**Neonatal amygdala microstructure mediates the relationship between gestational glycemia and offspring adiposity**

**Short running title – Amygdala mediate glycemia linked child adiposity**

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Word count: 4060(excluding tables)

Figures:2

Tables:3

Supplemental Material: 3 Figures, 1 Table

**Abstract**

**Introduction:** To determine if variations in the neonatal amygdala mediate the association between maternal antenatal glycemia and offspring adiposity in early childhood.

**Research Design and Methods:** 123 non-obese pregnant women with no pregnancy complications aside from gestational diabetes, did a 75-g 2-h oral glucose tolerance test at 26-28 weeks’ gestation. Volume and fractional anisotropy (FA) of the neonatal amygdala (5-17 days old) were measured by magnetic resonance imaging (MRI). Body mass index (BMI) z-scores and sum of skinfold thickness (subscapular and triceps) of these children were tracked up to 60 months of age (18, 24, 36, 48, 54 and 60 months).

**Results:** Maternal fasting glucose levels were positively associated with the offspring’s sum of skinfold thickness at age 48 [β=3.12 (95% CI 0.18 to 6.06)mm] and 60 months [β=4.14 (0.46 to 7.82) mm] and BMI z-scores at 48 [β=0.94 (0.03 to 1.85)], 54 [β=0.74 (0.12 to 1.36)] and 60 months [β=0.74 (0.08 to 1.39)]. Maternal fasting glucose was negatively associated with the offspring’s FA of the right amygdala [β= -0.019 (-0.036 to -0.003)]. Right amygdala FA was negatively associated with sum of skinfold thickness in the offspring at age 48 months [β= -56.95 (-98.43 to -15.47) mm], 54 months [β= -46.18 (-88.57 to -3.78) mm], and 60 months [β= -53.69 (-105.74 to -1.64)mm]. The effect sizes mediated by right amygdala FA between fasting glucose and sum of skinfolds were estimated at β=5.14 (0.74 to 9.53) mm (p=0.022), β=4.40 (0.08 to 8.72) (p=0.049) mm and β=4.56 (-0.17 to 9.29) mm (p=0.059) at 48, 54 and 60 months respectively.

**Conclusions:** In the offspring of non-obese mothers, gestational fasting glucose concentration is negatively associated with neonatal right amygdala FA and positively associated with childhood adiposity. Neonatal right amygdala FA may be a potential mediator between maternal glycemia and childhood adiposity.

Key Messages

What is already known about this subject?

* Maternal hyperglycemia increases risk of offspring adiposity later in life

What are the new findings?

* Maternal fasting glucose was negatively associated with the offspring’s fractional anisotropy (FA) of the right amygdala, a brain region that regulates appetite and feeding behavior.
* Right amygdala FA was negatively associated with sum of skinfold thickness in the offspring at age 48, 54 and 60 months
* Mediation analyses suggest neonatal right amygdala FA may be a potential mediator between maternal glycemia and childhood adiposity.

How might these results change the focus of research or clinical practice?

* This is a novel pathway by which maternal hyperglycemia can potentially contribute to subsequent risk of offspring adiposity.

**INTRODUCTION**

Obesity and gestational diabetes pose major public health challenges as their prevalence increase rapidly worldwide.(1; 2) Maternal hyperglycemia not only results in excessive fetal growth (3) but can also predispose the offspring to risk for adiposity later in life.(4-7) Indeed, our study group has previously shown that antenatal fasting glucose was significantly associated with offspring adiposity (8) amongst non-obese women, which was consistent with previous findings. (9) Metabolic imprinting and epigenetic modification have been suggested to contribute to childhood obesity. (10; 11) Some studies have used magnetic resonance imaging (MRI) in neonates and infants to study offspring adiposity. One study observed that volume of brain region like insula, to be inversely related to percentage body fat in babies from birth to six months postpartum (12) while another reported negative association between maternal adiposity (a risk factor of offspring adiposity) and global as well regional fractional anisotropy in neonates.(13) However, little research has examined if the brain regions which regulate appetite and feeding behavior, may play a role in offspring adiposity associated with maternal glycemia.

Food intake is regulated by hormones (14) and neural signals, with most studies focusing on the hypothalamus.(15; 16) Animal studies showed that amygdala lesions can affect appetite, food intake (i.e. hyperphagia) and weight gain (17-20)Many human studies support that the amygdala may be an important site for food intake regulation (21) and may be linked to obesity.(22-24) Children who exhibited greater brain response in anticipation of food relative to money, within the appetite related regions, such as amygdala, also ended up having more food intake.(25) Graham et al. demonstrated that greater neonatal right amygdala volume and connectivity were associated with lower impulse control for a snack delay task at two years of age.(26) Indeed, the amygdala volume has been previously linked to preference for fat intake in young adults (27) as well as body mass index (BMI) in young adults (28) and children. (23; 24) Functional magnetic resonance imaging (fMRI) of obese children demonstrated hyper-responsiveness to food rewards in their amygdala compared to normal weight children.(29) Schur et al. showed that obese children (9 to 11 years old) with greater reduction in brain activity in the appetite-processing brain regions (including the amygdala) when shown visual cues of high calorie foods, had greater BMI z-scores reduction.(30) Others have observed increased amygdala connectivity with the ventromedial prefrontal cortex was observed in lean individuals compared to obese adult participants.(31) The amygdala is susceptible to maternal environment during early development.(32-35)

However, to date most studies have emphasized on peripheral metabolism, with little focus on the possibility that *in utero* maternal effects may be associated with neural mechanisms that regulate energy imbalances associated adiposity. Maternal hyperglycemia has been shown to have long term effects in the offspring neurodevelopment,(36; 37) with a lot of focus on memory function in infants (38-40) due to the pathophysiology of maternal hyperglycemia (fetal hyperglycemia, fetal hypoxemia and iron deficiency).(41) Gestational diabetes has been linked to increased fetal hypothalamic activation towards glucose (42) and slower fetal postprandial brain responses.(43) Maternal insulin sensitivity, in the absence of gestational diabetes, was also associated with slower fetal brain responses.(44) Animal studies showed that regions of the brain, such as the striatum and hippocampus, are vulnerable to prenatal iron deficiency.(45; 46) However, to the best of our knowledge, little is known about maternal glycemia and how it affects neonatal amygdala. In this study, we examined if variations in the neonatal amygdala, in terms of its volume and fractional anisotropy (FA), mediate the association between maternal glycemia and offspring adiposity. The amygdala volume is a measurement of amygdala size while FA is used to characterize the microstructure organization of the amygdala.(47) We will focus on offspring of non-obese women, as we previously showed that maternal fasting glucose and offspring adiposity were significantly associated in these women.(8) It has been suggested that the effect of maternal glycemia on offspring adiposity is pronounced in non-obese women as maternal obesity shares common biological pathways with hyperglycemia and presence of maternal obesity may attenuate the association between maternal glycemia and offspring adiposity.(48; 49) We hypothesize that higher antenatal fasting blood glucose levels may be associated with decreasein volume and FA of the neonatal amygdala, which mediate the positive association between maternal glycemia and offspring adiposity in non-obese mothers. We did not hypothesize that there will be any association between 2h post OGTT glucose levels and offspring adiposity measures, in view of earlier null findings in the first three years of life, by our study group.(8) This study benefited from the unique opportunity to assess neonatal brain structure at birth and a prospective, longitudinal follow up of growth.

**RESEACH DESIGN AND METHODS**

**Participants**

Pregnant women were recruited in their first trimester from the KK Women's and Children's Hospital and National University Hospital in Singapore between June 2009 and September 2010 to participate in the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort study.(50) The children were born either at KK Women's and Children's Hospital or National University Hospital between November 2009 and May 2011.

Recruitment of neonates for MRI was previously described.(51) Neonatal brain MRI was done between 5 to 17 days after birth in 189 singleton, naturally conceived neonates (52) who all went through the T2-weighted MRI scans. A subset of 124 neonates had the diffusor tensor imaging (DTI) scans. Participants with poor image quality were excluded due to large head motion that caused misalignment across slices in T2-weighed MRI and signal loss in DTI (T2-weighted scans: n=7; DTI: n=2). Twenty-one neonates were excluded because they were part of multiple birth and/or had gestational age <37 weeks, birth weight <2500 g, a 5-minute Apgar score <9, born to mothers with pregnancy complications other than gestational diabetes (e.g., pre-eclampsia, intra-uterine growth retardation, Type 2 diabetes). Eight were excluded because they did not have antenatal maternal blood glucose data and another 30 neonates were excluded as their mothers had first trimester BMI of ≥30kg/m2. A total of 123 mother-child dyads (T2-weighted: n=123; DTI: n=89) were included in this analysis. All this information is summarized in Supplementary Figure 1.

This study was approved by the Institutional Review Board of the Singapore National Health care Group (B/2014/00411) and the Centralized Institutional Review Board of SingHealth (2009/785/A). All the women gave written consent for themselves and their children before their participation.

**Oral glucose tolerance test**

At 26-28 weeks gestation, all the women who came for the clinic visit were offered the 75g oral glucose tolerance test (OGTT), after overnight fasting. Blood glucose levels were measured at fasting and 2h post glucose test. The 1999 World Health Organisation (WHO) diagnostic criteria (53) were used to diagnose gestational diabetes mellitus (GDM): ≥ 7.0 mmol/L for fasting glucose and/or ≥7.8 mmol/L for 2-hour post-glucose. Amongst women diagnosed with GDM were treated as per standard hospital protocol.

**MRI acquisition and analysis**

MRI acquisition in the neonates was previously detailed in Qiu et al.(52) In brief, neonates underwent fast spin-echo T2- weighted MRI and single-shot echo-plan DTI scans using a 1.5-Tesla GE scanner (GE Healthcare) at the KK Women's and Children's Hospital, between 5 to 17 days after birth. The neonates were scanned while they were asleep and immobilized using an immobilization bag, without any sedation. The imaging protocols were i) fast spin-echo T2-weighted MRI (TR=3500 ms; TE=110 ms; FOV=256mm x 256mm; matrix size=256 x 256; 50 axial slices with 2.0 mm thickness); ii) single-shot echo-planar DTI (TR=7000 ms; TE=56 ms; flip angle = 90°, FOV=200mm x 200 mm; matrix size=200 x 200; 40 to 50 axial slices with 3.0 mm thickness; 19 diffusion weighted images (DWIs) with b=600 sec/mm2; 1 baseline image with b=0 sec/mm2). For T2-weighted MRI, 50 axial slices with 2.0 mm thickness were acquired parallel to the anterior-posterior commissure line. Two T2-weighted images were acquired per subject. For DTI, 40 to 50 axial slices with 3.0mm thickness were acquired parallel to the anterior–posterior commissure line. 19 diffusion weighted images (DWIs) with b=600 sec/mm2 and 1 baseline with b=0 sec/mm2 were obtained.

The delineation of the amygdala was done automatically using Markov random field (MRF).(54) We also randomly selected 20 T2-weighted MRI datasets for manual delineation and the intra-class correlation was 0.77. The leave-one-out validation approach was also used to confirm the MRF accuracy of the amygdala segmentation and the accuracy was found to be 0.75. The volume of the amygdala was calculated by multiplying the number of voxels in the structural mask and the image resolution.

The DTI analysis was described in detail previously.(35) Briefly, geometric distortion of the DTI due to B0-susceptibility differences across the brain was corrected as per Huang et al.(55) The diffusion tensor was derived by multivariate least-square fitting. FA was computed based on the three eigenvalues of the tensor. Mean FA was calculated within the amygdala mask for individual participants when the amygdala mask in the T2-weighted images was applied.

**Child anthropometric measurements**

All child anthropometric measurements were obtained as per previously described.(56) The child’s weight and height were measured at ages 18, 24, 36, 48, 54 and 60 months (Supplementary Fig 1). Sex and age specific BMI z-scores were derived using weight measurements and either length at 18 and 24 months or height measurements from 36 months onwards, with the WHO Anthro macro version 3.2.2.(57) Skinfold thicknesses (triceps and subscapular) were measured at birth and at 18, 24, 36, 48, 54 and 60 months using Holtain skinfold calipers (Holtain Ltd, Crymych, UK). The sum of skinfold is the summation of triceps and subscapular skinfold thickness.

**Additional data**

Demographic data such as ethnicity, maternal age and education as well as breastfeeding duration information were collected with interviewer administered questionnaires. At 26-28 weeks’ gestation, participants came for a clinic visit where their height and weight were measured and body mass index (BMI) derived. They were also given a self-administered Edinburgh Postnatal Depression Scale (EPDS) questionnaire to fill in to assess their mood. Birth outcomes (e.g, birth weight, sex of child, Apgar score) were recorded by midwives at delivery. BMI at the first trimester and GDM treatment were obtained from the medical records.

**Statistical analysis**

Independent t-test and chi-square test were used to compare the neonatal and maternal characteristics of participants. Linear regression models were run to assess the associations between (1) maternal blood glucose levels (predictor: fasting or 2h post OGTT) and child anthropometric measurements (outcome: BMI z-scores or sum of skinfold thickness), (2) maternal blood glucose level(s) [predictor: blood glucose level(s) significantly associated with child anthropometric measurement in (1)] and amygdala measurements (outcome: volume or fractional anisotropy), amygdala measurements (predictor: amygdala measurement significantly associated with maternal blood glucose levels in (2)] and child anthropometric measurements (outcome: BMI z-scores or sum of skinfold thickness). All regression models were adjusted for ethnicity, maternal age, maternal education, EPDS score and maternal BMI at 26-28 weeks’ gestation. Household income is not included in the model, due to the high correlation with maternal education. Our group has shown previously (58) that maternal glycemia was positively associated with offspring birth weight. At the same time, other studies have shown that birth weight is an important marker associated with structural differences throughout the brain(59), as well as childhood adiposity and obesity (60). Thus, birth weight may potentially lie on the causal pathway between maternal glycemia and child adiposity, as well as between maternal glycemia and neonatal amygdala. Conditioning on birth weight in these analyses could potentially introduce collider-stratification bias (61) hence birth weight is not included in the model. For all models involving child anthropometric measurements, only one anthropometric measurement (BMI z-scores or sum of skinfold thickness) was included each time. Likewise, for all models involving maternal blood glucose level, only one blood glucose concentration (fasting or 2h post OGTT) was included each time. For models with skinfold thickness as outcomes, sex of child and age at anthropometric measurement were added as additional covariates. This was not done for models with BMI z-scores as outcomes since the z-scores were already derived based on sex and age at measurement. For models involving amygdala measurements, post-conceptual age at MRI visit (gestational age + age at MRI visit) and total brain volume were included as additional covariates. These covariates were chosen because they are known to affect maternal metabolism, amygdala microstructure and/or offspring adiposity. For example, we adjusted for antenatal EPDS as it was previously found by our group to affect the amygdala microstructure.(35) Before testing for mediation, potential interactions between fasting blood glucose and amygdala measurements on offspring adiposity were checked using the general linear model. Mediation effect by the amygdala was estimated by regression-based mediation (62) using the PARAMED macro (63) in STATA (StataCorp LP) which uses a counterfactual-based approach to mediation. Data were missing on maternal education in 0.8% (n=1), antenatal EPDS score in 5.7% (n=7) and 26 weeks BMI in 1.6% (n=2) of the participants included in this analysis. Listwise deletion was used to handle missing data. Missing variables were found to be missing completely at random by Little’s MCAR test. In view of our relatively small sample size, no multiple comparison correction was applied for all analyses to minimize type II error,(64) to avoid missing out potential important findings.

Sensitivity analyses were done with additional adjustment for GDM treatment and breastfeeding duration, on top of the covariates listed above. The results were similar and therefore not presented. All analyses were carried out by using SPSS version 24.0 (IBM) unless otherwise stated.

**Data and Resource Availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Role of funding source**

The sponsor had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**RESULTS**

**Participant characteristics**

One hundred and twenty-three mother-child dyads were included in this analysis; these participants were comparable with those who did no undergo MRI, in terms of sex distribution of offspring and fasting blood glucose levels (Table 1). However, neonates included in this study had

**Table 1. Maternal and child characteristics of participants**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Participants Included (n=123)** | **Participants Excluded (n=972)** | **P-value** |
| **Infant variables** |  |  |  |
| **Gestational age (weeks)** | 39.0 ± 1.0 | 38.7 ± 1.6 | 0.003 |
| **Sex of child (Male), n (%)** | 68 (55.3) | 504 (51.9) | 0.473 |
| **Birth weight (g)** | 3149 ± 367 | 3071 ± 476 | 0.035 |
| **Ethnicity, n (%)** |  |  |  |
| Chinese | 60 (48.8) | 538 (55.3) | 0.001 |
| Malay | 49 (39.8) | 242 (24.9) |  |
| Indian | 14 (11.4) | 192 (19.8) |  |
| **Maternal variables** |  |  |  |
| **Maternal Education, n (%)** |  |  |  |
| Primary /No education | 4 (3.3) | 41 (4.2) | 0.014 |
| Secondary | 45 (36.6) | 251 (25.8) |  |
| Diploma / Technical education | 50 (40.7) | 336 (34.6) |  |
| University and above | 23 (18.7) | 331 (34.0) |  |
| Missing data | 1 (0.8) | 15 (1.5) |  |
| **Maternal age (years)** | 29.4 ± 5.5 | 30.6 ± 5.1 | 0.013 |
| **Antenatal EPDS score** | 8.46 ± 4.27 | 7.36 ± 4.54 | 0.013 |
| **Maternal First Trimester BMI (kg/m2)** | 22.2 ± 3.5 | 23.8 ± 4.9 | <0.001 |
| **Maternal 26-28 weeks BMI (kg/m2)** | 25.0 ± 3.4 | 26.4 ± 4.6 | <0.001 |
| **Antenatal fasting glucose (mmol/L)** | 4.34 ± 0.39 | 4.35 ± 0.46 | 0.947 |
| **Antenatal 2h glucose (mmol/L)** | 6.09 ± 1.43 | 6.57 ± 1.45 | 0.001 |
| **Diagnosed with GDM, n (%)** | 14 (11.4) | 185 (19.0) | 0.033 |

Data presented as mean ± standard deviation. EPDS-Edinburgh Postnatal Depression Scale; BMI-Body mass index; GDM- Gestational Diabetes Mellitus.

greater gestational age, higher birth weight and more likely to be of Malay ethnicity. The mothers were younger, less likely to have university or higher education and had higher EPDS scores. They also had lower first trimester and late second trimester BMI, lower 2h post OGTT blood glucose levels and less likely to be diagnosed with GDM. Of the 123 women included in this analysis, 14 were diagnosed with GDM, of which 3 (21.4%) did not undergo any treatment, 10 (71.4%) had dietary counselling and one (7.1%) received insulin treatment.

**Maternal blood glucose levels and childhood adiposity**

We observed that maternal antenatal fasting glucose was positively associated with the offspring’s sum of skinfold thickness at age 48 [β=3.12 (95% CI 0.18 to 6.06) mm]and 60 months [β=4.14 (0.46 to 7.82) mm](Fig 1A) and BMI z-scores at 48 [β=0.94 (0.27 to 1.61)], 54 [β=0.74 (0.12 to 1.36)]and 60 months [β=0.74 (0.08 to 1.39)] (Fig 1B) in offspring of non-obese mothers. A similar positive trend was observed with sum of skinfold thickness at 54 months [β=2.85 (-0.04 to 5.74) mm]. No obvious trends were observed between fasting glucose and childhood adiposity measurements in the earlier time points between 18 to 36 months of age. No significant associations were observed between maternal 2h post OGTT glucose levels and offspring adiposity measures (Supplementary Table 1).

**Maternal fasting glucose levels and neonatal amygdala microstructure**

The mean and standard deviations of the amygdala volume and FA of the left and right hemispheres are 214 ± 32 mm3, 0.15 ± 0.02; 187 ± 32 mm3, 0.16 ± 0.03 respectively. Higher maternal fasting glucose level was associated with a significantly lower FA of the right amygdala in the offspring (Table 2) of non-obese women. A similar trend was observed in the left amygdala FA but did not reach statistical significance (Table 2). Maternal fasting glucose was not significantly associated with the amygdala volume (Table 2).

**Table 2. Associations of maternal antenatal fasting blood glucose concentrations with volume and fractional anisotropy of the neonatal amygdala in offspring born to non-obese women**

|  |  |  |  |
| --- | --- | --- | --- |
| **All participants** | **N** | **Unadjusted β (95% CI)** | **Adjusted βa (95% CI)** |
| **Left Amygdala FA** | 89 | -0.007 (-0.016 to 0.003) | -0.005 (-0.018 to 0.007) |
| **Right Amygdala FA** | 89 | **-0.023 (-0.037 to -0.009)** | **-0.019 (-0.036 to -0.003)** |
| **Left Amygdala Volume (mm3)** | 123 | 9.68 (-4.93 to 24.29) | 5.48 (-12.69 to 23.64) |
| **Right Amygdala Volume (mm3)** | 123 | 3.59 (-11.24 to 18.42) | 3.36 (-14.96 to 21.70) |

Data presented as unstandardized β (95% confidence interval)

**a**Adjusted for post-conceptual age at MRI, ethnicity, sex of child, maternal age, maternal education, 26 weeks EPDS score, total brain volume and maternal BMI at 26 weeks

**Neonatal amygdala FA and childhood adiposity**

Figure 2 shows the association of the right amygdala FA and adiposity measures, including the sum of skinfold thickness (Fig 2A) and BMI z-scores (Fig 2B) up to 60 months. The neonatal right amygdala FA was negatively associated with sum of skinfold thickness at 48 [β= -56.95 (-98.43 to -15.47) mm], 54 [β= -46.18 (-88.57 to -3.78) mm] and 60 months [β= -53.69 (-105.74 to -1.64) mm] (Fig 2A). For BMI z-scores, although there was no significant association, the same negative pattern was observed (Fig 2B).

**Mediation by the right amygdala FA on maternal fasting glucose linked childhood adiposity**

Significant interaction was observed between maternal fasting glucose and the right amygdala FA on sum of skinfolds (48 months: p=0.005; 54 months: p<0.001; 60 months: p=0.027). This exposure-mediator interaction was taken into consideration in the regression-based mediation. The effect sizes mediated by the right amygdala FA between fasting glucose and sum of skinfolds were estimated at β=5.14 (0.74 to 9.53) mm (p=0.022), β=4.40 (0.08 to 8.72) mm (p=0.049) and β=4.56 (-0.17 to 9.29) mm (p=0.059) at 48, 54 and 60 months respectively (Table 3). In other words, the effects of 1 mmol/L change in maternal fasting glucose on offspring skinfolds that are attributed to variations in neonatal amygdala FA are 5.14mm, 4.40mm and 4.56mm at ages 48, 54 and 60 months respectively.

**Table 3. Estimated effect mediated by neonatal right amygdala fractional anisotropy in the association between antenatal fasting blood glucose and offspring adiposity.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Age (months)** | **Sum of skinfolds** | | | **BMI z-scores** | | |
| **β (95% CI)** | **p-**  **value** | **Proportion of total effect\*** | **β (95% CI)** | **p-value** | **Proportion of total effect\*** |
| 48 | **5.14 (0.74 to 9.53)** | **0.022** | 0.65 | 0.39 (-0.17 to 0.95) | 0.171 | 0.42 |
| 54 | **4.40 (0.08 to 8.72)** | **0.049** | 0.68 | 0.47 (-0.09 to 0.57) | 0.097 | 0.57 |
| 60 | 4.56 (-0.17 to 9.29) | 0.059 | 0.52 | 0.46 (-0.12 to 1.03) | 0.123 | 0.52 |

Data presented as unstandardized β (95% confidence interval)

\*Proportion of effect mediated by neonatal right amygdala FA compared to total effects of antenatal fasting glucose on offspring adiposity.

The proportions of total effect contributed by the mediator were estimated to be 0.65, 0.68 and 0.52 at 48, 54 and 60 months respectively (Table 3). No significant mediation by the right amygdala FA was found between fasting glucose and BMI z-scores score up to 60 months of age, although a marginal trend was observed at 54 months with β=0.47 (-0.09 to 1.03) (p=0.097) (Table 3).

**DISCUSSION**

We found that higher maternal antenatal fasting glucose concentrations were associated with increased sum of skinfolds in the offspring of non-obese Asian women, at 48 and 60 months as well as higher offspring BMI z-scores at 48, 54 and 60 months. Increased maternal antenatal fasting glucose concentrations were also correlated with lower FA in the right amygdala. The right amygdala FA was, in turn, negatively associated with offspring skinfold thicknesses from 48 months onwards. Mediation analyses suggest that right amygdala FA may be a potential mediator in the pathway between maternal antenatal fasting glucose and offspring adiposity as measured by the sum of skinfold thicknesses.

The amygdala is linked to food intake and obesity.(21; 22; 65) The human amygdala is known to respond to food cues (66; 67) and amygdala responses have been associated with subsequent consumption of high-fat food.(68) Amygdala volume has also been associated with fat intake (27) in adolescents and body mass index in young adults(28) and children (23; 24) while increased amygdala connectivity with ventromedial prefrontal cortex has been observed in lean individuals compared to obese adult participants.(31) Greater neonatal right amygdala volume and connectivity have also been associated with lower impulse control for a snack delay task at two years of age.(26) Functional magnetic resonance imaging (fMRI) of obese children showed hyper-responsiveness to food rewards in their amygdala compared to normal weight children.(29) Lower fractional anisotropy have been previously reported in many brain regions of obese individuals (elderly, adults and children), (69; 70) including brain regions involved in appetite, inhibitory control and reward such as amygdala. (71) While a couple of studies have observed positive association between both right and left amygdala volume and BMI or obesity,(24; 72) we found correlation only with the right side of the amygdala, similar to Orsi et al. who reported correlation between the right amygdala volume and BMI.(28) Another study also showed right amygdala volume and connectivity to be linked to poorer impulse control for a food task in two year old toddlers.(26) Although we do not have a full explanation of the lateralization differences, differences in hemispheric processing between left and right amygdala have been previously suggested in human (73) and animals.(74) Van der Laan et al.(75) found that hunger modifies the activation of the right amygdala towards images of food.

Overall, while we observed similar trend with both measurements of adiposity, we had more consistent findings with sum of skinfolds which is a surrogate for total adiposity. BMI has been regarded as a more crude measurement of adiposity as it can be influenced by fat free mass, especially in children.(76) It is noteworthy that previous studies showed associations between the amygdala and BMI in adolescents or older adults.(27; 28; 31)

Our group (8) and others (7; 77) previously reported higher antenatal fasting glucose to be associated with greater adiposity in children, particularly those born to non-obese mothers. In fact, our group has also examined longitudinal effect of antenatal fasting glucose on weight and BMI trajectory in the offspring and shown that higher antenatal fasting glucose was positively associated with weight and BMI trajectory in the first 36 months amongst offspring of non-obese mothers.(8) We did not observe significant association between 2h post OGTT glucose and offspring adiposity, possibly because of the large variation in 2h OGTT glucose, hence likelihood of larger errors. Moreover 2h OGTT blood glucose may be attenuated through exaggerated glucose steal due to fetal hyperinsulinemia,(78) a condition in the offspring of mother with hyperglycemia, which is also a driver of fetal fat accretion. As a result, some mothers may have “normal” glucose tolerance even though their offspring may exhibit diabetic fetopathy.(78) Maternal obesity and hyperglycemia share common biological pathways such as state of inflammation, impairment in regulation of energy and excess of fuel substrates.(48; 49) Hence maternal obesity may reduce the independent association observed between maternal glycemia and offspring adiposity. Our results also showed that significant differences in maternal glycemia associated offspring become apparent from 48 months of age onwards. This is consistent with earlier reports of null findings at 2 years of age (79) and a study of women with overt diabetes during pregnancy, where the maternal hyperglycemia related obesity resolved within first one to two years of life.(80) Many studies showed that the association between maternal diabetes and offspring obesity recur at a later part of childhood.(5; 81-83) This is not surprising if food intake and response to food cues are involved. During infancy and toddlerhood, food choices and portion size are largely determined by parents and caregivers while older children have more autonomy in food choices and portion sizes when they start attending preschool.

The prospective nature of our study is a strength as we examined the association of *in utero* glucose exposure on microstructure of the neonatal amygdala and subsequent adiposity in toddlerhood and early childhood. We are also, to the best of our knowledge, amongst the first to study the mediating role of differences in the amygdala, in the pathway between maternal glycemia and offspring adiposity. Another strength of our study is the large number of covariates considered, including maternal mental well-being. A limitation of our study is the small number of participants, which limited our statistical power. Replication of this study with a larger sample size is necessary. We also recognize that the blood glucose levels were measured once during pregnancy and may not be representative of the *in utero* exposure throughout pregnancy, especially post GDM diagnosis and treatment. Insulin levels were also not measured during the OGTT, as such we were unable to study maternal insulin sensitivity in relation to neonatal amygdala or child adiposity. We acknowledge that a large number of outcomes were investigated and we did not adjust for multiple comparisons, hence we cannot rule out the possibility of chance findings. However, the findings were in the same direction for both BMI z-scores and sum of skinfolds and consistently at similar time points. We acknowledge that there could be potential selection bias in the participants who agreed to do the MRI and were included in this study. For example, they were more likely to be older, have lower education, more depressed and of lower BMI. However, these factors were adjusted, where appropriate, in our analysis.

**CONCLUSIONS**

Our study shows that maternal blood glucose level during pregnancy associates with early childhood adiposity and this may be mediated by differences in microstructure of the amygdala, a brain structure implicated in feeding behaviors. These findings provide a novel pathway by which antenatal maternal blood glucose might influence the later risk for adiposity in the offspring.

**Acknowledgement**

We thank the staff and participants of the GUSTO study as well as members of the GUSTO study group. The GUSTO study group includes Allan Sheppard, Amutha Chinnadurai, Anne Eng Neo Goh, Anne Rifkin-Graboi, Arijit Biswas, Bee Wah Lee, Birit F.P. Broekman, Boon Long Quah, Borys Shuter, Chai Kiat Chng, Cheryl Ngo, Choon Looi Bong, Christiani Jeyakumar Henry, Cornelia Yin Ing Chee, Yam Thiam Daniel Goh, Doris Fok, Fabian Yap, George Seow Heong Yeo, Helen Chen, Hugo P S van Bever, Iliana Magiati, Inez Bik Yun Wong, Ivy Yee-Man Lau, Jeevesh Kapur, Jenny L. Richmond, Jerry Kok Yen Chan, Joanna D. Holbrook, Joshua J. Gooley, Keith M. Godfrey, Kenneth Kwek, Krishnamoorthy Niduvaje, Leher Singh, Lin Lin Su, Lourdes Mary Daniel, Mark Hanson, Mary Foong-Fong Chong, Mary Rauff, Mei Chien Chua, Mya Thway Tint, Neerja Karnani, Ngee Lek, Oon Hoe Teoh, P. C. Wong, Pratibha Agarwal, Rob M. van Dam, Salome A. Rebello, Seang-Mei Saw, Shang Chee Chong, Shu-E Soh, Sok Bee Lim, Chin-Ying Stephen Hsu, Victor Samuel Rajadurai, Walter Stunkel, Wee Meng Han, Wei Wei Pang, Yin Bun Cheung and Yiong Huak Chan.

Contributorship

SC and AQ conceptualize the idea, analyzed the data and wrote the manuscript. All other authors were involved in supervision of data collection, derivation/cleaning of the data and/or design of the cohort study. SC did statistical analysis for the paper and takes responsibility for the accuracy of the analysis All authors critically reviewed the manuscript. AQ had full access to all of the data in the study and takes responsibility for the integrity of the data. AQ is the guarantor of this article.

Funding

This research is supported by the Singapore National Research Foundation under its Translational and Clinical Research (TCR) Flagship Programme and administered by the Singapore Ministry of Health’s National Medical Research Council (NMRC), Singapore - NMRC/TCR/004-NUS/2008; NMRC/TCR/012-NUHS/2014. Additional funding is provided by the Singapore Institute for Clinical Sciences, Agency for Science Technology and Research (A\*STAR), Singapore. The funding organizations had no role in the design and conduct of the study; collection, management, analysis and interpretation of the data; preparation, review or approval of the manuscript and the decision to submit the manuscript for publication.

Competing Interests

PDG, KMG, and YSC have received lecture fees from companies that sell nutritional products. They are part of an academic consortium that has received research funding from Abbott Nutrition, Nestec, and Danone. KMG is supported by the UK Medical Research Council (MC\_UU\_12011/4), the National Institute for Health Research (NIHR Senior Investigator (NF-SI-0515-10042) and the NIHR Southampton Biomedical Research Centre) and the European Union (Erasmus+ Programme Early Nutrition eAcademy Southeast Asia-573651-EPP-1-2016-1-DE-EPPKA2-CBHE-JP). The other authors have nothing to disclose.

Ethics approval

This study was approved by the Institutional Review Board of the Singapore National Health care Group (B/2014/00411) and the Centralized Institutional Review Board of SingHealth (2009/785/A).

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**Figure 1.** Adjusted regression coefficient (with 95% confidence interval) of maternal antenatal fasting blood glucose levels (per 1 mmol/L) on child adiposity measures in the first 60 months of life for offspring of non-obese mothers - (A) sum of skinfolds (triceps and subscapular) (mm) and (B) body mass index z-scores. a p<0.05, b p<0.10

**Figure 2.** Adjusted regression coefficient (with 95% confidence interval) of neonatal right amygdala fractional anisotropy on child adiposity measures in the first 60 months of life for offspring of non-obese mothers - (A) sum of skinfolds (triceps and subscapular) (mm) and (B) body mass index z-scores. a p<0.05, b p<0.10

**Supplementary Figure 1.** Flow chart describing participant inclusion. a Exclusion criteria include multiple birth, gestational age <37 weeks, birth weight <2500 g, a 5-minute Apgar score <9, born to mothers with pregnancy complications other than gestational diabetes (eg pre-eclampsia, intra-uterine growth retardation, Type 2 diabetes). b First trimester BMI >30kg/m2. MRI- magnetic resonance imaging; DTI-diffusor tensor imaging; FA- fractional anisotropy; SSF- sum of skinfolds; BMI-z - BMI Z-score

**Supplementary Figure 2.** Timeline of main measurements. Oral glucose tolerance test (OGTT) was done at 26-28 weeks gestation; neonatal magnetic resonance imaging (MRI) at 5-17 days postpartum; anthropometric measurements of the child at 18, 24, 36, 48, 54 and 60 months of age.

**Supplementary Figure 3**. Axial slices from three infants’ brains from our sample, visualized with T2-weighted magnetic resonance imaging (MRI). The blue contour indicates the amygdala.