DSX 19 (2025) 103222

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Maternal serum folate status during early pregnancy: Sex-specific association with neonatal adiposity

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ARTICLE INFO

Keywords:
Folate
Pregnancy
Anthropometry
Adiposity
Infant
Gestational diabetes mellitus

ABSTRACT

Background & aims: Early pregnancy folate has been associated with GDM and possible adiposity in the newborn. The present study examined associations between maternal early pregnancy folate levels and sex-specific neonatal anthropometry. We further explored possible mediation by maternal glycemia on the association between folate and neonatal adiposity.
Methods: Sub-group data ($n = 511$) from a UK multi-ethnic early pregnancy longitudinal study (micronutrients in Pregnancy as a Risk factor for gestational Diabetes and Effects on mother and baby; PRiDE) was used. Maternal serum folate was assessed during early pregnancy (Mean ± SD = 12.5 ± 1.6 gestational weeks) and infant anthropometry including skinfold thickness (SFT) and mid-upper arm circumference (MUAC) at birth. Multiple linear regression was performed to analyse the relationship between maternal folate and infant adiposity indices. Interaction analysis was used to identify maternal glucose mediation of this relationship.
Results: Excess folate levels (≥45 nmol/l) were found in 40.3 % pregnant women ($n = 206$). Early pregnancy folate (1 SD unit) was positively associated with male newborn triceps SFT (std $\beta = 0.17$ (95 % CI: 0.06, 0.29; $p < 0.05$)) after adjusting for key maternal and infant confounders in multiple comparisons using Benjamini-Hochberg procedure. However, no associations were seen in female newborns. No influence of maternal fasting (FPG) and 2-h plasma glucose (2 h-PG) were detected on the association between folate and newborn anthropometry.
Conclusion: Our findings suggest a potential sex-specific influence of maternal folate on infant anthropometric indices. The association between early pregnancy folate on newborn adiposity was not mediated by maternal FPG and/or 2 h-PG at 24–28 weeks.

1. Introduction

Adequate maternal folate is crucial for optimal foetal growth and preventing congenital anomalies such as neural tube defects and congenital heart disease [1,2]. Folate plays a pivotal role in various cellular processes, including DNA synthesis, amino acid metabolism and epigenetic regulation [3]. Therefore, prenatal folic acid (FA) supplementation (at a daily dose of 400 μg) has become a standard practice to account for increased foetal requirements during pregnancy [4,5]. For this reason, some countries have adopted FA fortification as a strategy to enhance maternal folate levels [6]. Recent studies have shown that excess folate and unmetabolized folic acid (UMFA) is present in maternal and umbilical cord blood [7,8]. Concerns have also been raised that elevated gestational folate levels might be linked to an increased risk of masking B12 deficiency, DNA repair dysregulation and gestational diabetes mellitus (GDM) that might lead to metabolic imbalances in the offspring [7,9].

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https://doi.org/10.1016/j.dsx.2025.103222
Received 15 July 2024; Received in revised form 21 March 2025; Accepted 24 March 2025
Available online 1 April 2025

Abbreviations:

FA	Folic acid
SFT	Skinfold thickness
MUAC	Mid-upper arm circumference
HC	Head circumference
CC	Chest circumference
AC	Abdominal circumference
GDM	Gestational diabetes mellitus
OGTT	Oral glucose tolerance test
FPG	Fasting plasma glucose
2 h-PG	2- hour plasma glucose

Excess maternal folate has been shown to be associated with an increased propensity for fat accumulation, insulin resistance and altered cardiometabolic health regulation in children, thought to be through adverse epigenetic programming [10–12]. However, the evidence is equivocal. Longitudinal cohort studies from India found that higher maternal folate was positively correlated with fat mass, percent fat mass and insulin resistance in school aged children [10,11]. In contrast, another study from the Netherlands found protective effects of maternal folate on child BMI and android-to-gynoid fat ratio [13]. Experimental studies in animals have suggested adverse alterations in adipose tissue gene expression and energy expenditure in offspring exposed to higher maternal folate levels [14,15]. Additionally, the observation of a direct correlation between higher folate and maternal glycemia needs further investigation, as poorly controlled hyperglycaemia is a known risk factor for higher infant adiposity and higher neonatal fat mass [7,16–19].

Infant adiposity is an indicator of foetal growth and development. Fat serves as an energy reserve to support the demands of the developing brain [20]. However, significantly higher neonatal adiposity is correlated with childhood overweight at the age of 5, thereby potentially predisposing individuals to obesity later in life [21]. Neonatal skinfold thickness (SFT) is a measure of subcutaneous fat at distinct locations in newborn infants, encompassing areas such as triceps, subscapular, and thigh regions and is commonly used to evaluate newborn nutritional status and body composition [22]. Specifically, triceps skinfold measurement offers insights into peripheral adipose tissue reserves, while the subscapular and thigh SFT reflects trunk and lower body adiposity respectively [23]. While it is commonly acknowledged that fat distribution is different for male and female neonates, more research is needed to unravel the intricacies of sexual dimorphism in the relationship between maternal excess folate on infant adiposity [14,15,24].

In the present analysis, using a multi-ethnic UK-based longitudinal birth cohort, we aim to examine whether maternal folate status is related to newborn adiposity, and whether this relationship demonstrates sexual dimorphism. We also aim to explore the interplay between maternal glycemia and folate on neonatal body fat.

2. Subjects, materials and methods

2.1. Participants

This analysis was conducted in a single-centre subgroup of the PRiDE study (micronutrients in Pregnancy as a Risk factor for gestational Diabetes and Effects on mother and baby). The PRiDE study recruited 4746 women in their first trimester between 2012 and 2018 at ten study centres across the UK. While all centres collected the primary outcome data, George Eliot Hospital (GEH) was the only centre that systematically collected and documented the secondary infant anthropometry outcomes as part of routine clinical practice. The study findings have been previously described [7]. Detailed information on participant demographics and their serum micronutrients status at less than 16 weeks of gestation, and their fasting (FPG) and 2-hr (2 h- PG) plasma glucose levels at 24–28 weeks of gestation at the time of a 75-g oral glucose tolerance test (OGTT) were collected. GDM diagnosis was based on NICE criteria, defined as FPG $\geq$5.6 mmol/l or 2 h-PG $\geq$7.8 mmol/l and International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, defined as FPG $\geq$5.1 mmol/l or 2 h-PG $\geq$8.5 mmol/l [25,26]. Of the infants of mothers from one study centre (George Eliot Hospital- NHS Trust, Nuneaton, UK; $n = 1637$), 511 were assessed for birth outcomes and evaluated for neonatal anthropometry indices with complete newborn anthropometry and skinfold thickness data. In this exploratory analysis, we aimed to identify the possible association between maternal early pregnancy folate and offspring anthropometry at birth. Therefore, no imputations were not performed, and participants were excluded primarily due to incomplete data(Supplementary Fig. 1). Maternal and infant characteristics of those excluded are provided in supplementary files (Supplementary Table 1). All participants provided written informed consent, and ethical approval was obtained from the National Research Ethics Committee.

2.2. Biochemical analysis

Blood sampling was done at a mean of 12.5 ± 1.6 weeks of gestation and samples were transferred to −80°c freezers within 30 min of collection. Serum B12 and folate levels were assessed using an electrochemiluminescent immunoassay (Roche Cobas analyser from Roche Diagnostics, Burgess Hill, UK). Plasma total homocysteine (tHcy) levels were estimated by stable isotopic dilution analysis using a Shimadzu HPLC system equipped with an auto-sampler, linked to the detection system of an API 6500 QTrap tandem mass spectrometer (liquid chromatography mass spectrometry [LCMS]) (Applied Biosystems, Warrington, UK). A detailed description of this biochemical procedure has been given elsewhere [7]. Early pregnancy serum folate deficiency was defined as <10 nmol/l and excess folate as >45 nmol/l [27]. B12 insufficiency in pregnancy was defined as <220 pmol/l [7].

2.3. Infant anthropometric assessment at birth

The current analysis was done in 511 mother-infant pairs of PRiDE cohort from George Eliot Hospital NHS Trust, UK, with complete neonatal anthropometry including birthweight, crown-heel length, occipitofrontal head circumference (HC), abdominal (AC) and chest circumference (CC) and mid-upper arm circumference (MUAC), triceps SFT, and subscapular SFT. These were conducted by trained research midwives. Birthweight was measured using a Seca infant scale with accuracy to the nearest 50 g and crown-heel length was measured using a portable length board with accuracy to the nearest 0.1 cm. Triceps and subscapular SFT on the left side of the body were measured using Harpenden skinfold callipers with a precision of 0.2 mm. Similarly, standardised techniques were used to measure infant HC, AC, CC, MUAC [28]. All measurements were taken within 72 h of birth. Each measurement was performed in duplicate, and the mean value was used.

2.4. Statistical analysis

All analyses were performed in R software, version 4.2.2 (2022-10-31) (https://www.R-project.org/) [29]. The infant anthropometric variables were found to be normally distributed(Supplementary Fig. 1). Continuous variables were reported as mean and standard deviation (SD), while percentages were used to report categorical variables. Student's t-test was used to compare group differences in continuous variables, while Chi-square tests (χ^2) were used to compare categorical variables between the groups. An unadjusted regression model was performed to explore the association between maternal folate and infant anthropometric measures, stratified by infant gender (Mode 1) 1. An *a priori* set of multiple linear regression models were then performed to investigate the sex specific effects of maternal folate on infant body

composition due to the marked differences in the effect estimates of male and female newborn triceps SFT, MUAC and AC measurements (Supplementary Fig. 2) following an interaction analysis between maternal folate and newborn anthropometry. Three additional models were tested adjusting for relevant covariates that thought to be related to infant anthropometry. Model 2 adjusted for maternal and infant covariates (maternal age, BMI, GA at booking visit of one carbon metabolite measurement including vitamin B12 and homocysteine, ethnicity, parity and infant birthweight, GA at birth, length, HC and CC at birth). As maternal lipids and glycaemia are also shown to be associated with infant adiposity, these were tested in model 3 (high density lipoprotein (HDL), triglycerides (TG) and total cholesterol (TC)) and in model 4 (FPG, 2 h- PG at OGTT and gestational weight gain). Multiple linear regression models were stratified based on newborn sex adjusting for potential covariates in unadjusted and adjusted models. Because of the strong collinearity between smoking status and folate levels (p-value = 0.03), we excluded maternal smoking status from the adjusted analysis to maintain statistical validity. As there is evidence of direct associations between early pregnancy folate levels and plasma glucose levels at 24–28 weeks of gestion, interaction analysis was performed to explore whether the association between maternal folate and neonatal anthropometry is mediated via maternal glucose levels during pregnancy [7, 30]. All analyses were two-sided, and a p-value of <0.05 was considered statistically significant in all models. Additionally, we performed Benjamini-Hochberg procedure to control the false discovery rate (FDR) to obtain adjusted p-value calculations for multiple comparisons in Model 2–4.

3. Results

3.1. Baseline characteristics

Maternal antenatal characteristics are summarized in Table 1. Excess folate (>45 nmol/l) was reported in a significant proportion of women (40.3 %) in this cohort. Folate deficiency (<10 nmol/l) was observed in 1.2 % and B12 insufficiency in 47.0 % (<220 pmol/l) of women in early pregnancy. Fifty-five (10.8 %) women were diagnosed with GDM at the time of OGTT by NICE criteria and eighty-four (16.4 %) by IADPSG criteria. These proportions were similar to that of the parent PRiDE study (Supplementary Table 2) [7]. There were no significant differences observed in maternal clinical characteristics of male and female infants (maternal age, BMI, FPG and 2 h- PG at OGTT and lipid measurements including HDL, total cholesterol, and triglyceride). Table 2 shows the comparison of birthweight and other neonatal anthropometric measures between male and female infants. As expected, birthweight, length, and HC were greater in male infants compared to female infants (Table 2).

3.2. Association between early pregnancy folate, B12 and infant birthweight

Early pregnancy folate (std β = 0.06; 95 % CI: −0.01, 0.13; *p*-value = 0.10) and B12 (std β = −0.07; 95 % CI: −0.14, 0.002; *p*-value = 0.06) were not associated with infant birthweight after adjusting for infant sex and gestational age at birth. However, excess folate (>45 nmol/l) was positively associated with infant birthweight after adjusting for gender and GA at birth. No significant association was observed between insufficient B12 levels at <220 pmol/l and birthweight(Supplementary Table 3).

3.3. Association between early pregnancy folate and infant anthropometric indices

Maternal early pregnancy folate was positively associated with triceps SFT, subscapular SFT and MUAC in the unadjusted model (model 1; Supplementary Table 4) in male but not in female infants. Fig. 1 and

Table 1
Maternal antenatal characteristics stratified by infant sex.

Maternal characteristics	All (n = 511)	Female (n = 269)	Male (n = 242)	*p*-value
Age (years)	29.9 ± 5.3	29.9 ± 5.1	30.0 ± 5.5	0.98
Height (cm)	165 ± 6.8	164 ± 7.0	166 ± 6.6	0.24
Weight (kg)	87.1 ± 21.4	87.1 ± 22.6	87.2 ± 20.0	1.00
BMI (kg/m^2)	31.8 ± 7.2	32.0 ± 7.5	31.7 ± 6.7	0.92
GA at booking (weeks)	12.5 ± 1.6	12.4 ± 1.6	12.6 ± 1.5	0.67
Waist circumference (cm)	103 ± 16.7	102 ± 17.3	104 ± 16.0	0.46
Ethnicity				0.99
Caucasian	419 (82.0 %)	219 (81.4 %)	200 (82.6 %)	
[a]Other	27 (5.3 %)	14 (5.2 %)	13 (5.4 %)	
South Asian	65 (12.7 %)	36 (13.4 %)	29 (12.0 %)	
Parity	1.3 ± 0.8	1.3 ± 0.7	1.4 ± 0.9	0.91
Primigravida	402 (78.7 %)	211 (78.4 %)	191 (78.9 %)	0.99
Folate (nmol/l)	48.2 ± 33.1	47.0 ± 32.2	49.6 ± 34.0	0.69
Excess folate (>45 nmol/l)	206 (40.3 %)	104 (38.7 %)	102 (42.1 %)	0.71
B12 (pmol/l)	247 ± 97.6	245 ± 88.8	249 ± 107	0.89
B12 insufficiency at <220 pmol/l	240 (47.0 %)	121 (45.0 %)	119 (49.2 %)	0.64
Homocysteine (μmol/l)	12.8 ± 5.7	12.7 ± 6.0	12.8 ± 5.4	0.97
Cholesterol (mmol/l)	4.8 ± 0.8	4.8 ± 0.8	4.9 ± 0.9	0.41
HDL (mmol/l)	1.7 ± 0.4	1.6 ± 0.4	1.7 ± 0.4	0.95
Triglyceride (mmol/l)	1.4 ± 0.6	1.4 ± 0.6	1.4 ± 0.6	0.85
GA at OGTT (weeks)	26.9 ± 2.5	27.1 ± 2.5	26.8 ± 2.6	0.59
[b]Weight gain (kg)	5.9 ± 4.8	5.8 ± 4.9	6.1 ± 4.6	0.78
FPG (mmol/l)	4.6 ± 0.5	4.6 ± 0.5	4.5 ± 0.5	0.68
2 h- PG (mmol/l)	5.9 ± 1.5	5.9 ± 1.5	5.8 ± 1.4	0.44
GDM treatment	51 (10.0 %)	30 (11.2 %)	21 (8.7 %)	0.65

Data are presented as mean ± SD or *n* (%) unless otherwise indicated.
Abbreviations: GA- Gestational age; OGTT- Oral glucose tolerance test; FPG- Fasting plasma glucose; 2 h-PG- 2 h plasma glucose in OGTT; HDL- High density lipoprotein.
p-value <0.05 was considered significant.
[a] Other ethnic group includes North African, Black African, Caribbean, Asian, Southeast Asian, Middle Eastern, and mixed ethnicity.
[b] Gestational weight gain was defined by 'kg' weight gained by a pregnant woman at the time of OGTT since prenatal booking visit.

Supplementary Table 4 shows the sex-specific association between early pregnancy folate and infant anthropometric indices. The association remained significant for triceps SFT in adjusted model for key maternal and newborn anthropometric indices in Model 2 but attenuated for MUAC and subscapular SFT in Model 2 after correcting for Benjamini-Hochberg procedure. It was not significant for AC and no association was seen in female newborns across all anthropometry measurements. No such associations were found between maternal early pregnancy B12 or homocysteine levels (Supplementary Table 5). The positive associations between folate and infant anthropometry indices in males remained significant after adjusting for potential confounders (models 2; Supplementary Table 4). An interaction analysis showed statistically significant interactions (Infant sex*folate) in relation to triceps skinfold and AC (p < 0.05), but this was not significant for MUAC (p = 0.07), and subscapular skinfold thickness (p = 0.15) (Supplementary Fig. 2).

3.4. Exploring the effect of maternal glucose levels on the association between folate and infant anthropometry

Maternal glycemia showed no significant relationship with infant anthropometric indices in an adjusted model. Sub-group analysis of women who did not develop GDM at 24–28 weeks (n = 456; male: n =

Table 2
Infant anthropometric indices measured at birth stratified by sex.

Infant anthropometry	All (n = 511)	Female (n = 269)	Male (n = 242)	p-value
Birthweight (gms)	3420 ± 540	3350 ± 536	3510 ± 533	0.004
GA at birth (weeks)	39.3 ± 1.5	39.3 ± 1.6	39.3 ± 1.5	0.93
Length (cm)	50.5 ± 3.7	49.9 ± 3.8	51.2 ± 3.5	0.001
Ponderal Index (gm/cm^3)	2.7 ± 0.5	2.7 ± 0.6	2.7 ± 0.5	0.30
Head circumference (cm)	34.7 ± 1.6	34.5 ± 1.5	35.0 ± 1.7	<0.001
Chest circumference (cm)	34.3 ± 2.5	34.1 ± 2.6	34.6 ± 2.4	0.16
MUAC (cm)	11.2 ± 1.5	11.1 ± 1.5	11.4 ± 1.5	0.21
Abdominal circumference (cm)	33.4 ± 2.6	33.3 ± 2.7	33.6 ± 2.6	0.40
Triceps SFT (mm)	5.9 ± 1.9	5.9 ± 2.0	5.8 ± 1.9	0.66
Subscapular SFT (mm)	5.7 ± 1.9	5.6 ± 1.9	5.7 ± 1.9	0.98
Subscapular: Triceps SFT ratio	1.0 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	0.73
Infant Birth size				
LGA at birth (>90th percentile)	61 (11.9 %)	31 (11.5 %)	30 (12.4 %)	0.98
SGA at birth (<10th percentile)	41 (8.0 %)	21 (7.8 %)	20 (8.3 %)	

Data are presented as mean ± SD or n (%) unless otherwise indicated.
Abbreviations: LGA- Large for gestational age; SGA- Small for gestational age. LGA and SGA outcomes were calculated based on WHO (World Health Organisation) growth charts.
p-value <0.05 was considered significant.

221 and female: n = 235) also showed no association between maternal FPG and 2-hr PG with infant anthropometry (Supplementary Tables 6 and 7) in male and female infants respectively). The interaction analysis between early pregnancy folate and maternal FPG and 2-hr PG showed no significant association with triceps and subscapular SFT and MUAC (Data not shown).

4. Discussion

The present study examined the association between early pregnancy folate and neonatal anthropometric indices (triceps and subscapular SFT, MUAC, and AC) and the role of maternal glycemia as a possible mediator in this association. Our study, involving a multi-ethnic cohort of 511 mother-neonate pairs, had two main findings. Firstly, maternal folate was positively associated with infant anthropometric indices in a gender specific manner where significant associations were only observed in male infants. Secondly, the associations were present even after adjusting for maternal pre-pregnancy BMI and infant birthweight, significant predictors of newborn anthropometry. To our current knowledge, there have been no prior studies that have examined the association of maternal folate in early pregnancy on neonatal anthropometry and subcutaneous fat in a gender-specific manner.

Newborns' subcutaneous fat deposits serve as indicators of their nutritional status, providing survival benefits by acting as an energy reserve and regulating body temperature [20]. However, previous studies have reported that subcutaneous SFT predicts neonatal fat mass and may increase the risk of adiposity and insulin resistance [31]. Studies from India have shown that higher maternal folate levels during pregnancy were associated with greater childhood adiposity [10,11]. Given the sexual dimorphism in neonatal body fat stores, our study explored sex-specific association of maternal folate and adiposity measures at birth (triceps SFT and MUAC). Studies done in children suggest that MUAC predicts obesity and body fat distribution and could be a useful screening tool for central adiposity [32,33]. Our data indicate that folate positively predicted triceps SFT and MUAC but the association with subscapular SFT was not observed when adjusted for potential maternal and newborn confounders.

The sex-specific associations of early pregnancy folate on neonatal anthropometric indices raise questions about whether this observation demonstrates increased adipocyte proliferation or is due to the amplification of lipogenic transcription factors in the offspring [34,35]. Furthermore, body fat distribution in male infants may involve a more intricate process, with contributions from adipokines such as leptin and adiponectin [36]. Notably, boys with lower cord blood leptin at birth were found to have higher adiposity (SFT measurements) at age 3 [37]. We hypothesize that this sex-specific growth expansion occurred in the later stages of pregnancy, where maternal micronutrient-rich foods accelerate fat accumulation in the male fetus [38]. Currently, there are no plausible mechanisms that can explain the sex-specific relationship of maternal folate status with neonatal adiposity in humans. However, the following animal studies suggest possible mechanisms. First, male offspring of excess FA fed dams had higher bodyweight change and insulin resistance compared to female pups [15]. They also exhibited altered appetite regulatory gene expression such as, proopiomelanocortin (POMC), leptin receptor, neuropeptide Y, and agouti-related protein. Second, maternal folate dependent DNA methylation and epigenetic changes were more pronounced in male offspring compared to females. In their landmark randomised controlled study in sheep, Sinclair et al. showed that 53 % of adiposity, insulin resistance and blood pressure associated loci in the affected male offspring genome compared to 12 % in females, albeit with a 'low methylating cocktail diet' [39]. In a recent study by Schoonejans et al., sex-specific expression of proinflammatory genes were observed in male rodents exposed to maternal obesity and metformin highlighting the sexual dimorphism in developmental programming [40]. This study also showed while these adverse changes were obvious in male offspring at birth, similar changes were seen much later in life in female offspring. It is plausible that similar sex-specific changes may happen in humans but will require longer term studies. It has been shown that periconceptional multivitamin intake had sex-specific DNA methylation profiling with differential methylation levels of IGF2R in girls, and GTL2 in boys [41]. Finally, fetal growth is influenced by other factors such as placental characteristics, hormone levels, paternal anthropometric influences and maternal micronutrients interact in complex ways to affect the growth and metabolic programming of the fetus [19,42].

The results from our mother-infant pairs are consistent with previous findings that male neonates tend to be slightly heavier, longer with slightly larger head circumference and higher lean mass than female neonates [24,43]. Maternal folate status has been linked to higher blood glucose levels and risk of GDM in pregnancy [7,31,37]. Hence, we explored whether the association between folate and neonatal adiposity was mediated by maternal glycemia. Our findings did not support this, suggesting the folate association with maternal glycaemia and offspring adiposity may be due to different underlying pathophysiological mechanisms.

4.1. Strengths

The key strength of our study is the inclusion of the PRiDE cohort dataset, a large, multi-ethnic, prospective study that enhances the generalizability of our findings. Furthermore, the availability of accurate micronutrient status, maternal glycemia and lipid measurements enabled adjustment for key maternal confounders in our models.

4.2. Limitations

This study has the following key limitations. First, we used triceps and subscapular SFT as a surrogate measure of neonatal adiposity, instead of more robust methods such as air displacement plethysmography or magnetic resonance imaging. However, all the measurements were done within 72 h of birth which is shown to have higher accuracy

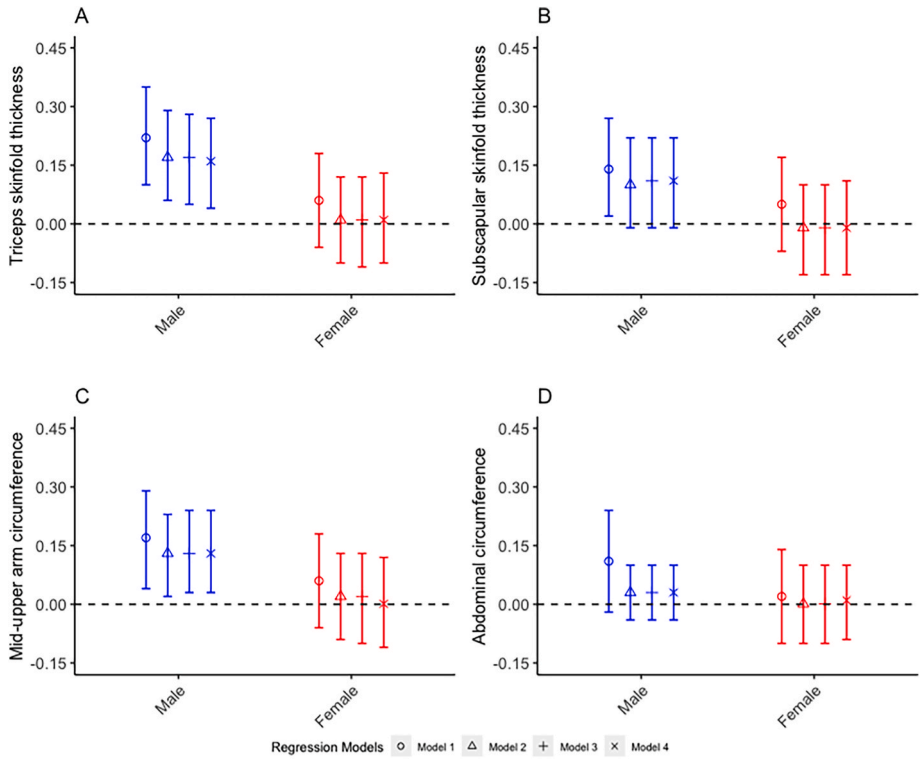

Fig. 1. Association between maternal folate and infant triceps and subscapular skinfold thicknesses, MUAC, AC and estimated percent fat mass stratified by gender Linear regression models showing the association between 1 SD unit change in maternal folate and estimates of neonatal triceps and subscapular skinfold thicknesses, MUAC and AC measured at birth and estimated percent fat mass. Blue lines showed the coefficients, and 95 % confidence intervals of male infant anthropometry; Red lines showed the coefficients and 95 % confidence intervals of female infant anthropometry. A: Association between maternal folate and triceps skinfold thickness (mm). B: Association between maternal folate and subscapular skinfold thickness (mm) C: Association between maternal folate and infant mid-upper arm circumference (cm). D: Association between maternal folate and infant abdominal circumference (cm).
Regression models were adjusted for the covariates as follows:
Model 1: Unadjusted model
Model 2: Adjusted for maternal age, BMI, gestational age at booking visit, ethnicity, parity, vitamin B12, homocysteine levels and infant birthweight length, head, and chest circumference measured at birth, and gestational age at birth
Model 3: as model 2, with adjustments to maternal HDL, triglyceride, and total cholesterol
Model 4: as model 3, with additional adjustments to fasting and 2-h plasma glucose at the time of OGTT, gestational age at the time of OGTT, weight gain, and GDM treatment. The blue and red dots represent the coefficient estimates from each model and the vertical lines represents 95% confidence intervals for the standardised regression coefficient (β) for male and female newborn respectively.

than later on in infancy [22]. Second, we did not have access to lower limb SFT, such as the thigh or calf. Including these measurements could have enabled to calculate the subcutaneous fat mass. Third, the present study is limited by its observational design, which prevents us from establishing causal relationships. Finally, we did not have any data on pre-pregnancy, mid- and late pregnancy and cord blood folate levels, therefore could not ascertain their role in relation to maternal early pregnancy folate levels. Moreover, the original PRiDE cohort was constrained by its focus on maternal characteristics, and we recognise that paternal anthropometry and adiposity may also impact neonatal adiposity. While there is currently no evidence suggesting that these factors may introduce bias to our results, it would be valuable for future studies to investigate and clarify their respective roles on neonatal adiposity (maternal and cord blood folate levels). A significant limitation of our study is that newborn anthropometric measurements were only available from a single study centre within the larger PRiDE cohort, which could have affected our findings. Nevertheless, the key maternal and newborn characteristics have been similar between the groups (single centre vs. other centres data) including early pregnancy folate levels, infant birth weight, and head circumference.

5. Conclusion

In conclusion, our study indicated that higher maternal folate levels may be associated with adverse metabolic programming of adiposity in males. It can be potentially explained by the notion that the male fetus is more responsive to maternal nutrition, glycemia and pre-pregnancy BMI and are more vulnerable to experiencing adverse neonatal outcomes during any *intra-uterine* disruption [40,44]. Additional larger cohort and randomised controlled trial studies are required to understand whether any causal, adverse relationship exist in a gender-specific manner between excess folate and offspring adiposity and metabolic health. In the meantime, it is important to strike a balance between adequate prenatal folate status vs. excess folate levels in early pregnancy. Maternal serum levels are easy to measure in early pregnancy and folic acid supplements should be stopped if adequate/excess folate levels are observed in early pregnancy.

Authorship

N.P., N.S., Y.W.S., C.Y., C.F. and P.S. conceptualized the analysis, N. P. conducted statistical analysis, N.P., Y.W.S., N.S. and P.S. interpreted the results, N.P wrote the first draft of manuscript, A.A. performed biochemical analysis, P.S. had primary responsibility for final content. All authors read and approve the final manuscript.

Ethics of human subject participation

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving research participants were approved by the National Research Ethics Committee (12/WM/0010). Written informed consent was obtained from all participants.

Data availability

The data that support the findings of this study are available on request from the corresponding author (PS) on reasonable request.

Declaration of generative AI and AI-assisted technologies in the writing process

None of the authors used any Generative AI and AI-assisted technologies in the writing process of manuscript.

Financial Support

Nishanthi Periyathambi is funded by Chancellor's International Scholarship for Postgraduate research by the University of Warwick. Ponnusamy Saravanan, Nithya Sukumar and Yonas Ghebremichael-Weldeselassie were part funded by Medical Research Council (MRC), UK (MR/J000094/1). The funders did not have any role in the study design, data collection, analysis and interpretation of the findings, manuscript writing, or decision to submit the manuscript for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We are grateful to all the GEH clinical and research staffs (Ilona Goljan, Selvin Selvamoni, Joanne Wilson, Gail Pounder, Judith Plester, Hema Venkataraman, and Christos Bagias), Amitha Gopinath-research co-ordinator of Diabetes centre-GEH and collaborators involved in PRiDE study. We like to thank all mothers and newborns for their co-operation and without which this study would not have been possible.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2025.103222.

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