), dSAT: 0.65 (0.44, 0.87) and IAT: 0.64 (0.42, 0.86) in neonates. Similarly, each 1 mmol/L increase in 2-hour OGTT glucose was

associated with higher neonatal sSAT, 0.11 (0.03, 0.19) and dSAT, 0.09 (0.00, 0.17). These associations were stronger in female neonates but only persisted in girls between fasting glucose, and sSAT and dSAT at 4.5 years.

Conclusions:

A positive association between maternal glycemia and neonatal abdominal adiposity was observed across the whole range of maternal mid-gestation glucose concentrations. These findings may lend further support on efforts of optimizing maternal hyperglycemia during pregnancy. The study also provides a suggestive evidence on sex differences of the impact of maternal glycemia which merits further confirmation in other studies.

Key words: Maternal Glucose, Mid-gestation, Pediatrics, Offspring's abdominal adipose tissue compartment, early infancy.

Introduction

Greater abdominal adiposity (AA) is an independent risk factor for adverse cardio-metabolic outcomes in adulthood (1). Similar to adults, AA in older children and adolescents appears more strongly related to insulin resistance, cardiovascular and diabetes risk factors than total body fat (2, 3). Most of the previous studies used waist circumference (WC) as a surrogate marker of AA (4, 5). South Asians have greater AA and are more insulin resistant as compared to Caucasians of similar body mass index (4). This thin-fat phenotype is present already at birth (5). Moreover, studies tracking AA in children observed that WC and waist-hip ratio categories; <25th; 25–75th, and >75th percentile of children at 7 years of age remained in the same categories at 15 years of age (6). Concomitantly with the increasing prevalence of childhood obesity and the prevalence of the metabolic syndrome among children and adolescents, it is important to better understand early life factors influencing AA in order to tackle the emerging epidemic of type 2 diabetes especially in the young.

Animal studies demonstrate that exposure to hyperglycemia in utero results in fetal programming towards a diabetes-prone phenotype in the offspring (7). Infants born to mothers with hyperglycemia are likely to have greater adiposity already at birth independent of infant's birth weight (BW) and maternal obesity. Human epidemiological studies suggest that the association between maternal gestational glucose concentrations and offspring's risk for large for gestational age (LGA) and excessive adiposity (>90th percentile of body fat measured by air displacement plethysmography or skinfold thickness) is a continuum even for glucose concentrations below diagnostic thresholds for gestational diabetes mellitus (GDM) (8-10). These early life changes may increase offspring susceptibility to the development of obesity and adverse metabolic risks, and seem to persist from childhood until adulthood (11, 12). There is limited information on the effects of maternal hyperglycemia during pregnancy on offspring AA at birth measured by Magnetic Resonance Imaging (MRI).

Moreover, no previous studies have explored these associations longitudinally from early infancy to preschool years.

Emerging data suggests that the impact of an adverse in utero environment on offspring health may have sex-specific effects, such as maternal overnutrition on offspring's adiposity. Potential biological mechanisms include fetal hyperinsulinemia resulting from maternal hyperglycemia which facilitates the conversion of glucose into free fatty acid. This leads to storage of excess glucose as glycogen and fat deposition in female fetus. On the other hand in male fetus, endogenous insulin acts as the dominant growth factor which leads to faster growth in utero than fat deposition (13-15).

However, findings from such studies have been inconsistent and conducted mainly in Western populations (12, 16-21) with sparse data in Asians, a high risk population for metabolic diseases, which forms 60 % of the global population. To address these research gaps, this study aimed to determine associations between maternal glycemic status during pregnancy and offspring's AA as measured by MRI in the neonatal period and at pre-school years. Further, we investigated whether such associations differed by offspring sex.

Subjects and Methods

The study was based on mother-offspring pairs from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study, a prospective birth cohort study in Singapore (22). Pregnant women aged 18 years and above were recruited between June 2009 and September 2010 during the first trimester of pregnancy (< 14 weeks' gestation based on dating ultrasound scan) from two public maternity units in Singapore; KK Women's and Children's Hospital and National University Hospital. Pregnant women self-identified their homogenous ethnicity; Chinese, Malay or Indian i.e. same ethnicity of the subject, their partners, and both sides of parents.

Details of the study have been presented previously (23). 333 healthy neonates born ≥34 weeks gestation with BW ≥2000g had abdominal adipose tissue compartment (AAT) volumes quantified using images of MRI performed within two weeks after delivery. 19 neonates born less than 37 completed weeks of gestation and 9 neonates of mothers with no oral glucose tolerance test (OGTT) performed were excluded (Supplementary figure 1). A total of 305 mother-neonate pairs remained for this analysis. 8 neonates in this study were born from IVF pregnancy. 119 children who attended neonatal MRI visit came back for 4.5-year MRI visits. Further, additional 154 children born term with available maternal OGTT participated in this visit. A total of 273 children had AAT volumes quantified at preschool age, 4.5 years (Supplementary table 1).

Ethics

This study was approved by the Institutional Review Board of the Singapore National Healthcare Group and the Central Institutional Review Board of SingHealth. Parents of the neonates gave voluntary written informed consent.

Maternal and infant characteristics

Demographic data, lifestyle, obstetric and medical history of the pregnant women were collected at 11-12 and 26-28 gestational week antenatal visits using interviewer administered questionnaires. Self-reported pre-pregnancy weights of mothers were recorded.

Maternal oral glucose tolerance test

Pregnant women underwent a 2-hour 75g OGTT at 26-28 weeks gestation. Plasma glucose concentrations were measured by a glucose oxidase method using Advia 2400 Chemistry system (Siemens Medical Solutions Diagnostics) and Beckman LX20 Pro analyzer (Beckman Coulter)]. GDM was diagnosed using 1999 World Health Organization (WHO) criteria: ≥7.0 mmol/L for fasting plasma glucose (FPG) concentrations or ≥7.8 mmol/l for 2-hour plasma glucose (2hPG) concentrations (24). Mothers diagnosed as GDM (N=46) were

treated according to clinical protocols at KK Women's and Children's Hospital and National University Hospital. 37 (80.4%) of 46 GDM mothers were treated with diet modification, 6 (13.0%) were on insulin. Information on treatment of GDM for 3 mothers (6.5%) was not available.

MR image acquisition and quantification of abdominal adipose tissue compartments

MRI of neonates was performed by GE Signa HDxt 1.5 T MR scanner (GE Healthcare) within 2 weeks after delivery. AAT volumes were quantified from images from neonatal MRI scans. The details have been described previously (23). The AAT was categorized into superficial (sSAT), deep (dSAT) subcutaneous and internal (IAT) adipose tissue. Neonatal MRI images were initially processed by an in-house semi-automated quantitative analysis algorithm using MATLAB 7.13 software (The MathWorks Inc., Natick, Massachusetts, USA) followed by manual optimization by 2 trained analysts who were blinded to all subject information. The mean inter-observer coefficients of variations (%) were 1.6% for sSAT, 3.2% for dSAT, and 2.1% for IAT. The mean intra-observer coefficients of variations (%) were 0.9%, 2.1% and 4.0% for sSAT, dSAT, and IAT, respectively.

MRI was also performed when children were around 4.5 years using a 3T MR scanner (Siemens Skyra, VE11A). Axial images with a 5 mm slice thickness and in-plane resolution of 0.94 x 0.94 mm were acquired using a water-suppressed half-Fourier-acquired single-shot turbo spin echo (HASTE) sequence (repetition time = 1000 ms, echo time = 95 ms) from the top of the liver to the top of the sacrum to be consistent with the definition of abdominal region for neonatal MRI. Abdominal subcutaneous adipose tissue (SAT) and IAT compartments were segmented from the abdominal MR images using a fully automated graph theoretic segmentation algorithm (25, 26) developed in MATLAB R2016 software (The MathWorks Inc., Natick, Massachusetts, USA). The SAT compartment was then

subclassified into dSAT and sSAT by manually drawing a boundary along the fascial plane by a trained analyst who was blinded to participant information. The volumes of each fat compartment were computed by summing the voxels and multiplying by the image resolution. The details of the MRI scans and the image analysis for 4.5 year children have been described elsewhere (26). The MATLAB software used for image analyses at 4.5 year was an upgraded version of the one used at the neonatal period. This upgraded MATLAB software was applied to MR images of all children at 4.5 year follow up visit.

Statistical analysis

Multivariable regression analyses were used to examine the associations of maternal glycemia with the offspring's sSAT, dSAT and IAT. Maternal glucose concentrations were considered as both continuous (FPG or 2hPG) and categorical (GDM status) variables. The primary outcome variables, AAT compartment volumes were converted to standard deviation scores (SD) for better comparison of magnitude of differences among 3 compartments and at two different time points. Covariates were controlled for based on prior knowledge from the literature about factors that might confound the associations between maternal glycemia and offspring adiposity. For the neonatal period, ethnicity, maternal educational attainment, tobacco exposure during pregnancy (27), parity, maternal age at recruitment, pre-pregnancy BMI, neonatal sex and age on MRI day were adjusted in regression analysis. Maternal gestational weight gains and gestational age (GA) at delivery were additionally adjusted in the regression models as a sensitivity analyses to test the robustness of the associations. At 4.5 years, models were adjusted for ethnicity, maternal educational attainment, maternal age at recruitment, child's sex and age on MRI day. BW was also included as a covariate for taking account the adiposity of the child at birth. Since BW is highly correlated with maternal BMI and also on the causal pathway between maternal BMI and childhood adiposity, maternal height was included in the models to account for maternal size. As the interactions

were significant between FPG and sex for two of 3 AAT compartments, stratified analyses were performed. Interactions between maternal pre-pregnancy BMI groups (28) and glycemia were also tested to determine if the associations between maternal glycemia and offspring AAT outcomes were modified by the level of maternal adiposity. All statistical analyses used SPSS Statistics for Windows, Version 21.0. (IBM Corp., Armonk, NY).

Results

Supplementary figure 1 shows the flow chart of this study. **Table 1** shows characteristics of mothers and offspring divided by sex. A total of 305 mother-neonate pairs were included in this study; 135 Chinese (44.3%), 115 Malays (37.7%) and 55 Indians (18.0%) mothers. In general, mothers of male and female neonates had similar characteristics except that a greater proportion of mothers of female neonates were nulliparous. There were 163 males (53.4%), 142 females (46.6%). Female neonates had greater sSAT and dSAT despite having lower BW and shorter birth length. They were born at marginally longer gestational age, compared to male neonates. Characteristics of mothers and children participated at 4.5-year visits between boys and girls were similar to those of children who attended during the neonatal period **(**Table 1**)**. Most of the characteristics of children who attended both neonatal and 4.5 year MRI visits were also similar to those of children who attended during the neonatal period. However, the greater proportion of mothers of boys had higher education attainment and girls has greater AAT of all 3 compartments. Characteristics of participants vs. non-participants in this study were also compared and presented in the **supplementary table 1**. In this study, there were relatively less Chinese and more Malay mothers compared to those who did not participate. Lower proportion of mothers in this study had university or higher education and no tobacco exposure. They were marginally younger, had higher prepregnancy BMI, higher FPG and lower 2hPG compared to non-participants.

Neonates in this study had higher BW (80g) and marginally longer GA and compared to neonates who did not participate in the study.

Maternal glycemic status and abdominal adiposity in early infancy

Table 2 shows the positive associations between maternal glycemia as a continuous variable and neonatal AA. Maternal FPG and 2hPG were positively associated with AA in the neonates. Each 1 mmol/L increment in FPG was associated with increases in sSAT, difference in SD, B (95% CI), 0.66 (0.45, 0.86), dSAT 0.65 (0.44, 0.87) and IAT 0.64 (0.42, 0.86) respectively. Similarly, each 1 mmol/L increment in 2hPG was associated with greater sSAT and dSAT, 0.11 (0.03, 0.19) and 0.09 (0.00, 0.17), respectively (Table 2). We also observed that the above-mentioned associations varied by offspring sex (Table 2). Interaction terms between maternal glucose and sex were significant for 4 of the 6 associations examined between FPG/2hPG and sSAT/dSAT/IAT (Table 2). The interaction (maternal glycemia and sex) coefficients in the regression models showed the slope difference in these associations between offspring sex; the slope differences in the associations between maternal FPG and neonatal AAT volumes between male and female neonates were -0.59 (-0.99, -0.19) SD for sSAT, -0.45 (-0.88, -0.03) SD for dSAT and -0.33 (-0.78, 0.11) SD for IAT, and -0.23 (-0.38, -0.08) SD for sSAT, -0.15 (-0.31, 0.02) SD for dSAT and -0.20 (-0.36, -0.40) SD for IAT for the associations between maternal 2hPG and neonatal AAT volumes (**Figure 1**).

Stronger associations were seen in female neonates, each 1 mmol/L increase in FPG was associated with higher AAT SD; 0.93 (0.62, 1.23) in sSAT, 0.89 (0.59, 1.19) in dSAT and 0.79 (0.52, 1.06) in IAT. In male neonates, the association between FPG and IAT was significant but weaker compared to associations in female neonates (Table 2). Likewise, higher 2hPG was associated with greater AAT SDs only in female neonates; each 1 mmol/L increase in 2hPG was associated with 0.27 (0.12, 0.41), 0.20 (0.05, 0.34) and 0.20 (0.07,

0.33) increases in sSAT, dSAT and IAT, respectively. Associations between 2hPG and AAT appeared to be weaker compared to those between FPG and AAT. By contrast, among male neonates, no significant associations were observed between 2hPG and neonatal AAT volumes. Female neonates appeared to be more sensitive to maternal glycemia compared to male neonates (Figure 1).

The above described associations became stronger when all models (for both FPG and 2hPG) were additionally adjusted for maternal gestational weight gain groups and GA at delivery **(Supplementary table 2)**. Also, the association between 2hPG and IAT for the whole cohort became significant (Supplementary table 2).

There were no interactions between maternal pre-pregnancy BMI groups and glycemia (both FPG and 2hPG) on associations between maternal glycemia and neonatal AAT; (all P=>0.05).

Maternal glycemic status and abdominal adiposity at preschool years (4.5 years)

There was a substantial increase in AAT at preschool years compared to the neonatal period (N=119). The magnitude of change in AAT was mean (SD); 4.4 (2.8) fold for sSAT, 11.5 (11.9) fold for dSAT and 8.2 (4.6) fold for IAT. Compared to 4.7 (1.0) fold change in weight, the increase in dSAT and IAT was disproportional over first 4.5 years. At the early neonatal period, VAT or intraperitoneal fat within IAT such as mesenteric fat (fat around intestines), liver and pancreas was minimal compared to retro-peritoneal fat. However, there was a substantial increase in intra-abdominal VAT within IAT at 4.5 years **(Figure 2)**.

Adjusting for ethnicity, maternal age, maternal education, maternal height, BW, neonatal sex and age on MRI day, maternal FPG and 2hPG showed no association with child's AAT volumes (**Table 3**). However, interaction terms were significant between maternal FPG and sex on child's all AAT at 4.5 years. Therefore, stratified analyses were

241 performed and the associations were present only in girls between FPG and SAT (SD); 0.47
242 (0.04, 0.89) and 0.47 (0.04, 0.90) for sSAT and dSAT respectively (Table 3). However, no
243 associations were observed between 2hPG and AA of preschool children among girls.
244
245 ***Maternal GDM status and abdominal adiposity***
246 Maternal GDM status was characterized based on WHO 1999 criteria.
247 S**upplementary table 3** showed characteristics of participants by GDM status. Higher
248 proportion of mothers with GDM had lower tobacco exposure. They were older and had
249 higher BMI and higher glucose concentrations (both FPG and 2hPG). AAT volumes were not
250 different in the whole cohort. BW and proportion of neonates with macrosomia in GDM vs.
251 non-GDM mothers were similar i.e. 3.14 (0.39) vs. 3.18 (0.50) kg and 2.3% vs. 2.2%
252 respectively. However, sex modified the associations between maternal GDM status and
253 child's AAT (p for interaction: 0.011 for both sSAT and IAT). In the stratified analyses by
254 sex of children, associations between sSAT and IAT in the neonatal period were significantly
255 greater for female neonates who were born from GDM pregnancies than those born from
256 pregnancies uncomplicated by GDM, differences in SD of AAT (95%CI); 0.78 (0.11, 1.45)
257 and 0.76 (0.17, 1.35) respectively. The differences were greater when models were
258 additionally adjusted for maternal GWG and GA at delivery; differences (95%CI): 0.97
259 (0.28, 1.67) for sSAT and 0.86 (0.23, 1.48) for IAT. There was no association between
260 maternal GDM status and AAT when children were at preschool age.
261
262 **Discussion**
263 This prospective multiethnic Asian study with a large number of longitudinal MRI
264 measurements at the neonatal period and preschool age reports on associations between
265 maternal gestational glycemia and offspring AA. A positive association between maternal

gestational glycemia and AAT of neonates measured by MRI was observed during first two weeks of life. These associations were continuous across the whole range of both FPG and 2hPG concentrations and independent of maternal pre-pregnancy BMI. Female neonates were particularly susceptible to maternal glycemia and more prone to have higher AAT, and persisted in girls for sSAT and dSAT at preschool years independent of maternal size and child's BW. sSAT is suggested to be metabolically different in Asians (29). Ethnic differences in adipose tissue partitioning was observed in a study on adipose tissue volumes measured by MRI comparing British and Indian neonates. Indian neonates had greater AAT in all 3 compartments and significantly lower non-abdominal SAT (such as SAT from head, chest, pelvis and extremities) at birth compared to British neonates. dSAT has been studied only recently. dSAT is suggested to be strongly related to insulin resistance and cardio-metabolic risk factors in a similar manner to VAT (30-32).

The continuous positive association observed between maternal glycemia and neonatal AAT in this study is generally consistent with previous findings based on BW and skinfold thickness (SFT) measurements in the GUSTO cohort (8), as well as with findings in the HAPO study (9) which both demonstrated that each one SD increase in maternal mid-gestation glycemia was associated with increasing odds of excessive neonatal adiposity defined by large-for-gestational-age, >90th percentile of sum of SFT or percentage fat (8, 9). Recently, The HAPO Follow-up Study (HAPO-FUS) reported that exposure to higher concentrations of glucose in utero is independently associated with childhood adiposity at 10-14 years; being overweight/obese according to age and sex specific cut-offs based on International Obesity Task Force or >85th percentile for SFT, waist circumference and percent body fat measured by air displacement plethysmography (10). However, mothers diagnosed with GDM received treatment in the present study, unlike HAPO-FUS in which mothers did not receive treatment. These findings of continuous associations may have

clinical implications not only in controversies on best cut-off points for screening maternal glycemia but also on future metabolic health of the offspring.

We observed stronger associations between maternal glycemia and neonatal AA among female neonates. However, a sex difference in associations between maternal glycemia and offspring's total adiposity in previous studies seemed to be distinct between Western and Asian populations. In Western populations, such associations were observed primarily in boys at early infancy throughout adolescence (16, 18, 21, 33) although one Norwegian study showed that maternal fasting plasma glucose at week 30-32 was significantly associated with BW among girls only (34). A prospective study in Australia reported that maternal FPG was the major predictor of adiposity as measured by air displacement plethysmography in male neonates of GDM mothers but had little effect in female neonates (16). Similarly, the association of GDM with offspring obesity risk from late childhood through early adulthood was observed among boys in a large prospective study of 15,009 U.S. individuals (21). By contrast, previous studies in Asian cohorts showed associations of maternal GDM on offspring adiposity in girls of preschool/school age. In a study in Indian children, female offspring of diabetic mothers had larger SFT than offspring of non-GDM mothers at 5 and 9.5 years of age (19, 20). Maternal hyperglycemia in pregnancy was independently associated with offspring's risk of abnormal glucose tolerance, obesity, and higher blood pressure at 7 years of age only in girls in a total of 970 Chinese mothers in Hong Kong (12). Our findings support and strengthen the previous findings in Asians.

The significant and positive associations of maternal FPG with offspring sSAT and IAT during preschool years persisted for girls despite having similar weight compared to boys, and independent of maternal size. This may reflect at least in part a contribution of maternal glycemia to long-term health consequences among the offspring in addition to

potential environmental influence of an obesogenic environment. With the disproportional increase in dSAT and substantial increase in VAT at 4.5 years which are metabolically active, our findings may reflect an unfavorable metabolic phenotype of the offspring especially among Asian girls.

Previous studies have shown the association between maternal glycemia and offspring total adiposity, and also reported sex differences. However, a unique strength of our study is the use of AAT measurement by MRI in a large number of neonates with a follow up MRI. MRI is the most accurate and only available method without radiation to quantify AA (29). In addition, GUSTO is a prospective study of Asian mothers and their offspring, including three Asian ethnic populations (Chinese, Malay, Indian) which all are at high risk of type 2 diabetes. We also explored sex difference in AA in association with maternal glycemia which is little studied. The timing of MRI scans for neonates was within 2 weeks after delivery thus the observation would largely reflect the developmental influences on the offspring before any environmental exposures. Similarly, the variation in postnatal environmental influences among children were relatively less at preschool age of 4.5 years. Several potential limitations merit discussion. First, only a subset of eligible children whose parents gave consent for their children's MRI were included in this study therefore care should be taken when generalizing our findings. Future studies are warranted to confirm these findings in larger study populations. Secondly, the MATLAB software used at 4.5 year analyses was an upgraded version of the one used at the neonatal period. However, as this same upgraded MATLAB software was applied to MR images of all children at the follow up 4.5 years visit, the upgrade cannot explain the observed associations between maternal glycemia and offspring abdominal adiposity. Lastly, as with any observational study, we cannot exclude the possibility of bias due to residual confounding although we carefully controlled for a number of major potential confounders. However, our findings, in line with those from

341 others, indicated that the association between maternal glycemia with offspring AA is in
342 continuum.

343 Taken together, our findings reinforce the impact of maternal glycemia even below
344 the threshold for the diagnosis of gestational diabetes, during fetal development on
345 offspring's AA. These findings lend further support on efforts of optimizing glycemic status
346 of the mothers during pregnancy. The suggestive evidence on sex differences of the impact of
347 maternal glycemia merits further confirmation in other studies.
348

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374 ***Conflict of Interest***

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383 that could appear to have influenced the submitted work.

384

385 ***Authors' contributions***

386 MTT supervised collection of data, performed image analysis of neonates, analyzed the data
387 and wrote the manuscript. SAS, NM and SSV performed image analysis of preschool
388 children. MVF supervised image acquisition and image analysis. CZ, JGE and YSL provided
389 statistical advice and made critical revision of the manuscript for important intellectual
390 content. KMG, SYC, FY, KHT and LPCS, SES and IMA contributed to discussion and
391 reviewed the manuscript. MTT and YSL had primary responsibility for final content. YSC,
392 PDG, KMG and YSL conceptualized and designed the study.

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Table 1 Maternal and offspring characteristics among participants in the GUSTO study stratified by offspring's sex at neonatal and preschool age

	Neonatal Period				Preschool age			
	All (N=305)	Male (N=163)	Female (N=142)	P	All (N=273)	Boys (N=124)	Girls (N=149)	P
Maternal characteristics								
Ethnicity, N(%)				0.523				0.548
Chinese	135 (44.3)	70 (42.9)	65 (45.8)		139 (50.9)	63 (50.8)	76 (51)	
Malay	115 (37.7)	66 (40.5)	49 (34.5)		81 (29.7)	40 (32.3)	41 (27.5)	
Indian	55 (18.0)	27 (16.6)	28 (19.7)		53 (19.4)	21 (16.9)	32 (21.5)	
Mother highest education groups , N(%)				0.386				0.322
Below secondary	129 (43.0)	63 (39.4)	66 (47.1)		102 (37.8)	44 (36.4)	58 (38.9)	
GCE, ITE, Diploma	111 (37.0)	64 (40.0)	47 (33.6)		93 (34.4)	38 (31.4)	55 (36.9)	
University and above	60 (20.0)	33 (20.6)	27 (19.3)		75 (27.8)	39 (32.2)	36 (24.2)	
Parity, N(%)				0.023				0.033
Nulliparous	123 (40.3)	56 (34.4)	67 (47.2)		103 (37.7)	38 (30.6)	65 (43.6)	
Multiparous	182 (59.7)	107 (65.6)	75 (52.8)		170 (62.3)	86 (69.4)	84 (56.4)	
Maternal tobacco exposure, N(%)				1.000				0.714
No exposure	113 (39.6)	61 (39.6)	52 (39.7)		133 (52.0)	62 (53.0)	71 (51.1)	

Exposed with Cotinine level <level of detection	90 (31.6)	49 (31.8)	41 (31.3)		74 (28.9)	35 (29.9)	39 (28.2)	
Exposed with cotinine level <14 ng/ml	71 (24.9)	38 (24.7)	33 (25.2)		41 (16.0)	18 (15.4)	23 (16.6)	
Exposed with cotinine level >14 ng/ml	11 (3.9)	6 (3.9)	5 (3.8)		8 (3.13)	2 (1.7)	6 (3.1)	
Mother age (years)	30 (5)	30 (5)	29 (6)	0.407	31 (5)	30 (5)	31 (5)	0.457
Mother pre-pregnancy BMI (kg/m^2)	23.3 (5.1)	23.0 (4.7)	23.7 (5.6)	0.310	23.2 (4.6)	22.6 (4.2)	23.6 (4.8)	0.083
Fasting Plasma Glucose (mmol/L)	4.4 (0.6)	4.4 (0.6)	4.4 (0.6)	0.625	4.3 (0.4)	4.3 (0.4)	4.3 (0.4)	0.802
2 Hour OGTT Glucose (mmol/L)	6.3 (1.5)	6.4 (1.6)	6.2 (1.4)	0.316	6.3 (1.3)	6.3 (1.3)	6.4 (1.4)	0.589
Offspring's characteristics								
Macrosomia				0.127				1.000
No	298 (97.7)	157 (96.3)	141 (99.3)		271 (99.3%)	123 (99.2%)	148 (99.3%)	
Yes	7 (2.3)	6(3.7)	1 (0.7)		2 (0.3%)	1 (0.8%)	1 (0.7%)	
Size at birth				0.917				0.153
Large for gestational age	52 (17.0)	28 (17.2)	24 (16.9)		44 (16.1)	18 (14.5)	26 (17.4)	
Small for gestational age	36.0 (11.8)	18 (11.0)	18 (12.7)		27 (9.9)	8 (6.5)	19 (12.8)	
Birth Weight (kg)	3.1 (0.4)	3.2 (0.4)	3.1 (0.4)	0.023	3.1 (0.4)	3.2 (0.3)	3.1 (0.4)	0.105
Birth Length (cm)	48.6 (2.0)	48.8 (2.0)	48.3 (2.1)	0.032	48.6 (1.9)	48.8 (1.8)	48.5 (1.9)	0.295
Gestational age (weeks)	38.9 (1.0)	38.8 (1.0)	39.1 (1.0)	0.035	39.0 (1.0)	38.8 (1.0)	39.1 (1.0)	0.003
Age on the day of MRI at early infancy (days)	10 (3)	10 (3)	10 (3)	0.408	--	-	-	

Age on the day of MRI at preschool age (years)	-	-	-		4.6 (0.1)	4.6 (0.1)	4.6 (0.1)	0.413
Weight at age 4.5Yr MRI (kg)	-	-	-	-	17.6 (3.0)	17.7 (3.0)	17.5 (3.1)	0.550
Height at age 4.5Yr MRI (cm)	-	-	-	-	105.5 (4.1)	105.6 (4.5)	105.4 (4.1)	0.714
BMI at age 4.5Yr MRI (kg/m^2)	-	-	-	-	15.7 (1.8)	15.8 (1.8)	15.7 (1.9)	0.546
Superficial subcutaneous adipose tissue (ml)	79.40 (21.67)	75.67 (19.11)	83.68(23.63)	0.001	337.77 (160.84)	310.35 (127.09)†	377.84 (221.19)†	<0.001
Deep subcutaneous adipose tissue (ml)	13.66 (5.64)	12.78 (5.15)	14.67 (6.02)	0.003	111.25 (105.69)	85.23 (81.36)†	136.49 (131.70)†	<0.001
Internal adipose tissue (ml)	23.10 (7.51)	23.13 (7.69)	23.06 (7.33)	0.934	177.32 (73.99)	185.60 (171.53)†	171.53 (71.18)†	0.161

Data shown are N (%) for categorical variables or mean ± SD for continuous variables unless otherwise stated. P-values are based on between group comparisons of offspring sex using ANOVA for continuous variables and $\chi 2$-test for categorical variables. † indicates that the data shown are median (IQR) and P values were based on non-parametric Mann-Whitney U test.

Abbreviations: sSAT abdominal superficial subcutaneous adipose tissue, dSAT abdominal deep subcutaneous adipose tissue, IAT, abdominal internal adipose tissue.

Table 2 Change in standard deviation scores of abdominal adipose tissue compartment volumes per 1 mmol/L increment in maternal glucose concentrations in mid-gestation at neonatal period in mother-neonate pairs

	Fasting plasma glucose (mmol/L)			2 hour plasma glucose (mmol/L)		
	sSAT (SD) β (95% CI)	dSAT (SD) β (95% CI)	IAT (SD) β (95% CI)	sSAT (SD) β (95% CI)	dSAT (SD) β (95% CI)	IAT (SD) β (95% CI)
All (N=305)	0.66 (0.45, 0.86) P<0.001[1]	0.65 (0.44, 0.87) P<0.001[1]	0.64 (0.42, 0.86) P<0.001[1]	0.11 (0.03, 0.19) P=0.009[1]	0.09 (0.00, 0.17) P=0.042[1]	0.07 (-0.02, 0.15) P=0.135[1]
Male (N=163)	0.23 (-0.03, 0.49) P=0.088[2]	0.25 (-0.06, 0.57) P=0.110[2]	0.38 (0.02, 0.74) P=0.036[2]	0.01 (-0.10, 0.07) P=0.736[2]	0.01 (-0.10, 0.09) P=0.885[2]	0.05 (-0.16, 0.06) P=0.350[2]
Female (N=142)	0.93 (0.62, 1.23) P<0.001[2]	0.89 (0.59, 1.19) P<0.001[2]	0.79 (0.52, 1.06) P<0.001[2]	0.27 (0.12, 0.41) P<0.001[2]	0.20 (0.05, 0.34) P=0.008[2]	0.20 (0.07, 0.33) P=0.003[2]
P for interaction	P=0.004	P=0.037	P=0.139	P=0.003	P=0.075	P=0.017

Coefficients (β) shown are differences in standard deviation scores (95% confidence intervals) of AAT volumes (ml) with each 1mmol/L increment in maternal glucose concentrations; fasting plasma glucose or 2-hour plasma glucose. P-values were determined with the use of multivariable regression models.

[1]: Models adjusted for ethnicity, parity, maternal age, maternal education, tobacco exposure and pre-pregnancy BMI, age on MRI day and neonatal sex.

[2]: Models adjusted for ethnicity, parity, maternal age, maternal education, tobacco exposure and pre-pregnancy BMI, and age on MRI day.

Abbreviations: Abdominal adipose tissue compartment (AAT) volumes: dSAT: abdominal deep subcutaneous adipose tissue, IAT: abdominal internal adipose tissue. sSAT: abdominal superficial subcutaneous adipose tissue, SD: Standard deviation

Table 3 Changes in standard deviation scores of abdominal adipose tissue compartment volumes per 1mmol/L increment in maternal glucose concentrations at preschool years in mother-child pairs

	Fasting plasma glucose (mmol/L)			2 hour plasma glucose (mmol/L)		
	sSAT (SD) β (95% CI)	dSAT (SD) β (95% CI)	IAT (SD) β (95% CI)	sSAT (SD) β (95% CI)	dSAT (SD) β (95% CI)	IAT (SD) β (95% CI)
All (N=273)	0.22 (-0.07, 0.51) P=0.140[1]	0.20 (-0.09, 0.49) P=0.178[1]	0.13 (-0.17, 0.42) P=0.393[1]	0.03 (-0.06, 0.12) P=0.558[1]	0.03 (-0.06, 0.17) P=0.542[1]	0.00 (-0.09, 0.09) P=0.963[1]
Sex						
Male (N=124)	-0.11 (-0.50, 0.27) P=0.563[2]	-0.16 (-0.55, 0.22) P=0.397[2]	-0.14 (-0.52, 0.24) P=0.480[2]	-0.04 (-0.15, 0.08) P=0.514[2]	-0.04 (-0.16, 0.07) P=0.448[2]	-0.02 (-0.13, 0.10) P=0.743[2]
Female (N=149)	0.47 (0.04, 0.89) P=0.031[2]	0.47 (0.04, 0.90) P=0.032[2]	0.33 (-0.11, 0.76) P=0.138[2]	0.08 (-0.06, 0.21) P=0.258[2]	0.08 (-0.05, 0.22) P=0.226[2]	0.03 (-0.10, 0.17) P=0.648[2]
P for interaction	P=0.021	P=0.012	P=0.016	P=0.138	P=0.114	P=0.314

Coefficients (β) shown are differences in standard deviation scores (95% confidence intervals) of AAT volumes (ml) with each 1mmol/L increment in maternal glucose concentrations; fasting plasma glucose or 2-hour plasma glucose. P-values were determined with the use of multivariable regression models.
[1]: Models adjusted for ethnicity, maternal age, maternal education, maternal height, age on MRI day, birth weight and neonatal sex.
[2]: Models adjusted for ethnicity, maternal age, maternal education, maternal height, age on MRI day and birth weight.
Abbreviations: Abdominal adipose tissue compartment (AAT) volumes: dSAT: abdominal deep subcutaneous adipose tissue, IAT: abdominal internal adipose tissue. sSAT: abdominal superficial subcutaneous adipose tissue, SD: Standard deviation

Figure legend

Figure 1 Scatter plots of abdominal adipose tissue compartment volumes in relation to maternal mid-gestation glucose concentrations stratified by sex

1A. Associations between maternal fasting plasma glucose concentrations and neonatal adipose tissue compartment volumes stratified by sex
P for interaction between maternal mid-gestation fasting plasma glucose concentrations and sex on neonatal adipose tissue compartment volumes were 0.004, 0.037 and 0.003 for sSAT, dSAT and IAT respectively. The slope differences (95%CI) are -0.59 (-0.99, -0.19) SD for sSAT, -0.45 (-0.88, -0.03) SD for dSAT and -0.33 (-0.78, 0.11) SD for IAT.

1B. Associations between maternal 2 hour OGTT glucose concentrations and neonatal adipose tissue compartment volumes stratified by sex

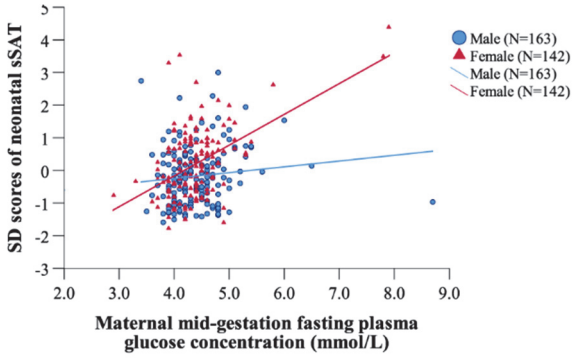
P for interaction between maternal mid-gestation 2 hour OGTT glucose concentrations and sex on neonatal adipose tissue compartment volumes were 0.003, 0.075 and 0.017 for sSAT, dSAT and IAT respectively. The slope differences (95%CI) are -0.23 (-0.38, -0.08) SD for sSAT, -0.15 (-0.31, 0.02) SD for dSAT and -0.20 (-0.36, -0.40) SD for IAT

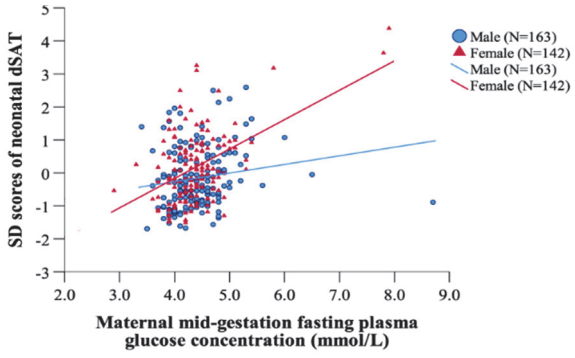
Abbreviations: SD scores: Standard Deviation scores; dSAT: Abdominal adipose tissue compartment volumes; IAT: abdominal internal adipose tissue; sSAT: abdominal superficial subcutaneous adipose tissue

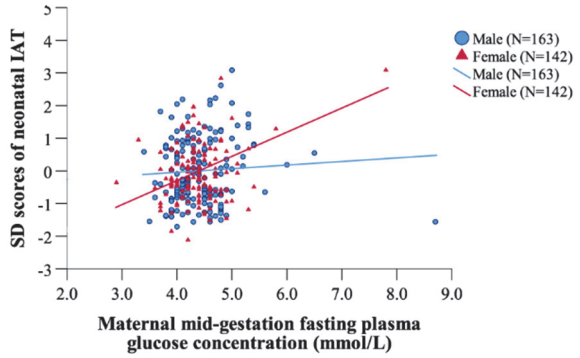
Figure 2 Original abdominal water-suppressed magnetic resonance images (left column) and segmented images (right column) at neonatal period and preschool years. In segmented images, each abdominal adipose tissue compartment is color coded: red denotes superficial subcutaneous tissue, green denotes the deep subcutaneous tissue, and blue denotes the internal adipose tissue.

There was a substantial increase in abdominal adipose tissue for all three compartments at preschool years compared to the neonatal period. At neonatal period (A and B), the intra-peritoneal fat within internal abdominal adipose tissue such as mesenteric fat (fat around intestines), liver and pancreas was minimal compared to retro-peritoneal fat but increase substantially at preschool years (C and D), and is indicated in yellow arrow.

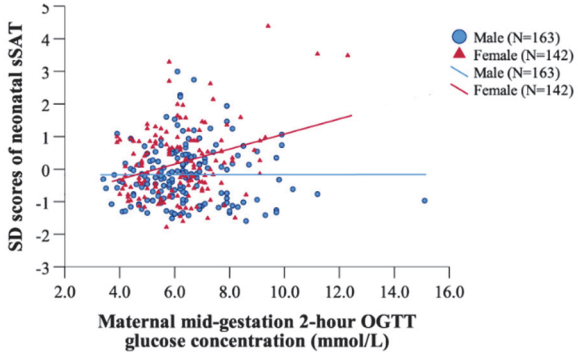
1A

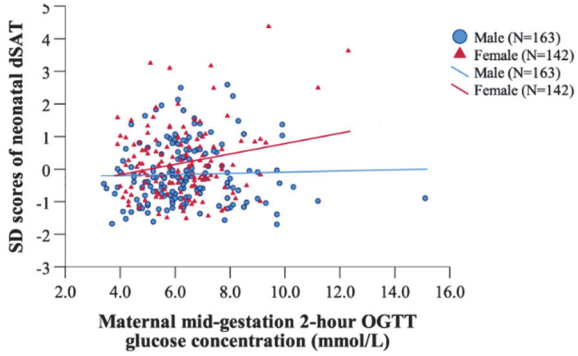

SD scores of neonatal sSAT
Male (N=163)
Female (N=142)
Male (N=163)
Female (N=142)
Maternal mid-gestation fasting plasma glucose concentration (mmol/L)

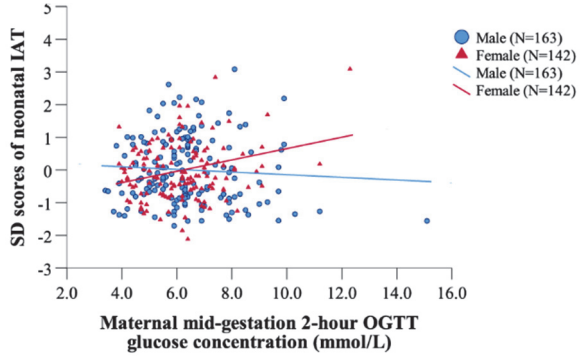

SD scores of neonatal dSAT
Male (N=163)
Female (N=142)
Male (N=163)
Female (N=142)
Maternal mid-gestation fasting plasma glucose concentration (mmol/L)


SD scores of neonatal IAT
Male (N=163)
Female (N=142)
Male (N=163)
Female (N=142)
Maternal mid-gestation fasting plasma glucose concentration (mmol/L)

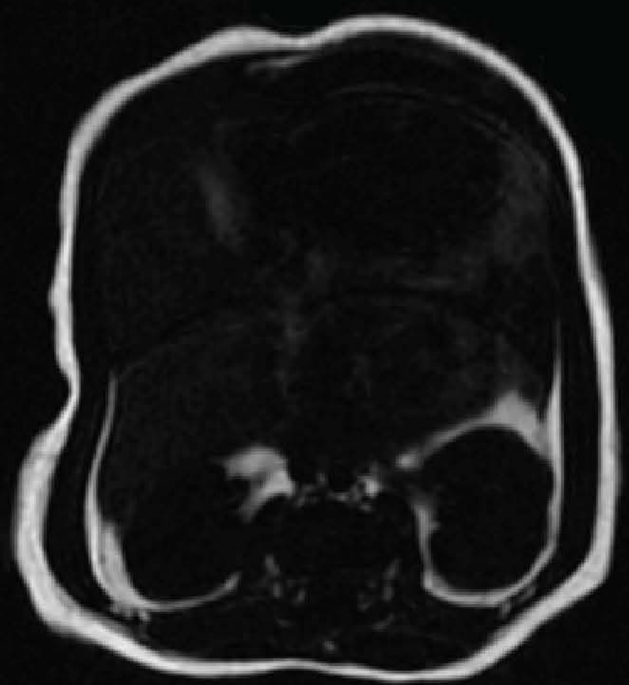
1B


SD scores of neonatal sSAT
Male (N=163)
Female (N=142)
Male (N=163)
Female (N=142)
Maternal mid-gestation 2-hour OGTT glucose concentration (mmol/L)

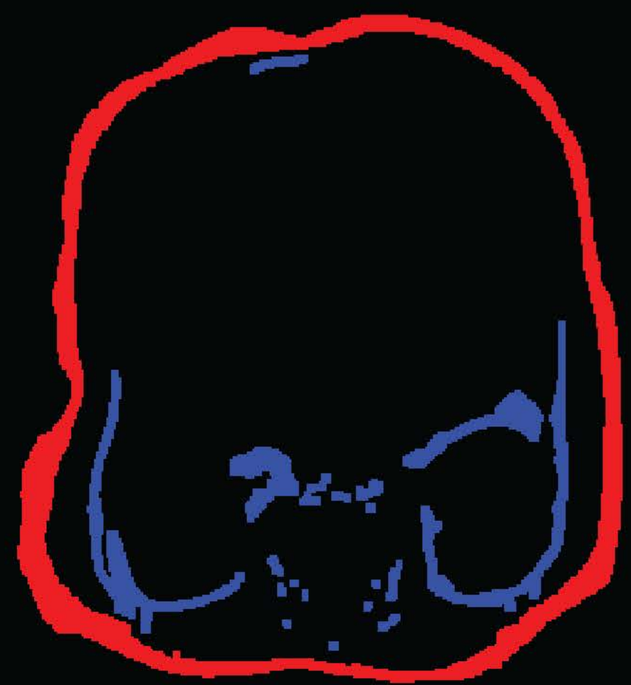

SD scores of neonatal dSAT
Male (N=163)
Female (N=142)
Male (N=163)
Female (N=142)
Maternal mid-gestation 2-hour OGTT glucose concentration (mmol/L)


SD scores of neonatal IAT
Male (N=163)
Female (N=142)
Male (N=163)
Female (N=142)
Maternal mid-gestation 2-hour OGTT glucose concentration (mmol/L)

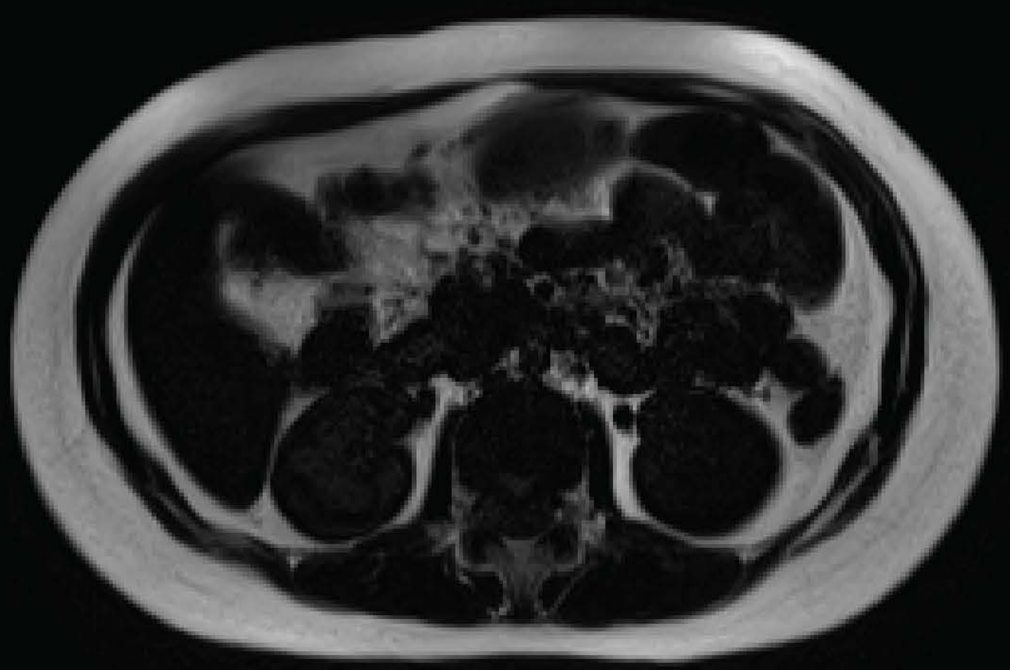
A



B



C



D