**Prospective associations of maternal choline status with offspring body composition in the first five years of life in two large mother-offspring cohorts: the Southampton Women’s Survey cohort and the Growing Up in Singapore Towards healthy Outcomes cohort.**

Linde van Lee†, Sarah R Crozier†, Izzuddin M Aris, Mya T Tint, Suresh Anand Sadananthan, Navin Michael, Phaik Ling Quah, Sian M Robinson, Hazel M Inskip, Nicholas C Harvey, Mary Barker, Cyrus Cooper, Sendhil S Velan, Yung Seng Lee, Marielle V Fortier, Fabian Yap, Peter D Gluckman, Kok Hian Tan, Lynette P Shek, Yap-Seng Chong, Keith M Godfrey†\*, Mary FF Chong†\*

**1**Singapore Institute for Clinical Science, Agency for Science, Technology, and Research, Singapore. (LvL, IMA, MTT, SAS, NM, PLQ, SSV, YSL, MVF, PDG, LPS, YSC, MFFC)

2MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom. (SRC, SMR, HMI, NCH, MB, CC, KMG)

3Department of Obstetrics & Gynaecology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore. (IMA, MTT, YSC)

4Laboratory of Molecular Imaging, Singapore Bioimaging Consortium, Agency for Science Technology and Research, Singapore. (SSV)

5Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore. (SSV)

6Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore. (YSL, LPS)

7Khoo Teck Puat-National University Children’s Medical Institute, National University Health System, Singapore, Singapore. (YSL)

8Department of Diagnostic and Interventional Imaging, KK Women’s and Children’s Hospital, Singapore, Singapore. (MVF)

9Duke-NUS Medical School, Singapore, Nanyang Technological University, Singapore, Singapore. (FY)

10Department of Pediatrics, KK Women’s and Children’s Hospital, Singapore, Singapore. (FY)

11Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore. (FY)

12Liggings Institute, University of Auckland, New Zealand. (PDG)

13Department of Reproductive Medicine, KK Women’s and Children’s Hospital, Singapore, Singapore (KHT)

14NIHR Southampton Biomedical Research Centre, University of Southampton and Hospital Southampton NHS Foundation Trust, United Kingdom. . (SMR, HMI, NCH, MB, CC, KMG)

15Clinical Nutrition Research Centre, Agency for Science, Technology, and Research, Singapore. (MFFC)

16Saw Swee Hock School of Public Health, National University of Singapore, Singapore (MFFC)

†authors contributed equally to this work

**\*Corresponding authors:**

Dr. Mary FF Chong (mary\_chong@nus.edu.sg)

Saw Swee Hock School of Public Health, National University of Singapore

Tahir Foundation Building,

12 Science Drive 2, #09-01Q

Singapore 117549

Tel: +65 6516 4969

Fax: +65 6779 1489

Professor Keith Godfrey (kmg@mrc.soton.ac.uk)

MRC Lifecourse Epidemiology Unit

(University of Southampton)

Southampton General Hospital, Mail point 95

Southampton SO16 6YD

Tel: +44 (0)23 80777624

Fax: +44 (0)23 80704021

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**Running title:** Maternal choline status on offspring growth

**Abbreviations:**

SWS: Southampton Women’s Survey

GUSTO: Growing Up Towards healthy Outcomes

BMI: Body Mass Index

CV: Coefficients of Variation

DXA: Dual energy X-ray Absorptiometry

MRI: Magentci Resonance Imaging

dSAT: deep subcutaneous abdominal adipose tissue

sSAT: superficial subcutaneous abdominal adipose tissue

IAT: Internal abdominal adipose tissue

TAAT: Total abdominal adipose tissue

TMA: Trimethylamine

**Clinical Trial Registration:**

The GUSTO cohort is registered under the clinical trials identifier NCT01174875

http://www.clinicaltrials.gov/ct2/show/NCT01174875?term=GUSTO&rank=2

**Abstract** (255 words)

**Background:** Choline status has been positively associated with weight and fat mass in animal and human studies. As evidence examining maternal circulating choline concentrations and offspring body composition in human infants/children is lacking, we investigated this in two cohorts.

**Methods:** Maternal choline concentrations were measured in the UK Southampton Women’s Survey (SWS; serum, n=985, 11 weeks’ gestation) and Singapore Growing Up Towards healthy Outcomes (GUSTO); n=955, 26-28 weeks’ gestation) mother-offspring cohorts. Offspring anthropometry was measured at birth and age up to 5 years. Body fat mass was determined using DXA at birth and age 4 years for SWS, and using air displacement plethysmography at birth and age 5 years for GUSTO. Linear regression analyses were performed adjusting for confounders.

**Results:** In SWS, higher maternal choline concentrations were associated with higher neonatal total body fat mass [β=0.60 SD/5 µmol/L maternal choline (95% CI 0.04-1.16)] and higher subscapular skinfold thickness [β=0.55 mm/5 µmol/L (0.12-1.00)] at birth. In GUSTO, higher maternal choline concentrations were associated with higher neonatal BMI-for-age z-score [β=0.31 SD/5 µmol/L (0.10-0.51)], and higher triceps [β=0.38 mm/5 µmol/L (0.11-0.65)] and subscapular skinfold thicknesses [β=0.26 mm/5 µmol/L (0.01-0.50)] at birth. No consistent trends were observed between maternal choline and offspring gain in BMI, skinfold thicknesses, abdominal circumference, weight, length/height, and adiposity measures in later infancy and early childhood.

**Conclusion:** Our study provides evidence that maternal circulating choline concentrations during pregnancy are positively associated with offspring BMI, skinfold thicknesses and adiposity at birth, but not with growth and adiposity through infancy and early childhood to ages 5 years.

**Keywords:** Choline, pregnancy, offspring, body composition, birth size

**Key messages:**

* As evidence examining maternal circulating choline concentrations and offspring body composition in human infants/children is lacking, we investigated this in two cohorts.
* Maternal circulating choline concentrations during pregnancy are positively associated with offspring BMI, skinfold thicknesses and adiposity at birth.
* Maternal circulating choline concentrations during pregnancy are not associated with offspring growth and adiposity at early childhood.

**Introduction**

Maternal nutritional status during pregnancy has an important influence on the intra-uterine environment, with implications for fetal development (1), postnatal growth (2) and the risk of childhood and adult obesity (3, 4). Prenatal and early postnatal life are considered critical periods as these developmental influences can have profound long-term consequences on risk of excess weight gain, by determining the biological set-points for appetite and body weight (5, 6). Understanding these early life environmental exposures may halt the growing global epidemic of obesity. Recently, maternal choline status during pregnancy has gained interest for its potential beneficial effect on offspring cognitive function, and neural tube closure (7, 8). However, the impact of choline on other aspects of offspring postnatal development such as growth has been little studied.

Choline is a semi-essential nutrient that is largely found in animal-based food such as eggs, beef, pork and liver (9, 10) and can also be synthesized endogenously (9, 11). Choline is involved in methylation processes and is the backbone for phosphatidylcholine, which is, in turn, essential for cell membrane formation, myelination of nerve axons, cell division and lipid transport (8, 12, 13). During pregnancy, the placenta transfers large amounts of choline to the fetus, and choline concentrations are 3-5 times higher in fetal cord blood as compared with maternal choline concentrations (8). The Institute of Medicine recommends an adequate intake of 450 mg/day of dietary choline during pregnancy (9). However, studies in USA and Canada reported that the majority (77 to 90%) of pregnant women did not adhere to this recommendation (14-16), which poses the question of whether choline supplementation is warranted (8, 17).

To date, few studies have investigated the effect of maternal choline status or maternal dietary choline intake on offspring birth size and growth measures. A small randomized controlled trial in 26 US women showed that a maternal dietary intake of 930 mg choline per day during the last trimester of pregnancy did not affect offspring birth size as compared with the lower dietary choline intake (480 mg/d) (18). Moreover, maternal choline status assessed between 30-34 weeks gestation (19) or just before delivery (20, 21) showed no association with offspring birth weight. In contrast, lower choline concentrations in umbilical cord blood have been observed in low birth weight (22), and in growth restricted neonates (23) as compared to controls. An experimental study in rats reported that maternal supplementation with choline, betaine, folic acid, and vitamin B12 during pregnancy and lactation lowered offspring fat mass content at postnatal day 3 and lowered offspring weight at day 21 and week 12 as compared to controls (24).

In summary, studies on the association between maternal choline concentrations and offspring size are limited, conflicting and predominantly confined to size at birth. To date, no study has included analyses of human offspring growth and adiposity measurements in the first years of life. Here, we examined these associations using two mother-offspring cohorts. We hypothesize that higher circulating choline concentrations during pregnancy are associated with greater gain in offspring BMI and adiposity as depicted by skinfold measurements, which were our a-priori primary endpoints.

**Methods**

*Study design and population*

We used data from the UK Southampton Women’s Survey (SWS) (25) and the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) (26) mother-offspring cohorts. The SWS cohort recruited 12 583 women aged 20-34 years from the general population between April 1998 and December 2002 through General Practices across Southampton, UK. Women who subsequently became pregnant were thereafter recruited for the pregnancy phase of the SWS. At 11 weeks’ gestation, participants underwent a venipuncture, and lifestyle assessments. The offspring were followed up at ages 6 months, 1, 2, 3 and 4 years and beyond. Medical ethical approval for the study was obtained from the Southampton and South West Hampshire Local Research Ethics Committee.

The GUSTO cohort recruited 1247 pregnant (<14 weeks of gestation) Chinese, Malay or Indian Singapore citizens or permanent residents from June 2009 to September 2010 from two major public hospitals in Singapore. Inclusion criteria were age 18-50 years, intention to live in Singapore for the following five years, intention to deliver in one of the two major maternity units in Singapore, willingness to donate cord, cord blood, and placenta, and the participants’ parents and spouse’s parents were of the same ethnic origin. Women with a serious health condition such as type 1 diabetes mellitus were excluded. At 26-28 weeks of gestation, participants underwent anthropometric measurements, a venipuncture and completed questionnaires on lifestyle and demographics. The offspring were followed up at ages 6 months, 1, 2, 3, 4, and 5 years for physical measurements of growth and development. The study protocol was approved by the National Healthcare Group Domain Specific Review Board and Sing Health Centralized Institutional Review Board. Written informed consent was obtained from all participants from both cohorts.

*Blood concentrations assessment*

A venipuncture was performed by trained staff following a standardized protocol and samples were stored at -80 ⁰C for SWS and -20 ⁰C for GUSTO before further analyses. For the SWS, measurements of serum choline were available for a limited number of blood samples obtained in the first trimester of pregnancy (11 weeks’ gestation), with samples being selected on the basis of completeness of childhood follow-up data and availability of resource for the assays. Serum choline concentrations were analyzed by automated colorimetric analyses using a Konelab20 analyzer at the NIHR Southampton Biomedical Research Centre in 2013; between-assay coefficients of variation (CV) were 3.7%. Late pregnancy serum folate and vitamin B12 concentrations were also measured by automated chemiluminescent immunoassay systems (Beckman Dxl 800); between-assay CV varied between 3.8 and 12.4%.

For GUSTO, mid-pregnancy (26-28 weeks gestation) plasma choline concentrations were analyzed in 2015 using HPLC (1100 series, Agilent Technologies, USA) and mass-spectrometry (API 3000, AB Sciex, USA), as described by Midttun *et al.*(27); the within- and between-day imprecision CVs was <8%. Plasma vitamin B12 and folate concentrations were assessed by competitive electrochemiluminescence immunoassay (ADVIA Centaur Immunoassay System, Siemens, Germany); between-assay CV varied between 4 and 9% for vitamin B12 and 6 and 12% for folate concentrations.

*Anthropometric measurements*

In both cohorts, offspring weight was measured to the nearest g using calibrated scales (SECA corp. Germany). Crown-to-heel length was measured using a neonatometer (SWS: CMS Ltd, UK, to the nearest 1 mm) or a mobile measuring mat (GUSTO: SECA model 210, SECA Corp. Germany, to the nearest 5 mm); standing height was measured with a stadiometer (SWS: Leicester height measure; CMS Ltd, UK; GUSTO: SECA model 213, SECA Corp. Germany). Abdominal circumference was measured with a non-flexible tape to the nearest 0.1 cm. Skinfold thickness of triceps and subscapular regions were recorded to the nearest 0.2 mm using Harpenden (Baty Int. UK) calipers for SWS or Holtain calipers (Holtain Ltd) for GUSTO.

Offspring total body fat (%) at birth was measured with dual energy x-ray absorptiometry (DXA; SWS: Lunar DPX-L instrument, GE Corp, USA) in a subsample of SWS infants (n=437) and with air displacement plethysmography (GUSTO: PEA POD® Life Measurement Inc. USA) in a subsample of GUSTO infants (n=290). During childhood a Hologic Discovery DXA instrument was used for SWS at age 4 years (Hologic Inc. USA) and BOD POD® for GUSTO at age 5 years (Life Measurement Inc. USA).

Another subsample of the GUSTO offspring underwent a magnetic resonance imaging (MRI) scan (1.5 Tesla MRI scanner, GE Healthcare, USA) within 14 days after birth (n=307) and additionally at age 4.5 years (n=120) to assess abdominal adipose tissue compartments from the diaphragm to the superior aspect of the sacrum. Superficial subcutaneous (sSAT) and internal abdominal adipose tissue (IAT) volumes were semi-automatically processed using Matlab software version 7.13 (the Mathworks Inc.) and manually optimized. Deep subcutaneous adipose tissue (dSAT), separated from sSAT by fascial plane, was manually defined and processed using Matlab software (28). At 4.5 years, abdominal adiposity (between liver dome and upper sacrum) of children at 4.5 years was assessed using the Siemens Skyra 3T MR scanner. Segmentation and quantification of the adipose tissue compartments was done by a fully automated graph theoretic segmentation algorithm (29). The boundary along the fascial plane was traced manually to separate the dSAT and sSAT depots. The final segmentation was edited to remove misclassified structures.

*Covariates*

Maternal lifestyle habits (e.g. smoking habits, physical activity) and characteristics (e.g. education level, ethnicity, age) were obtained by questionnaires. Pre-pregnancy weight was measured (SWS) or self-reported (GUSTO), and height was measured in both cohorts. Pre-pregnancy BMI was calculated as weight (kg) divided by squared height (m). Gestational diabetes (yes/no) was obtained from medical reports in SWS; in GUSTO it was assessed by an oral glucose tolerance test at 26-28 weeks gestation using the WHO definition (30). Information on infant’s characteristics including gender, date of delivery, and birth order was abstracted from obstetric records.

*Statistical analyses*

The pregnancy phase of the SWS cohort included 3158 women who delivered a live born singleton infant (**Figure 1**). In total, 1247 participants were recruited for the GUSTO study of which 55 participants dropped out mainly due to personal reasons; a further 15 participants were lost to follow-up and 95 participants who underwent in-vitro fertilization or were having twins were excluded. For this report, we excluded participants who did not have information on maternal choline blood concentrations (SWS n=2156; GUSTO n=112) or had early preterm babies (≤34 weeks gestation; SWS n=17; GUSTO n=15), resulting in two analytic samples of 985 SWS and 955 GUSTO mother-offspring dyads.

Offspring BMI at all time points was standardized to age and sex-adjusted z-scores using UK child growth standards (31) for SWS and using the WHO child growth standards (32) for GUSTO. Length/height and weight age- and sex-adjusted z-scores were based on the WHO-UK child growth standards (33) for SWS and based on the WHO child growth standards (32) for GUSTO. Abdominal circumferences were internally standardized for each cohort using the LMS method for SWS (34) and mean divided by SD for GUSTO.

Maternal and offspring characteristics are presented by quintiles of maternal circulating choline concentrations. The p-values for trend were calculated using the choline concentrations continuously.

Linear models were used to describe the association between the circulating choline concentrations exposure and offspring anthropometric measurements outcomes. At time points beyond birth, all measurements up to that time point were included as predictors in the regression model; the regression coefficient can therefore be interpreted as the conditional growth in the interval up to the time point of interest additional to that which would be expected from measurements prior to that time point. Adjusted models were additionally adjusted for maternal height (m), maternal age (y), maternal pre-pregnancy BMI (kg/m2), maternal educational level (SWS: primary/secondary, higher national diploma, degree) GUSTO: Primary/secondary, Postsecondary, University), gestational age (weeks), offspring sex, and maternal folate (nmol/L) and vitamin B12 (µmol/L) concentrations. Folate and vitamin B12 were included because they have been shown to influence choline concentrations (35, 36) and growth (37). For the GUSTO study, we additionally adjusted for ethnicity (Chinese, Malay, and Indian), which was not necessary for the SWS as it predominantly consisted of white Caucasians (97.5%). These analyses were repeated while using the quintiles of maternal choline concentrations as exposure in the statistical models to explore the linearity of the association. Additional adjustment for the potential confounders of smoking and alcohol use during pregnancy did not alter our findings and was therefore not included in the final statistical model. No adjustment was done for multiple testing because we considered our analyses exploratory, generating new hypotheses that need to be confirmed in future studies (38).

Missing values for covariates maternal height (SWS n=1, GUSTO n=9), pre-pregnancy BMI (SWS n=7, GUSTO n=72), educational level (SWS n=2, GUSTO n=16), folate (SWS n=176), and vitamin B12 concentrations (SWS n=158) were imputed using multiple imputation analyses by chained equations on 20 imputed datasets (39). These regression models additionally included maternal choline status, offspring anthropometric measurements and all covariates for better prediction. The results of the 20 datasets were pooled. All statistical analyses were performed using Stata software version 14.2 (StataCorp LP, Chicago, USA).

**Results**

Mean choline concentrations were 6.03 µmol/L (SD 0.86) for SWS and 9.2 µmol/L (SD 1.6) for GUSTO. Across both cohorts, higher concentrations of choline were seen in women who were older, had a higher pre-pregnancy BMI, and delivered infants with thicker subscapular and triceps skinfolds at birth in both cohorts (**Table 1**). In addition, SWS participants with higher choline concentrations were more likely to be multiparous and had infants with larger total body fat at birth as compared to women in the lowest quintile of choline concentrations, whereas GUSTO participants with higher choline concentrations were more likely to be shorter, of Indian ethnicity, to gain more weight during pregnancy, to have gestational diabetes mellitus, less often physically active and to have delivered infants with greater BMI at birth,

Higher maternal choline concentrations were associated with a higher offspring BMI z-score at birth for both cohorts [SWS: 0.32 SD (95% CI -0.02, 0.67) per 5 µmol/L choline, GUSTO: 0.30 SD (95% CI 0.08, 0.51) per 5 µmol/L choline]. After adjustment for all covariates, this association remained in GUSTO, but attenuated in SWS (**Table 2**). In fully adjusted models, higher maternal choline concentrations were associated with thicker neonatal subscapular skinfolds [SWS: 0.55 mm (95% CI 0.12, 1.00); GUSTO: 0.26 mm (95% CI 0.01, 0.50) and triceps skinfolds [GUSTO: 0.38 mm (95% 0.11, 0.65)]. In GUSTO, we also observed a association with abdominal circumference at birth [0.21 SD (95% CI 0.00, 0.43) per 5 µmol/L choline]. Higher maternal choline concentrations were also associated with higher offspring total body fat at birth when adjusting for covariates [SWS: 0.60 SD (95% CI 0.04, 1.16) per 5 µmol/L choline, **Table 3**] only in the SWS cohort. This association slightly attenuated after additionally including birth length in the statistical model [model 2: 0.49 SD (95% CI 0.00, 0.98) per 5 µmol/L choline].

In both cohorts, we observed positive associations between maternal choline status and offspring conditional growth in BMI z-scores between two and three years in the unadjusted model, which attenuated to a trend in GUSTO [0.17 SD (95% CI -0.02, 0.36) per 5 µmol/L choline, p=0.085], but disappeared in SWS [0.19 SD (95% CI -0.07, 0.45) per 5 µmol/L choline; **Table 2**]. In SWS, higher maternal choline status was only associated with larger conditional growth in abdominal circumference between birth and 6 months [0.44 SD (95% 0.07, 0.82) per 5 µmol/L choline. Maternal choline status was not associated with growth in length/height or weight z-scores in the first 5 years of life in both cohorts (**Supplemental table 1**). No associations were observed between maternal choline concentrations and abdominal adipose tissue compartments in the GUSTO cohort in the first 5 years of life (**Supplemental table 2**). The sensitivity analyses to explore linearity of the associations confirmed a linear dose-response relationship (data not shown).

**Discussion**

To our knowledge, this is the first study to investigate the associations between maternal choline concentration and offspring adiposity and growth in the first 5 years of life. We found maternal choline status to be associated with greater offspring BMI and adiposity at birth reflected by abdominal and skinfold measures, with replication across two cohorts. No consistent relations were found between maternal choline status and subsequent conditional growth measures after birth or with body composition, weight or length/height z-scores in the first five years of life.

As was expected, the mean maternal choline concentrations in early pregnancy observed in SWS were lower as compared to those from mid-pregnancy observed in GUSTO (6.0 µmol/L vs. 9.2 µmol/L, respectively). It has previously been reported that maternal choline concentrations increase over the course of pregnancy (40), likely caused by the increased *de novo* synthesis induced by higher estrogen levels during pregnancy (17, 36). Nevertheless, the choline concentrations presented here were comparable to previously reported concentrations varying between 7.2-8.4 µmol/L at 12-16 weeks gestation (41, 42) and 7.0-9.4 µmol/L at 24-29 weeks gestation (18, 40).

Offspring total body fat at birth as assessed by dual energy x-ray absorptiometry in SWS showed

positive associations with maternal choline concentrations, whereas no association was observed for offspring total body fat measurements in GUSTO assessed by air-displacement plethysmography. A possible explanation for these null findings within GUSTO might be selection bias; the subsample (n=290), as compared to the total analytical GUSTO sample minus subsample (n=655), comprised mothers who had higher plasma choline concentrations, were older, less well educated, had lower plasma folate status, were more likely to smoke, less often suffered from gestational diabetes and were less physically active during pregnancy (supplemental table 3). Nevertheless, the associations between maternal choline concentrations and BMI, abdominal circumference and skinfolds in the total GUSTO population, collectively suggest greater adiposity in the GUSTO infants, which is consistent with the DXA total body fat findings in the SWS cohort.

Only four previous studies investigated the association between maternal choline concentrations and birth weight, with none showing an association (18-21), similar to our findings. However, lower choline concentrations from umbilical cord blood collected in modest sample sizes showed associations with lower birth size (22, 23). An explanation for this discrepancy might be that maternal status may not accurately represent fetal status due to possible disturbed placental metabolism, maternal-placental transfer or uptake by the fetus.

In both cohorts, maternal choline concentrations did not show a consistent trend with growth and weight gain measurements in the first five years of life. Similarly, null findings have also been reported in the literature examining choline status in relation to body size in children and adults (43-46). No cross-sectional differences in phosphatidylcholine concentration have been reported between overweight and normal weight 5-year-old children (43). Also, choline supplementation in children with cystic fibrosis showed no effect on height-for-age, weight-for-age, BMI-for-age, fat mass or fat-free mass as compared to placebo (44). In adults, higher citicoline supplementation (500 mg), an intermediate metabolite in the synthesis of phosphatidylcholine from choline, did not alter weight gain when compared to 200 mg supplementation in a 6-week intervention (45). Also, no difference in free choline concentrations has been found among normal weight or overweight adult men (46). In contrast, two experimental animal studies found an effect of choline on growth. During the lactation period, rats were fed a choline-deficient diet and their offspring received less choline in breast milk. After three weeks, the offspring with lower choline intake showed lower body weight as compared to those whose mothers were fed a choline-sufficient diet (47, 48). Overall, maternal choline status seems to have only a marginal independent role in growth and weight gain in the first years of life, probably overruled by the many other factors influencing growth and weight gain such as complementary foods, breastfeeding, and overall health status.

*Potential Mechanisms*

The precise mechanisms by which choline may affect neonatal adiposity remains unclear. However, some plausible mechanisms have been reported (12). Choline is the backbone for phosphatidylcholines that are critical for cell membrane integrity, signaling and synthesis, and lipoprotein assembly and secretion by the liver (36).

Choline is, besides folate, a methyl donor in homocysteine metabolism. Homocysteine can, thereafter, be formed to cysteine that has consistently been associated with greater BMI and body fat mass in cellular, animal and epidemiological studies (49). Furthermore, the methyl donor capacity of choline has been linked to offspring altered epigenetic marks that control gene expression (50). It has been reported that maternal choline supplementation altered transcript levels of methionine and lipid metabolism genes in offspring livers (50).

Secondly, studies have linked gut microbiota and obesity in humans (51). Gut microbiota are able to convert dietary choline and phosphatidylcholine into trimethylamine formation (TMA) (50); thereafter TMA can be oxidized to trimethylamine N-oxine through the action of the flavin mono-oxygenase 3 enzyme. This enzyme has been linked with obesity in humans and to impact the beiging of white adipose tissue (52). The molecular mechanisms are not yet fully understood and more research is required to confirm the relationship between choline, TMA, flavin mono-oxygenase 3, and obesity.

Lastly, acetylcholine, a neurotransmitter that can be synthesized from choline, might activate M3 receptors in the brain. This receptor is involved in regulation of glucose uptake and lipolysis in adipose tissue (11, 53). Moreover, M3 knockout mice fed a high-fat diet showed increased rates of resting and total energy expenditure as compared to controls (54). These studies may indicate that higher choline status could play a role in the development of adiposity; however, more research is necessary to establish this hypothesis.

*Strengths and limitations*

Strengths of the present study include the use of circulating nutrient markers. Plasma and serum choline concentrations have shown to reflect dietary choline intake (18, 55), and additionally reflect bioavailability, uptake, and metabolic processes such as *de novo* synthesis. In addition, plasma concentrations of folate and vitamin B12 that might alter choline availability and independently influence growth and adiposity were available to evaluate possible confounding and interactions. Secondly, we had an extensive set of body composition measurements, including DXA scans. Whole-body DXA scans are considered accurate and precise measurements of total body fat mass in infants (56). Lastly, the longitudinal design of this study limits the likelihood of reverse causation.

The present study is, however, limited by the lack of fetal choline status measurements, as maternal plasma choline concentrations may not reflect the nutrient status of the fetus during pregnancy due to disturbed placental metabolism, maternal-placental transfer or uptake by the fetus. Moreover, as choline concentrations increase over the course of pregnancy(40), multiple circulating choline concentrations measurements would be preferred for more precise estimations. A further limitation is the discordance between early pregnancy measurements of maternal choline status in SWS and mid-late pregnancy measurement in GUSTO. Moreover, as prenatal supplements are unlikely to contain a choline source, dietary choline intake and metabolic conversions in the mother are the major determinant of choline status. No information on maternal dietary choline intake was available to determine adequate intakes or maternal metabolic activity relating to choline. Loss to follow up was considerable in both cohorts over the 4 and 5 years of anthropometric measurements. While most of the maternal and offspring baseline characteristics were comparable between those with complete follow-up and those loss to follow-up; some differences were observed. In the SWS cohort, mothers who had complete follow-up at 4 years tended to have more children, were less educated, less likely to be in employment, and were more physically active than mothers who dropped out. In the GUSTO cohort, the mothers who complete follow-up tended to be Chinese, had higher pregnancy folate status, and tended to already have a first child. This may have resulted in selection bias, which would have confounded the results. Furthermore, total body fat mass was measured in a subsample of the participants of the SWS (n=437) and GUSTO study (n=290). Notably, in the GUSTO study, selection bias may have limited our power to detect significant associations. Lastly, despite our efforts to adjust for multiple confounders, we cannot exclude the possibility of residual confounding by unmeasured or poorly measured covariates.

*Conclusions and future research*

In conclusion, we observed that higher maternal choline concentrations were associated with greater offspring adiposity at birth. Maternal choline concentrations did not show consistent associations with conditional gain in adiposity, length/height and weight in the first 5 years of life. The associations with neonatal adiposity were observed in two independent mother-offspring cohorts, suggesting that our findings were robust and exist in Caucasian and Asian study samples. Future studies should confirm our results and ideally include offspring choline concentrations measurements to further investigate the effect of choline on growth and body composition.

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LvL, SRC, KMG, and MFFC: designed the research, wrote the manuscript, and have primary responsibility for the final content; LvL and SRC: performed the statistical analyses; IMA, MTT, SAS, NM, PLQ, SSV, YSL and MVF: contributed to the data collection and analysis; HMI, NCH, MB, CC, KMG designed and led the SWS study; YSL, MVF, FY, PDG, KHT, LPCS and YSC: designed and led the GUSTO study; and all authors: interpreted the results and critically reviewed and approved the final manuscript.

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| **Table 1.** Study sample characteristics according to quintiles of maternal choline in the SWS cohort and the GUSTO study | | | | | | | |  |
|  | SWS (n=985) | | |  | GUSTO (n=955) | | |  |
|  | Choline | |  |  | Choline | |  |  |
|  | Q1: 3.98-5.31 µmol/L  N=198 | Q5: 6.69-10.4  N=195 | p trend‡ |  | Q1: 5.38-7.69 µmol/L  N=191 | Q5: 10.6-14.8 µmol/L  N=179 | p trend‡ |  |
| **Maternal characteristics** |  |  |  |  |  |  |  |  |
| Choline (µmol/L) | 5.0 (2.7) | 7.3 (6.0) | - |  | 7.10 (5.6) | 11.7 (13.5) | - |  |
| Age (y) | 30.4 (3.7) | 31.4 (3.6) | 0.001 |  | 29.6 (4.8) | 31.2 (5.5) | 0.006 |  |
| Height (cm) | 164.4 (6.4) | 163.3 (6.1) | 0.098 |  | 158.5 (5.7) | 157.4 (5.6) | 0.035 |  |
| Pre-pregnancy BMI (kg/m2) | 24.3 (3.6) | 26.4 (5.2) | <0.001 |  | 21.7 (4.0) | 23.6 (4.5) | <0.001 |  |
| Pregnancy weight gain (kg)\* | 11.6 (6.2) | 13.0 (7.5) | 0.094 |  | 7.8 (4.1) | 10.0 (4.8) | <0.001 |  |
| Ethnicity (%) | NA | NA |  |  |  |  | 0.007 |  |
| Chinese | - | - |  |  | 46.7 | 52.4 |  |  |
| Malay | - | - |  |  | 35.6 | 21.1 |  |  |
| Indian | - | - |  |  | 17.7 | 26.6 |  |  |
| Educational level (%) |  |  | 0.629 |  |  |  | 0.135 |  |
| Low | 9.4 | 13.7 |  |  | 35.6 | 32.6 |  |  |
| Intermediate | 60.9 | 64.0 |  |  | 32.6 | 42.5 |  |  |
| High | 29.7 | 22.3 |  |  | 31.9 | 24.8 |  |  |
| Gestational diabetes mellitus (%) | 2.5 | 4.5 | 0.122 |  | 17.6 | 25.2 | 0.035 |  |
| Plasma folate (nmol/L) | 17.4 (4.4) | 17.4 (4.5) | 0.357 |  | 31.0 (19.9-41.9) | 43.9 (29.7-47.5) | 0.064 |  |
| Plasma vitamin B12 (pg/mL) | 410.3 (232.3) | 410.2 (200.0) | 0.943 |  | 203 (166-256) | 220.2 (165-256) | 0.999 |  |
| Birth order - first child (%) | 56.9 | 43.1 | 0.018 |  | 61.6 | 58.1 | 0.193 |  |
| Physically active during pregnancy (%) | 26.8 | 16.4 | 0.576 |  | 28.9 | 25.4 | 0.029 |  |
| Alcohol intake during pregnancy (%) | 89.9 | 87.2 | 0.570 |  | 0.0 | 0.9 | 0.593 |  |
| Employed (%) | 58.2 | 49.5 | 0.080 |  | 86.9 | 79.5 | 0.484 |  |
| Smoking during pregnancy (%) | 21.2 | 16.0 | 0.772 |  | 21.1 | 9.9 | 0.083 |  |
| **Infant characteristics** |  |  |  |  |  |  |  |  |
| Sex – male (%) | 55.6 | 57.1 | 0.192 |  | 58.9 | 52.2 | 0.352 |  |
| Gestational age (weeks) | 39.8 (1.7) | 39.8 (1.4) | 0.893 |  | 38.6 (1.4) | 38.6 (1.2) | 0.821 |  |
| Birth weight (SD) | 0.1 (1.0) | 0.3 (1.1) | 0.080 |  | 0.0 (1.0) | 0.1 (1.1) | 0.126 |  |
| Birth length (SD) | -0.2 (0.9) | -0.1 (0.8) | 0.345 |  | 0.14 (1.0) | 0.0 (1.0) | 0.433 |  |
| Birth BMI (SD) | 0.5 (0.9) | 0.8 (1.0) | 0.059 |  | -0.5 (1.1) | -0.2 (1.0) | 0.007 |  |
| Birth total body fat† (kg) | -0.2 (1.1) | 0.3 (0.9) | 0.008 |  | 0.3 (0.1) | 0.3 (0.1) | 0.658 |  |
| Abdominal circumference (SD) | 0.0 (1.0) | 0.2 (1.0) | 0.077 |  | -0.2 (1.0) | 0.0 (0.9) | 0.088 |  |
| Subscapular skinfolds (mm) | 4.9 (1.0) | 5.2 (1.2) | 0.004 |  | 4.7 (1.1) | 5.1 (1.2) | 0.003 |  |
| Triceps skinfolds (mm) | 4.6 (0.9) | 4.8 (1.0) | 0.022 |  | 5.3 (1.2) | 5.7 (1.3) | 0.001 |  |
| Values are mean (SD), median (25th-75th percentile) or % | | | | | | | |  |
| Quintiles SWS: Q1 3.98-5.31 µmol/L; Q2 5.32-5.75 µmol/L; Q3 5.76-6.14 µmol/L; Q4 6.15-6.68 µmol/L; Q5 6.69-10.4 µmol/L  Quintiles GUSTO: Q1 5.38-7.69 µmol/L; Q2 7.7-8.57 µmol/L; Q3 8.58-9.4 µmol/L; Q4 9.41-10.5 µmol/L; Q5 10.6-14.8 µmol/L | | | | | | | |  |
| \*SWS: Weight gain at 34 weeks’ gestation; GUSTO: weight gain at 26 weeks’ gestation | | | | | | | |  |
| †SWS: n=439; GUSTO: n=290 | | | | | | | |  |
| ‡P-values for trend were calculated using the choline concentrations continuously | | | | | | | |  |

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| **Table 2.** Multivariate linear associations between maternal plasma choline concentrations (5 µmol/L) and offspring size and conditional growth in first 5 years of life. | | | | | | | | | | | | | | | | | |
|  | SWS | | | | | | | |  | GUSTO | | | | | | | |
|  |  | Crude | | |  | Adjusted model | | |  |  | Crude | | |  | Adjusted model | | |
|  | n | β | 95% CI | p |  | β | 95% CI | p |  | n | β | 95% CI | p |  | β | 95% CI | p |
| **BMI (SD)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Birth | 955 | 0.32 | -0.02, 0.67 | 0.062 |  | 0.21 | -0.16, 0.58 | 0.262 |  | 953 | 0.30 | 0.08, 0.51 | 0.007 |  | 0.31 | 0.10, 0.51 | 0.004 |
| 6 months | 775 | 0.12 | -0.03, 0.53 | 0.574 |  | 0.16 | -0.30, 0.61 | 0.500 |  | 853 | -0.15 | -0.38, 0.09 | 0.221 |  | -0.09 | -0.33, 0.15 | 0.479 |
| 12 months | 724 | 0.26 | -0.02, 0.55 | 0.067 |  | 0.20 | -0.11, 0.52 | 0.203 |  | 722 | 0.02 | -0.15, 0.19 | 0.833 |  | 0.02 | -0.15, 0.19 | 0.788 |
| 2 years | 668 | 0.16 | -0.12, 0.45 | 0.263 |  | 0.17 | -0.13, 0.47 | 0.275 |  | 534 | 0.10 | -0.08, 0.29 | 0.271 |  | 0.09 | -0.10, 0.29 | 0.337 |
| 3 years | 654 | 0.21 | -0.03, 0.46 | 0.089 |  | 0.19 | -0.07, 0.45 | 0.150 |  | 451 | 0.19 | 0.01, 0.38 | 0.040 |  | 0.17 | -0.02, 0.36 | 0.085 |
| 4 years | 442 | 0.09 | -0.16, 0.35 | 0.473 |  | -0.05 | -0.32, 0.23 | 0.735 |  | 435 | 0.04 | -0.13, 0.21 | 0.659 |  | 0.03 | -0.15, 0.20 | 0.761 |
| 5 years |  |  |  |  |  |  |  |  |  | 378 | -0.06 | -0.20, 0.09 | 0.450 |  | 0.05 | -0.20, 0.10 | 0.518 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Abdominal circumference (SD)** | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Birth | 967 | 0.15 | -0.20, 0.49 | 0.400 |  | 0.17 | -0.18, 0.52 | 0.347 |  | 879 | 0.20 | -0.01, 0.42 | 0.058 |  | 0.21 | 0.00, 0.43 | 0.053 |
| 6 months | 795 | 0.69 | 0.24, 1.14 | 0.003 |  | 0.44 | 0.07, 0.82 | 0.019 |  | 734 | 0.04 | -0.18, 0.25 | 0.746 |  | 0.04 | -0.18, 0.27 | 0.717 |
| 12 months | 755 | 0.00 | -0.39, 0.38 | 0.997 |  | -0.16 | -0.46, 0.14 | 0.306 |  | 647 | -0.11 | -0.30, 0.08 | 0.241 |  | -0.11 | -0.30, 0.09 | 0.293 |
| 2 years | 668 | 0.21 | -0.21, 0.63 | 0.334 |  | 0.20 | -0.12, 0.53 | 0.218 |  | 496 | 0.12 | -0.07, 0.31 | 0.212 |  | 0.14 | -0.06, 0.33 | 0.182 |
| 3 years | 647 | 0.04 | -0.30, 0.38 | 0.806 |  | -0.16 | -0.44, 0.12 | 0.266 |  | 476 | 0.08 | -0.09, 0.25 | 0.364 |  | 0.01 | -0.16, 0.18 | 0.877 |
| 4 years |  |  |  |  |  |  |  |  |  | 427 | 0.05 | -0.10, 0.21 | 0.514 |  | 0.07 | -0.09, 0.23 | 0.384 |
| 5 years |  |  |  |  |  |  |  |  |  | 413 | -0.04 | -0.16, 0.07 | 0.459 |  | -0.06 | -0.18, 0.06 | 0.350 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Triceps skinfold (mm)** | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Birth | 965 | 0.40 | 0.06, 0.75 | 0.022 |  | 0.32 | -0.07, 0.70 | 0.108 |  | 799 | 0.44 | 0.18, 0.71 | 0.001 |  | 0.38 | 0.11, 0.65 | 0.006 |
| 6 months | 791 | 0.14 | -0.71, 0.98 | 0.754 |  | -0.03 | -0.98, 0.93 | 0.952 |  |  |  |  |  |  |  |  |  |
| 12 months | 741 | 0.39 | -0.48, 1.26 | 0.383 |  | 0.17 | -0.78, 1.13 | 0.719 |  |  |  |  |  |  |  |  |  |
| 2 years | 650 | 0.62 | -0.18, 1.42 | 0.130 |  | 0.50 | -0.37, 1.37 | 0.261 |  | 558 | 0.21 | -0.16, 0.59 | 0.269 |  | 0.22 | -0.17, 0.61 | 0.267 |
| 3 years | 615 | -0.63 | -1.50, 0.23 | 0.151 |  | -0.79 | -1.71, 0.14 | 0.095 |  | 527 | 0.03 | -0.41, 0.48 | 0.880 |  | -0.08 | -0.55, 0.38 | 0.720 |
| 4 years |  |  |  |  |  |  |  |  |  | 473 | 0.13 | -0.42, 0.68 | 0.639 |  | 0.08 | -0.48, 0.64 | 0.784 |
| 5 years |  |  |  |  |  |  |  |  |  | 347 | 0.24 | -0.40, 0.88 | 0.458 |  | 0.27 | -0.41, 0.94 | 0.439 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Subscapular skinfold (mm)** | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Birth | 965 | 0.59 | 0.19, 0.99 | 0.004 |  | 0.55 | 0.12, 1.00 | 0.012 |  | 799 | 0.37 | 0.12, 0.61 | 0.003 |  | 0.26 | 0.01, 0.50 | 0.038 |
| 6 months | 791 | -0.09 | -0.75, 0.58 | 0.800 |  | -0.20 | -0.95, 0.54 | 0.589 |  |  |  |  |  |  |  |  |  |
| 12 months | 745 | 0.56 | -0.02, 1.13 | 0.058 |  | 0.39 | -0.24, 1.03 | 0.230 |  |  |  |  |  |  |  |  |  |
| 2 years | 655 | 0.08 | -0.49, 0.65 | 0.781 |  | 0.18 | -0.46, 0.81 | 0.585 |  | 538 | 0.05 | -0.24, 0.33 | 0.755 |  | 0.09 | -0.20, 0.38 | 0.530 |
| 3 years | 615 | 0.08 | -0.52, 0.69 | 0.783 |  | 0.26 | -0.39, 0.91 | 0.435 |  | 511 | 0.23 | -0.16, 0.63 | 0.242 |  | 0.12 | -0.28, 0.52 | 0.546 |
| 4 years |  |  |  |  |  |  |  |  |  | 446 | 0.12 | -0.35, 0.58 | 0.628 |  | 0.09 | -0.38, 0.56 | 0.711 |
| 5 years |  |  |  |  |  |  |  |  |  | 314 | -0.04 | -0.56, 0.49 | 0.888 |  | -0.11 | -0.66, 0.44 | 0.704 |
| Crude: Adjusted for skinfold measurements at previous time points for time points after birth | | | | | | | | | | | | | | | | | |
| Adjusted model: Adjusted for maternal age, education level, height, pre-pregnancy BMI, plasma folate and vitamin B12 concentrations, fetal sex, and gestational age. The results from the GUSTO cohort were additionally adjusted for ethnicity. | | | | | | | | | | | | | | | | | |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 3.** Associations between maternal plasma choline concentrations (5 µmol/L) and offspring total body fat at birth and 5 years | | | | | | | | | | | | |
|  |  | Crude | | |  | Model 1 | | |  | Model 2 | | |
|  | n | β | 95% CI | p |  | β | 95% CI | p |  | β | 95% CI | p |
| **SWS – DXA** |  |  |  |  |  |  |  |  |  |  |  |  |
| Birth (kg) | 437 | 0.67 | 0.12, 1.19 | 0.016 |  | 0.60 | 0.04, 1.16 | 0.037 |  | 0.49 | 0.00, 0.98 | 0.048 |
| 4 years (kg) | 261 | 0.27 | -0.52, 1.06 | 0.491 |  | 0.33 | -0.42, 1.09 | 0.391 |  | 0.37 | -0.32, 1.05 | 0.299 |
| **Gusto – ADP** |  |  |  |  |  |  |  |  |  |  |  |  |
| Birth (kg) | 290 | -0.01 | -0.06, 0.04 | 0.648 |  | -0.07 | -0.16, 0.02 | 0.113 |  | 0.00 | -0.04, 0.01 | 0.972 |
| 4.5 years (kg) | 105 | 0.69 | -0.35, 1.73 | 0.192 |  | 0.83 | -0.18, 1.85 | 0.108 |  | 0.59 | -0.40, 1.57 | 0.241 |
| Crude: Adjusted for skinfold measurements at previous time points for time points after birth | | | | | | | | | | | | |
| Model 1: Crude model and additionally adjusted for maternal age, education, height, pre-pregnancy BMI, plasma folate and vitamin B12 concentrations, fetal sex, and gestational age. The results from the GUSTO cohort were additionally adjusted for ethnicity | | | | | | | | | | | | |
| Model 2: Model 1 and additionally adjusted for infant length/height at time of measurement | | | | | | | | | | | | |
| DXA: dual energy x-ray, ADP: air displacement plethysmography | | | | | | | | | | | | |

**Figure 1.** Flowchart of participants included for analysis from the SWS cohort and the GUSTO cohort.