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Systematic review

Effectiveness of folic acid supplementation in pregnancy on reducing the risk of small-for-gestational age neonates: a population study, systematic review and meta-analysis

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Objectives To assess the effect of timing of folic acid (FA) supplementation during pregnancy on the risk of the neonate being small for gestational age (SGA).

Design A population database study and a systematic review with meta-analysis including the results of this population study.

Setting and data sources A UK regional database was used for the population study and an electronic literature search (from inception until August 2013) for the systematic review.

Participants and included studies Singleton live births with no known congenital anomalies; 111 736 in population study and 188 796 in systematic review.

Outcome measures, data extraction and analysis The main outcome was SGA based on customised birthweight centile. Associations are presented as odds ratios (OR) and adjusted odds ratios (aOR), adjusted for maternal and pregnancy-related characteristics.

Results Of 108 525 pregnancies with information about FA supplementation, 92 133 (84.9%) had taken FA during pregnancy. Time of commencement of supplementation was recorded in 39 416 pregnancies, of which FA was commenced before conception in 10 036, (25.5%) cases. Preconception commencement of FA supplementation was associated with reduced risk of SGA <10th centile (aOR 0.80, 95% CI 0.71–0.90, $P < 0.01$) and SGA <5th centile (aOR 0.78, 95% CI 0.66–0.91, $P < 0.01$). This result was reproduced when the data were pooled with other studies in the systematic review, showing a significant reduction in SGA (<5th centile) births with preconception commencement of FA (aOR 0.75, 95% CI 0.61–0.92, $P < 0.006$). In contrast, postconception folate had no significant effect on SGA rates.

Conclusion Supplementation with FA significantly reduces the risk of SGA at birth but only if commenced preconceptually independent of other risk factors.

Systematic review registration This systematic review was prospectively registered with PROSPERO number CRD42013004895.

Keywords Fetal growth, folic acid, growth restriction, small for gestational age.

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Introduction

Folic acid (FA) has been shown to reduce the risk of neural tube defects (NTD).[1] Despite recommendations in many countries (including the UK)[2] for women to start FA supplementation preconceptually, uptake is low. Publications from France and the UK have shown rates of preconceptual uptake to be between 14.8 and 31% with lower uptake in the younger age groups and ethnic minorities.[3,4] Therefore, some countries have adopted a food fortification policy, although concern regarding masking of vitamin B12 deficiency has hindered the adoption of such a policy in

many countries[5] while still recognising the significance of the timing of FA supplementation in the context of reducing the risk of NTD.[5]

Being born small for gestational age (SGA), defined as birthweight <10th centile, is associated with increased neonatal morbidity and mortality[6] and has an association with chronic diseases in later life such as type 2 diabetes, hypertension, obesity, cardiovascular disease and mental health problems.[7–11] The use of centiles customised for maternal characteristics (maternal height, weight, parity and ethnic group) as well as gestational age at delivery and infant sex, identifies small babies at higher risk of morbidity and mortality than those identified by population centiles.[12] Hence, prevention of SGA fetuses has the potential to produce significant public health benefits.

A Cochrane review has evaluated FA use in pregnancy and reported that although it was associated with improvements in mean birthweight, there was no significant effect on birthweight <2500 g.[13] Similarly, a systematic review by Fekete et al.[14] demonstrates an effect of FA supplementation on absolute birthweight. The Generation R study has demonstrated that the lowest folate levels are significantly associated with lower placental weights and birthweights and the lowest folate quartile was also associated with an increased risk of SGA.[15] The above evidence suggests an association with FA supplementation and absolute birthweight and folate levels and SGA; nevertheless, there is no clear indication of an effect of FA supplementation on reducing the risk of SGA. Evidence suggests interaction between folate intermediates, DNA methylation at a global and gene-specific level, and birthweight centiles in humans.[10,16] Given the susceptibility of the fetal methylome during embryogenesis[17] and the critical role of folate in providing methyl groups for DNA synthesis, the timing of FA supplementation is particularly interesting.

Therefore, the main aim of this study was to assess the impact of timing of FA supplementation (preconception or postconception) on reducing the risk of SGA by analysing a large regional perinatal database and systematic appraisal followed by meta-analysis of the published literature.

Methods

This study consisted of a population study reported according to the STROBE guidelines[18] and then a protocol-driven systematic review and meta-analysis of all available literature, including the results of the population study as reported in this paper (called Morris 2014). This systematic review was performed in accordance with recommended methods[14] and reported according to the PRISMA guidelines.[19]

Population study

Maternal data collection and consent

The cohort represents deliveries between July 2009 and July 2012 to women within the West Midlands NHS region of the UK. We excluded stillbirths, multiple pregnancies and pregnancies with congenital anomalies. Data were prospectively recorded in the handheld maternity notes, which are in standard use throughout the region and entered retrospectively by trained data clerks on the NHSnet based 'perinatal episode electronic record' (PEER) that covered all 19 maternity units within the West Midlands. Regular on-site quality audits were undertaken to ensure the quality of the electronic data entry. Consent for data collection was obtained through the Perinatal Institute's confidentiality and consent protocol, which has been reviewed by UK Connecting for Health, the NHS and the Information Commissioner.

At the booking appointment, information recorded included maternal characteristics (age, parity, ethnic origin, and maternal height and weight), social factors (employment status of the mother and her partner, consanguinity and index of multiple deprivation). Pre-existing medical history and previous obstetric history (mental health problems, diabetes mellitus, hypertensive disorders and previous stillbirth), smoking status, alcohol consumption, nonprescription drugs, FA intake (timing and dose of supplementation), and time of first visit in pregnancy were collected plus additional information relating to pregnancy complications [gestational diabetes, antepartum haemorrhage, pregnancy-induced hypertension, and pre-eclampsia (pregnancy-induced hypertension with proteinuria)]. Neonatal information included sex, gestational age (based on early pregnancy dating scans) and birthweight. During the period of data collection 96.5% of pregnancies within the region had their estimated date of delivery confirmed by ultrasound in either the first or second trimester (<22 weeks).

Birthweight centiles were derived from the gestation-related optimal weight standard (GROW),[20] which defines the fetal growth potential by excluding pathological factors (e.g. smoking and diabetes) and by individual adjustment or 'customisation' for the baby's sex and the mother's height, weight, ethnic origin and parity. For the purpose of this analysis, we defined SGA as two categories below the 10th and 5th birthweight centiles according to the GROW standard.

Although undiagnosed fetal growth restriction has been shown to be a significant factor in the unexplained stillbirth population[12,21] it was felt that without post-mortem exclusion of other pathologies the inclusion of these pregnancies would introduce a significant bias.

Data synthesis and statistical analyses

A logistic regression model was employed to assess the effects of FA supplementation and its timing on SGA. The model output variable was SGA, with input variables those of known clinical relevance, including maternal age, parity, ethnic origin, body mass index, history of mental health problems, pre-existing hypertension, pre-existing diabetes, cardiac disease, previous stillbirths, smoking in pregnancy, alcohol consumption, antenatal FA intake, late booking (≥13 weeks), gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, antepartum haemorrhage and past history of SGA. In addition to the index of multiple deprivation, we included maternal and paternal employment status as social factors in the multivariable analysis. Variables were entered using the backward stepwise method. In the multivariable analysis all variables reaching a 0.05 significance level were retained in the model.

Systematic review

Identification of studies, study selection, data extraction and quality assessment

We searched MEDLINE (1966–2013), EMBASE (1980–2013), Cochrane Library (2013:3), CINAHL (inception to 2013), MEDION, SIGLE, Index of scientific and technical proceedings, DARE and the British Nursing Index for relevant citations. The MEDLINE search is shown in the Appendix S1, this search strategy was adapted for use in the other electronic databases and the last search was performed in August 2013. A comprehensive database was constructed using ENDNOTE (Thomson Reuters, New York, NY, USA). In addition the reference lists of all known primary and review articles were examined to identify cited articles not captured by electronic searches. No language restrictions were applied.

Initially, the database was scrutinised by two reviewers (RKM or VH, partly in duplicate), and full articles of all citations that were likely to meet the predefined selection criteria were obtained. Translations of articles in languages other than English were obtained. Final inclusion or exclusion decisions were made through examination by two reviewers (RKM and VH), strictly adhering to the following criteria:

1 *Population*: all pregnant women.
2 *Intervention*: any supplement given at any gestation containing FA.
3 *Comparator*: no supplement containing FA.
4 *Outcome*: any outcome for fetal or neonatal birthweight [absolute measures of birthweight or birthweight centile (measure of SGA)]. Growth restriction by any measure as defined by authors. Outcomes had to exclude fetuses with chromosomal and structural anomalies and include only live births.
5 *Study design*: randomised controlled trials, and controlled and uncontrolled observational studies. Case reports and case series of fewer than five were excluded.

Data were extracted on study characteristics namely: population (inclusion/exclusion criteria, level of risk), study design (details of blinding, eligibility if cohort), methods of assessing FA supplementation (dose, timing, compliance and whether food fortification with FA supplementation was in place), outcome measures (threshold, methods, timing of measurement and method of pregnancy dating). A decision was made to include observational studies as well as randomised controlled trials to allow contemporary studies to be included. In current day obstetrics it would be unethical to randomise women to receive no FA treatment because of its proven benefit in preventing neural tube defects.[1] It is recognised that observational studies have inherent unique biases and so these were accounted for within the quality assessment. Information on study characteristics, quality and data to construct 2 × 2 tables was inputted onto an Excel spreadsheet, extraction was done in duplicate by two reviewers (VH, RKM). The 2 × 2 tables compared intervention (FA supplementation) with no FA intake according to the outcome examined. To optimise the completeness of the data the authors of the included studies were contacted and asked to provide extra information to allow completion of 2 × 2 tables and this was provided for one study.[22] Any disputes regarding data extraction were resolved by input from a third reviewer (KI). For multiple and/or duplicate publication of the same data set only the most recent and/or complete study was included. Where studies reported adjusted odds ratios (aOR) these were also extracted and reported.

All included manuscripts were assessed for study and reporting quality using validated tools (Cochrane tool for randomised controlled trials[23] and Reporting of Observational Studies in Epidemiology (STROBE) statement[18] for observational studies). Quality scores were not applied and nor was a subgroup analysis based on quality performed because of the different types of studies included. However, a qualitative assessment of the impact of poor quality on individual results was undertaken.

For the systematic review and meta-analysis, 2 × 2 tables were used to compute odds ratios (ORs) and their 95% confidence intervals (95% CIs). Studies were included in a meta-analysis if they used the same intervention (dose and timing) and outcome (measurement and threshold) and had the same study design i.e. randomised controlled trials and cohort studies were not combined. We plotted summary odds ratio data in forest plots (aORs where possible) and assessed the between-study heterogeneity in prognostic effect of each test by estimating I^2 (the amount of variability in prognostic effects due to between-study heterogeneity)[24] and tau-squared.[25] Due to the small number of

included studies in each analysis it was not possible to perform meta-regression. There were no zero cells requiring adjustment. The analyses were performed using statistical package STATA 11 and StatsDirect (Statsdirect Ltd, Cheshire UK).

Results

Population study

A total of 134 884 cases were recorded on PEER during the 3-year collection period (July 2009 to July 2012). Of these, 23 148 were stillbirths, multiple pregnancies or congenital anomalies and were excluded, leaving a total of 111 736 normally formed, singleton, live births for analysis.

Table 1 lists the characteristics of the study population. The West Midlands population in our cohort demonstrates many of the demographic characteristics of a heterogeneous UK population, with a mean maternal age of 28.7 years (SD 5.9), median body mass index of 24.7 kg/m^2 [interquartile range (IQR) 8.7 kg/m^2] and 42.4% primipara. The majority of women were non-smokers (n = 89 925, 81.7%). The median length of pregnancy was 40.0 weeks (IQR 13 days) and the median birthweight was 3380 g (IQR 680 g). Non-European ethnic group constituted 23.7% and 40.5% lived in the most socially deprived areas (index of multiple deprivation category 5).

Table 2 shows data for FA supplementation including dose and timing. Of the 111 736 pregnancies, 108 525 had information on FA supplementation with 84.9% (n = 92 133) of women reporting taking some form of FA supplementation during the pregnancy and 15.1% (n = 16 392) taking no supplementation. The dose of folate was recorded in only 40 955 cases; of these women 95.5% took 400 μg (n = 39 110) and 1416 (3.5%) took 5 mg. Reasons for taking higher doses were not recorded so data from this subgroup were not analysed further. Timing of commencement of FA supplementation was recorded in 39 416 cases; of these women 25.5% (n = 10 036) commenced preconceptually and 74.5% (n = 29 380) postconception.

Table 1. Characteristics of the study population, based on 111 736 singleton pregnancies with live birth and no congenital anomalies in the West Midlands between July 2009 and July 2012

	Data field completed	*n*	%	Mean	SD	Median	IQR
Maternal age (years)	111 729			28.7	5.9	28.6	8.7
Age>35 years	111 729	17 744	15.9				
Primipara	111 682	47 383	42.4				
Non-European ethnic group	111 736	26 524	23.7				
Body mass index (BMI)	111 717			25.9	5.7	24.7	6.8
BMI > 30 kg/m^2	111 717	22 679	20.3				
Mother not employed	106 487	42 344	39.8				
Partner not employed	93 763	14 627	15.6				
No partner	106 572	4083	3.8				
High deprivation index (IMD Q 5)	107 094	43 363	40.5				
Baby's father is blood relation	101 982	5889	5.8				
Mother smoker	110 064	20 139	18.3				
Other smoker in household	103 514	31 607	30.5				
Nonprescription drug intake	109 208	1074	1.0				
Previous stillbirth	111 736	1175	1.1				
Pre-existing diabetes	110 454	776	0.7				
Pre-existing hypertension	110 670	2745	2.5				
Heart disease	110 663	2310	2.1				
Mental health problems	110 346	13 504	12.2				
Late booking (≥13 weeks)	110 056	16 502	15.0				
Pregnancy-induced hypertension	108 860	5576	5.1				
Pre-eclampsia	108 086	1102	1.0				
HELLP syndrome	107 803	72	0.1				
Gestational diabetes	108 877	3375	3.1				
Antepartum haemorrhage	109 098	8891	8.1				
Gestation (days)	111 605			277.8	12.8	280.0	13.0
Birthweight (g)	111 362			3362.5	551.9	3380.0	680.0

HELLP, haemolysis, elevated liver enzymes, low platelet count; IMD, index of multiple deprivation; IQR, interquartile range; SD, standard deviation.

Table 2. Folic acid supplementation, dose and timing (n = 111 736) women giving birth to a singleton, live birth with no congenital anomalies in the West Midlands between July 2009 and July 2012

		n	%
Folic acid taken	Intake recorded	108 525	100.0
Yes		92 133	84.9
No		16 392	15.1
Dose of folic acid	Dose recorded	40 955	100.0
400 μg		39 110	95.5
5 mg		1416	3.5
Other dose		429	1.0
Timing of folic acid intake	Timing recorded	39 416	100.0
Preconception		10 036	25.5
Postconception		29 380	74.5

The overall proportions of babies with a birthweight <10th and <5th customised centile were 13.4% (14957) and 7.0% (7783), respectively. Table 3 looks at the relationship between FA use and timing of commencement, in the pregnancies where the recommended dose of 400 μg was taken, with an SGA neonatal outcome. The highest rate of SGA occurred in pregnancies where no folate had been taken, with 16.3% <10th centile and 8.9% <5th centile. When comparing preconception and postconception FA supplementation, the prevalence of birthweight <10th customised centile was 9.9% and 13.8%, respectively while that of birthweight <5th customised centile was 4.8% and 7.1%, respectively. Therefore, preconceptual FA was associated with a reduced risk of BW <10th customised centile (OR 0.56, 95% CI 0.52–0.61) and birthweight <5th customised centile (OR 0.51, 95% CI 0.46–0.58). Commencing supplementation postconception had a lesser but still significant protective effect according to unadjusted odds ratios (OR 0.82, 95% CI 0.77–0.87) for birthweight <10th centile, and (OR 0.78, 95% CI 0.72–0.84) for birthweight <5th centile (Table 3).

Multivariable logistic regression analysis demonstrated that the protective effect of FA in reducing the risk of SGA remained after adjusting for all potential confounding variables (aOR 0.80; 95% CI 0.71–0.90; $P < 0.01$) for birthweight <10th customised centile; aOR 0.78 (95% CI 0.66–0.91) $P < 0.01$ for birthweight <5th customised centile. This relates to a number needed to treat (NNT) of 15 (birthweight <10th centile) and 24 (birthweight <5th centile). The beneficial effect was lost if supplementation commenced postconception in the adjusted analysis.

Systematic review

Literature identification and study characteristics

The process of literature searching and study selection is illustrated in Figure 1. There were 13 primary articles that met the selection criteria.[22,26–36] This gave a population of 188 796 neonates. The characteristics of the included studies are shown in Table 4. Of the 13 studies, three were randomised controlled trials,[30,31,36] nine cohort studies and one case–control study.[34] The intervention was any supplementation containing FA against the comparator of no supplementary FA intake. It was agreed a priori that the comparator group could be taking any other supplements or a multivitamin as long as it did not contain FA. Therefore, we included one study that compared an iron + FA preparation against iron[32] and another study that compared FA supplementation against a trace element group.[31] Only one study was performed in a country with food fortification in place at the time of recruitment.[28] The major-

Table 3. Preconception and postconception folate intake and SGA rates in pregnancies with 400 μg folate intake and time of commencement known (n = 29 857), versus no folate intake (n = 16 392)

	Folate commenced	*n*	SGA *n*	SGA %	OR	95% CI	*P*-value	aOR*	95% CI
SGA <10th centile	Preconception	7556	748	9.9	0.56	0.52–0.61	<0.01	0.80	0.71–0.90
	Postconception	22 301	3075	13.8	0.82	0.77–0.87	<0.01	0.96	0.89–1.04
	No folate taken	16 392	2678	16.3	Reference		Reference		
SGA <5th centile	Preconception	7556	362	4.8	0.51	0.46–0.58	<0.01	0.78	0.66–0.91
	Postconception	22 301	1581	7.1	0.78	0.72–0.84	<0.01	0.91	0.82–1.01
	No folate taken	16 392	1463	8.9	Reference		Reference		

Chi-square test with Yate's correction.

*Adjusted odds ratio from logistic regression analysis, adjusted for maternal age, ethnicity, parity, body mass index, mother's and partner's employment status, consanguinity, late booking, social deprivation (using index of multiple deprivation), active and passive smoking, non-prescription drug intake, previous stillbirth, pre-existing hypertension, pregnancy-induced hypertension; pre-eclampsia, pre-existing and gestational diabetes.

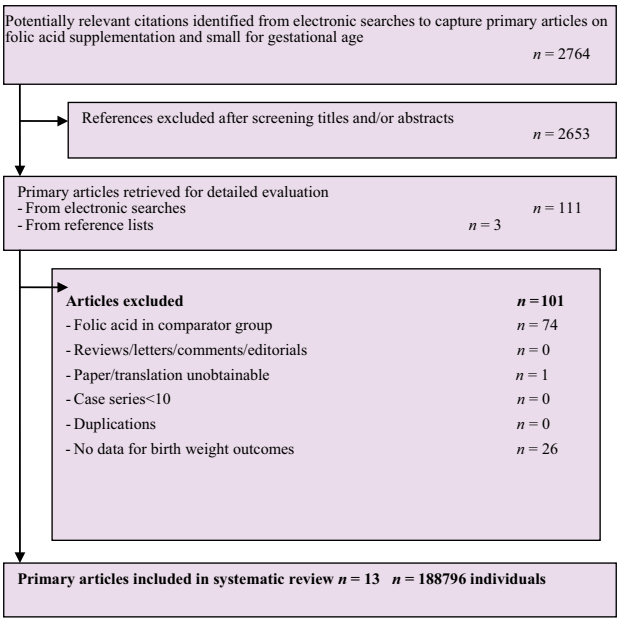

Figure 1. Process from initial search to final inclusion for folic acid supplementation and small for gestational age (up to August 2013).

ity of studies evaluated FA commenced after conception and four studies specifically included only preconception FA, these studies were Catov et al.[28,29], Timmermans et al.[22] and Morris 2014. We specifically evaluated the two Catov et al.[28,29] papers to confirm that there was no overlap of study participants to avoid duplication of data; the participants were drawn from two different population cohorts' databases. The dose of FA supplementation varied from 200 μg to 15 mg daily.

Study quality

The overall quality of included studies was variable. Table 5 summarises quality assessment for each of the individual studies. There are three randomised controlled trials in the included studies and all demonstrated a high risk of bias in masking of participants.[30,31,36] Rolschau et al.[36] particularly had difficulty reducing bias and were unable to randomise women fully to treatment groups, this was mainly because FA is an established treatment for the prevention of NTD and it would have been unethical to withhold FA treatment from women. The randomised controlled trial performed by Charles et al.[30] is a re-analysis of a randomised controlled trial performed in 1966/67 which was devised to primarily evaluate FA supplementation in the prevention of NTD, therefore, it was difficult to fully assess bias.

Included studies generally had an inadequate description of the population demographics and pregnancy risk assessment of participating women. We are aware that differences between populations can introduce bias into the results. Therefore, where the population was defined as 'high risk' such studies have only been included in the primary data collection and not in the meta-analysis, and hence allowing a broad overview of the literature on this topic. We reported all published adjusted odds ratios, allowing the reader to understand the potential bias in cohort studies and evaluate differences between adjusted odds ratios and odds ratios. In the majority of papers the adjustment of the odds ratios did not change the significance of the odds ratios and their 95% CI. However, in Catov et al. 2007,[28] the significant association between preconceptual FA supplementation and birthweight disappeared when this was adjusted for gestational age at delivery, smoking, education, parity and body mass index. Conversely, this effect was not lost when the data from Morris 2014 was adjusted.

The data that were meta-analysed in the preconceptual FA supplementation group were obtained from population cohorts and had to rely on maternal reporting with no monitoring of compliance and hence could have been subject to report bias.

Data analysis

The results for the individual studies are shown in the Supporting information (Table S1) along with the reported adjusted odds ratios. Due to variations in timing and dose range of FA across the included papers, it was only possible to perform four independent meta-analyses. On review of the reported odds ratios Table S1 (Supporting Information); timing of FA supplementation seems to have an impact on the results with preconceptual FA having a significant odds ratio and post-conception FA or mixed timing population groups having nonsignificant odds ratios. [Iyengar[32] demonstrated a significant odds ratio (0.54; 95% CI 0.32–0.89) for FA being commenced after conception but this effect size is likely to be confounded by the intervention being iron and FA versus iron]. We can only speculate that the inclusion of iron in the intervention and comparison group is likely to have its own effect on preventing SGA via its ability to prevent anaemia.[37] This paper was published in 1975 without reporting of adjusted odds ratios, and was not included in any meta-analysis.

Figure 2 shows the results for the two meta-analyses for the outcome of birthweight <5th centile. For preconceptual low-dose FA supplementation, there were four studies eligible but as one of them[28] was performed in the USA when food fortification was in place this was excluded from the meta-analysis. Hence, the analysis included three studies with a total of 112 510 fetuses. The chi-square test suggested significant heterogeneity, so sensitivity analyses were performed with Catov et al. 2011[29] excluded because of the intervention being a multi-vitamin-containing FA; again with Morris 2014

Table 4. Characteristics of included studies in systematic review of folic acid supplementation and birthweight outcome

Author and year	Country	Population	Design	Total included	Intervention	Comparison	Outcome and pregnancy dating
Alwan 2010[26]	United Kingdom	Low-risk mothers, 18–45 years. Recruited 8–12 weeks between 2003 and 2006	Prospective cohort interview and questionnaire (interviewer administered—1st/ 3rd trimester, self-administered 2nd trimester)	1259	22 different supplements. Variety of dosages. 'Most contain folic acid'	No supplementation	Birth weight <3rd customised centile (adjusted for maternal height, ethnicity, parity, neonatal gestation and sex). Dating via first trimester ultrasound
Banhidy 2010[27]	Hungary	Pregnant women with pre-eclampsia 1980–1996	Retrospective cohort study	2087	Folic acid average dose 5.6 mg daily	No supplementation	Birth weight <2500 g. Dating via LMP>
Catov 2007[28]	United States of America	Women <16 weeks, excluding pre-existing diabetes/ hypertension and multiple pregnancy 1997–2001 (Food fortification in place)	Prospective cohort	1823	Periconceptual (2 weeks before) multivitamin use	No supplementation	SGA <5th centile. Dating via early pregnancy ultrasound
Catov 2011[29]	Denmark	Danish population—all pregnant women 1997–2003	Retrospective cohort study with 30% of Danish population	35 897	Multivitamin containing folic acid	No supplementation	SGA birth weight – 2SD mean for a given gestational age. Dating via first trimester ultrasound
Charles 2005[30]*	United Kingdom	Re-analyses of data on folic acid to prevent neural tube defects, included all pregnant women booking for antenatal care under 30 weeks gestation. 1966–1967	Double blind randomised controlled trial. Post conception folic acid	1007	Folic acid 200 μg, Folic acid 5 mg.	No supplementation	LBW <2500 g. Dating via LMP
Czeizel 1994[31]	Hungary	Women with no history of delayed conception or infertility	Blind randomised controlled trial	4672	Multivitamin containing 0.8 mg folic acid	Trace element (manganese, zinc and vitamin C)	LBW no definition. Dating via LMP
Iyengar 1975[32]	India	Women 20–28 weeks pregnant, belonging to low-income groups	Prospective cohort	500	Iron 60 mg plus folic acid 500 μg daily.	Iron 60 mg	LBW <2500 g. Dating via LMP
Morris 2014	United Kingdom	All pregnant women July 2009–July 2012	Retrospective population-based cohort study	111 736	Folic acid 400 μg daily	No folic acid supplementation	SGA <10th customised centile SGA <5th customised centile. Dating via first-trimester ultrasound

Table 4. (Continued)

Author and year	Country	Population	Design	Total included	Intervention	Comparison	Outcome and pregnancy dating
Nilsen 2010[33]	Norway	Random cohort from women entered on Norwegian registry who had donated a blood sample at ultrasound appointment and had completed a food frequency questionnaire. July 2002 to December 2003	Retrospective cohort study	2934	Folic acid ≥400 μg	No supplementation	SGA <10th centile. Dating via combination of LMP and second trimester ultrasound
Palma 2008[38]	Spain	Delivery of LBW infant <2500 g with no congenital anomalies and received prenatal care. Women anaemic at booking were excluded. 1998–2002	Retrospective case–control	108	Folic acid 15 mg daily	No supplementation	LBW <2500 g. Dating was not described
Papadopoulou 2013[35]	Greece	Female residents of Heraklion, Crete who were pregnant for 12-month period from February 2007, >16 years of age, first visit 10–13 weeks gestation, singleton pregnancy	Prospective population-based cohort study	1122	Folic acid 5 mg daily	No supplement or iron supplementation	LBW <2500 g and SGA <10th centile. Dating via first trimester ultrasound
Rolschau 1999[36]	Denmark	Danish female citizens planning a pregnancy or pregnant. Previous neural tube defects were excluded. Singletons only. January 1983 to March 1986	Randomised control trial, not blinded	13 860	Folic acid 1 mg or folic acid 2.5 mg daily	No supplementation	LBW <2550 g and SGA defined by birthweight 2SD mean. Dating via LMP and first-trimester ultrasound
Timmermans 2009[22]	Netherlands	Women resident in study area (April 2002 and January 2006) singletons only	Prospective population-based cohort	8791	Folic acid 400–500 μg daily	No supplementation	LBW <2550 g and SGA defined by birthweight -2SD mean. Dating via first-trimester ultrasound

LBW low birth weight; LMP last menstrual period; SGA small for gestational age; SD standard deviation.
*Original trial performed between 1966–1967.

Table 5. Assessment of quality of included studies

Author and year	Quality measure	Outcome	Notes
Alwan 2010[26]	STROBE	Meets all 22 statements	
Banhidy 2010[27]	STROBE	Meets all 22 statements	
Catov 2007[28]	STROBE	21/22 statements STROBE meet criteria	Loss to follow up not explained
Catov 2011[29]	STROBE	21/22 statements STROBE meet criteria	Loss to follow up not explained
Charles 2005[30]	Cochrane risk of bias assessment	3/6 low-risk bias	Masking blinding of participants high-risk bias. Unsure of risk of bias as primary study and data collected in 1960s
Czeizel 1994[31]	Cochrane risk of bias assessment	1/6 low-risk of bias	High risk of bias in allocation concealment and masking. Unclear risk of bias in selective outcome reporting and other sources of bias
Iyengar 1975[32]	STROBE	19/22	Does not meet recommendations in statistics, participants, descriptive data and main results
Morris 2014	STROBE	Included in this paper. 20/22	Does not address bias and deficient in data sources/measurement
Nilsen 2010[33]	STROBE	Meets all 22 statements	
Palma 2008[34]	STROBE	21/22 statements STROBE meet criteria	Deficient in description of matching
Papadopoulou 2013[35]	STROBE	Meets all 22 statements	
Rolschau 1999[36]	Cochrane risk of bias assessment	1/6 low-risk bias	High risk of bias in allocation concealment and masking. Unclear risk of bias in selective outcome reporting and other sources of bias. Not all participants were randomised
Timmermans 2009[22]	STROBE	Meets all 22 statements	

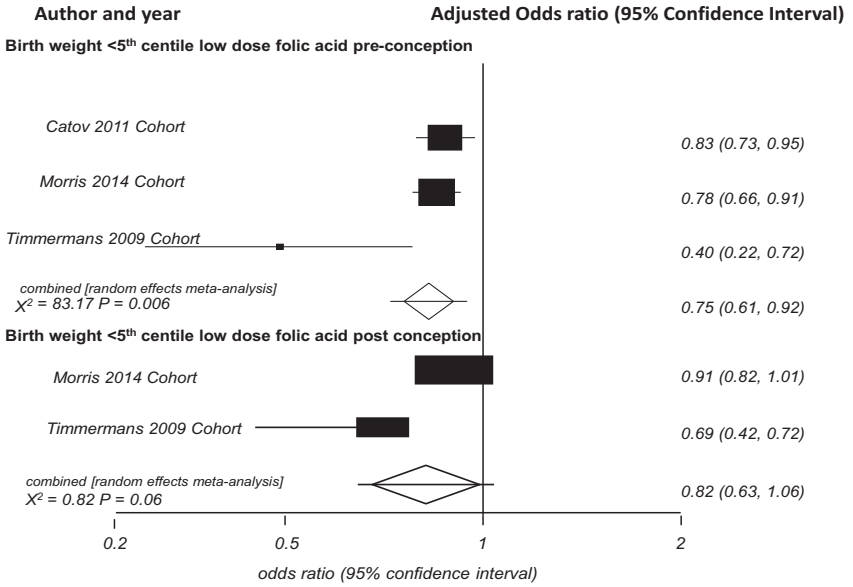

Figure 2. Meta-analysis folic acid supplementation and birthweight <5th centile.

excluded to assess the effect of smaller studies, these did not significantly change the overall result. Hence the meta-analysis for pre conceptual low-dose FA supplementation and SGA <5th centile demonstrated a significant adjusted odds ratio of 0.75 (95% CI 0.61–0.92; $P < 0.006$). A meta-analysis of postconceptual low-dose

FA and birthweight <5th centile, included two studies, Morris 2014 and Timmermans et al.[22] with 92 850 fetuses included giving a non-significant adjusted odds ratio of 0.82 (95% CI 0.63–1.06; $P = 0.06$).

Figure S1 (see Supporting information) shows the results for the meta-analysis of birthweight <10th centile, with low--dose FA started at any gestation. This meta-analysis included two studies, Morris 2014 and Nilsen et al.[33] with 72 474 fetuses and yielded an odds ratio of 0.98 (95% CI 0.94–1.02).

Figure S2 (see Supporting information) shows the meta-analysis for birthweight <2500 g, high-dose FA post conception, with two randomised controlled trials—Charles et al.[30] and Rolschau et al.[36]—evaluating 8682 fetuses with an odds ratio of 0.88 (95% CI 0.72–1.07) and one cohort study (not included in the meta-analysis)—Palma et al.[34]—evaluating 95 fetuses with an odds ratio of 1.26 (95% CI 0.45–3.44).

Discussion

Main findings

Our study examines the association of FA supplementation with the SGA neonate and the importance of timing of supplementation via a large multi-ethnic population study and a systematic review with meta-analysis. The population study is the largest database to demonstrate an effect of the timing of folate intake on the risk of a baby being SGA, with a significant reduction in this risk, only if FA supplementation is started before conception. Our systematic review is the first to focus on the timing of FA supplementation in the prevention of SGA and confirms the findings of our population study. Indeed, this meta-analysis demonstrated that preconceptual FA supplementation was associated with a significant reduction in the risk of SGA <5th centile (a category that is more likely to include babies that are growth restricted). This finding is of particular clinical relevance because growth restriction is known to be associated with poor outcomes in the short-term and long-term and there are no established preventive treatment options for such clinical conditions. Moreover, this study supports the view that fetal growth is mediated or modified through an epigenetic mechanism that can be explored further in basic science research.

Our data represent an unselected population within the West Midlands NHS region of the UK and correlates well with the recently published data from women undergoing Down syndrome screening within regions of the UK (excluding the West Midlands).[3] In our analysis, the protective effect of preconceptual FA remained significant after adjustment for other factors such as smoking. The protective effect of periconceptual FA in preventing SGA in smokers has also been reported in the Dutch generation R cohort.[38] This is of particular interest in view of the growing evidence that smoking seems to be associated with changes in the fetal DNA methylation.[39–41]

Strengths and limitations

The strengths of the population study are in the large size of the database, the use of customised growth centiles, the diversity of the population included and the large number of variables that could be adjusted for in the logistic regression analysis. SGA was previously defined via population centiles but the use of customised centiles (as previously described) has been shown to identify small babies at higher risk of morbidity and mortality than those identified by population centiles.[12,42] The database also includes information about factors known to influence fetal growth, which enabled us to adjust for these known confounders. Weaknesses include missing data regarding dosages, lack of information about reason for taking high-dose FA, lack of dietary information to determine folate availability, and reliance on participants' recall with regards to timing, dose and compliance with regards to FA supplementation. We also report only one pregnancy outcome (SGA) and the potential effect of FA on other pregnancy outcomes needs to be considered via randomised controlled trials. The Generation R study demonstrated that periconceptual FA supplementation was associated with higher systolic and diastolic blood pressures during pregnancy but neither the patterns of blood pressure change during pregnancy, nor the risk of gestational hypertension and preeclampsia differed between the FA categories.[43] The role of postconceptual FA in preventing preeclampsia is currently being examined via the FACT trial.[41] Previous studies have suggested that the increase in birthweight seen with FA supplementation in pregnancy[30] might lead to an increase in rates of macrosomia and hence caesarean section delivery.

The strength of the systematic review lies in the methodology employed. We are confident that we have included all available studies and have contacted authors for further data to produce the most comprehensive assessment possible. All studies within the meta-analysis for birthweight <5th centile also used first-trimester ultrasound to date pregnancies, improving the accuracy of SGA diagnosis.

We evaluated customised birthweight centile where possible and excluded congenital anomalies and stillbirths. The limitations of this review lie in the bias in the primary studies. Most of the information is derived from population cohorts where there is a risk of different sources of bias particularly with regards to confounders, case selection and information recall. Nevertheless, both studies in the preconceptual analysis (Timmermans et al.[22] and Morris) have adjusted the odds ratio to take account of these differences and exclude confounders. Indeed, with this adjust-

ment, the odds ratios show no effect with post-conception folate but remain significant when FA is commenced before conception.

Interpretation

Through one-carbon metabolism, FA is an important source of methyl groups and hence can influence DNA methylation. Studies reported on links between SGA and DNA methylation, both globally and at a gene-specific level.[16,17] It is also recognised that the epigenome is particularly susceptible during early stages of embryogenesis.[43] Although speculative, it is plausible that the effect of preconceptual FA supplementation on birthweight is mediated through a fetal epigenomic modification in the early stages of embryogenesis.

Due to unavailability of enough data, we were not able to investigate if there is a dose–response relationship with regards to preconceptual FA supplementation in our systematic review. However, we were able to analyse high--dose postconception FA against the outcome of birthweight <2500 g, which generated a nonsignificant OR. This suggests that even when a high dose is given post conception there is no effect on birthweight outcome. Rolschau et al. evaluated two different doses of FA (1 mg and 2.5 mg) commenced preconceptually and in the first 19 weeks on pregnancy outcomes.[36] Although they found no appreciable difference in outcomes between the different dose groups, their findings suggested that the timing of FA supplementation was important in preventing SGA. Therefore, although it is possible that there is additional benefit in higher dosages of FA prescribed preconceptually, particularly in groups at higher risk of SGA, one must be careful in drawing any conclusions regarding such policies without rigorous assessment and follow up, particularly because of the possibility that we might be influencing epigenetic changes that could be passed down to several generations even after the initial stimulus has stopped.[10]

Conclusion

Preconceptual FA supplementation is associated with a significant reduction in the risk of SGA. Although FA supplementation before pregnancy and throughout the first trimester is a standard recommendation in the UK to reduce the risk of NTD, it is a policy that is poorly followed. Interventions to improve the implementation of such policy can have significant public health implications given the negative consequences of SGA in the short and longer term. There is a need to understand the mechanism by which FA produces its effect on birthweight and to optimise the dose of FA supplementation in women considered to be at higher risk of SGA. Dose optimisation is particularly relevant to the on-going international debate about food fortification policies.

Practical and research recommendations

1 Folic acid supplementation is recommended preconceptually and uptake has been shown to be low in a population undergoing Down syndrome screening in the UK. Therefore we should evaluate strategies to increase preconceptual uptake.
2 Previous studies have shown an association between FA supplementation and increased absolute birthweight but not assessed effect on SGA births nor assessed timing and dose of supplementation. This should be an area for further research.

Disclosure of interests

The authors have no conflicts of interest to disclose.

Contribution to authorship

The idea of the population study and the systematic review was conceived by KI. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work were made by VAH, RKM, AF, JG and KI. VAH, RKM, AF, JG and KI were also responsible for drafting the work or revising it critically for important intellectual content and for final approval of the version to be published; they agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Details of ethics approval

Ethics approval for this study was not required because data for the population study were collected with patient consent and were pseudo anonymised before analysis.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Meta-analysis of folic acid supplementation and birthweight <10th centile (low dose, any gestation).

Figure S2. Meta-analysis of folic acid supplementation and birthweight <2500 g (high dose, post-conception).

Table S1. Folic acid supplementation and effect on birthweight outcomes, individual results from included studies

Appendix S1. Search strategy for systematic review. ■

References

1 Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992;327:1832–5.

2 Expert Advisory Group. *Folic acid and the prevention of neural tube defects: report from an expert advisory group*. Heywood: DOH Health Publication Unit; 1992. p. 21.

3 Bestwick JP, Huttly WJ, Morris JK, Wald NJ. Prevention of neural tube defects: a cross-sectional study of the uptake of folic acid supplementation in nearly half a million women. *PLoS ONE* 2014;9: e89354.

4 Tort J, Lelong N, Prunet C, Khoshnood B, Blandel B. Maternal and health care determinants of preconceptional use of folic acid supplementation in France: results from the 2010 National Perinatal Survey. *BJOG* 2013;120:1661–7.

5 Crider KS, Bailey LB, Berry RJ. Folic acid food fortification-its history, effect, concerns, and future directions. *Nutrients* 2011;3:370–84.

6 McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. *N Engl J Med* 1999;340:1234–8.

7 Waterland R. Is epigenetics an important link between early life events and adult disease? *Horm Res* 2009;71(Suppl 1):13–16.

8 Tosh DN, Fu Q, Callaway CW, McKnight RA, McMillen IC, Ross MG, et al. Epigenetics of programmed obesity: alteration in IUGR rat hepatic IGF1 mRNA expression and histone structure in rapid vs delayed postnatal catch-up growth. *Am J Physiol Gastrointest Liver Physiol* 2010;299:G1023–9.

9 Schlotz WPD, Phillips DI. Fetal origins of mental health: evidence and mechanisms. *Brain Behav Immun* 2009;23:905–16.

10 Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KM. Epigenetic control of fetal gene expression [Review] [127 refs]. *BJOG* 2008;115:158–68.

11 Weinstock M. The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain Behav Immun* 2005;19:296–308.

12 Clausson B, Gardosi J, Frances A, Cnaatingius S. Perinatal outcome in SGA births defined by customised versus population–based birthweight standard. *BJOG* 2001;108:830–4.

13 Lassi ZS, Salam RA, Haider BA, Bhutta ZA. Folic acid supplementation during pregnancy for maternal health and pregnancy outcomes. *Cochrane Database Syst Rev* 2013;3:CD006896.

14 Fekete K, Berti C, Trovato M, Lohner S, Dullemeijer C, Souverein OW, et al. Effect of folate intake on health outcomes in pregnancy: a systematic review and meta-analysis on birth weight, placental weight and length of gestation. *Nutr J* 2012;11:75.

15 Bergen NE, Jaddoe VW, Timmermans S, Hofman A, Lindemans J, Russcher H, et al. Homocysteine and folate concentrations in early pregnancy and the risk of adverse pregnancy outcomes: the Generation R Study. *BJOG* 2012;119:739–51.

16 Fryer AA, Emes RD, Ismail KM, Haworth KE, Mein C, Carroll WD, et al. Quantitative, high-resolution epigenetic profiling of CpG loci identifies associations with cord blood plasma homocysteine and birth weight in humans. *Epigenetics* 2011;6:86–94.

17 Fryer AA, Nafee TM, Ismail KM, Carroll WD, Emes RD, Farrell WE. LINE-1 DNA methylation is inversely correlated with cord plasma homocysteine in man: a preliminary study. *Epigenetics* 2009;4: 394–8.

18 von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;370:1453–7.

19 Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 2009;338:b2535.

20 GROW (Gestation Related Optimal Weight): customised antenatal growth chart software; Gestation Network, version .6.0(UK), 2014. [www.gestation.net]. Accessed 10 January 2014.

21 Gardosi J, Madurasinghe V, Williams M, Malik A, Francis A. Maternal and fetal risk factors for stillbirth: populaton based study. *BMJ* 2013;346:f108.

22 Timmermans S, Jaddoe VWV, Hofman A, Steegers-Theunissen RPM, Steegers EAP. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. *Br J Nutr* 2009;102:777–85.

23 Collaboration TC. *Cochrane Handbook for Systematic Reviews of Interventions: The Cochrane Collaboration*. Higgins JPT, Green S, editors. Collaboration TC, 2008. [http://www.cochrane-handbook.org.] Accessed September 2013

24 Higgins JP, Thompson SG, Deeks JJ. Measuring inconsistencies in meta-analyses. *BMJ (Clin Res Ed)* 2003;327:557–60.

25 Higgins JT. Heterogeneity in meta-analysis should be expected and appropriately quantified. *Int J Epidemiol* 2008;37:1158–60.

26 Alwan NA, Greenwood DC, Simpson NA, McArdle HJ, Cade JE. The relationship between dietary supplement use in late pregnancy and birth outcomes: a cohort study in British women. *BJOG* 2010;117:821–9.

27 Banhidy F, Dakhlaoui A, Dudas I, Czeizel AE. Birth outcomes of newborns after folc acid supplementation in pregnant women with early and late pre-eclampsia: a population based study. *Adv Prevent Med* 2011;127369.

28 Catov JM, Bodnar LM, Ness RB, Markovic N, Roberts JM. Association of periconceptional multivitamin use and risk of preterm or small-for-gestational-age births. *Am J Epidemiol* 2007;166:296–303.

29 Catov JM, Bodnar LM, Olsen J, Olsen S, Nohr EA. Periconceptional multivitamin use and risk of preterm or small-for-gestational-age Births in the Danish National birth cohort. *Am J Clin Nutr* 2011;94:01.

30 Charles DH, Ness AR, Campbell D, Smith GD, Whitley E, Hall MH. Folic acid supplements in pregnancy and birth outcome: re-analysis of a large randomised controlled trial and update of Cochrane review. *Paediat Perinat Epidemiol* 2005;19:112–24.

31 Czeizel AE, Dudas I, Metneki J. Pregnancy outcomes in a randomised controlled trial of periconceptional multivitamin supplementation. Final report. *Arch Gynecol Obstet* 1994;255:131–9.

32 Iyengar LR, Rajalakshmi K. Effect of folic acid supplement on birth weights of infants. *Am J Obstet Gynecol* 1975;122:332–6.

33 Nilsen RM, Vollset SE, Monsen ALB, Ulvik A, Haugen M, Meltzer HM, et al. Infant birth size is not associated with maternal intake and status of folate during the second trimester in Norwegian pregnant women. *J Nutr* 2010;140:572–9.

34 Palma S, Perez-Iglesias R, Prieto D, Pardo R, Llorca J, Delgado-Rodriguez M. Iron but not folic acid supplementation reduces the risk of low birthweight in pregnant women without anaemia: a case control study. *J Epidemiol Commun Health* 2008;62:120–4.

35 Papadopoulou E, Stratakis N, Roumeliotaki T, Sarri K, Merlo DF, Kogevinas M, et al. The effect of high-doses of folic acid and iron supplementation in early-to-mid pregnancy on prematurity and fetal growth retardation: the mother-child cohort study in Crete, Greece (Rhea study). *Eur J Nutr* 2013;52:327–36.

36 Rolschau J, Kristoffersen K, Ulrich M, Grinsted P, Schaumburg E, Foged N. The influence of folic acid supplement on the outcome of pregnancies in the county of Funen in Denmark. Part I. *Eur J Obstet Gynecol Reprod Biol* 1999;87:105–10.

37 Haider BA, Olofin I, Wang M, Spiegelman D, Ezzati M, Fawzi WW. Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis. *BMJ (Clin Res Ed)* 2013;346:f3443.

38 Bakker R, Timmermans S, Steegers EA, Hofman A, Jaddoe VW. Folic acid supplements modify the adverse effects of maternal smoking on fetal growth and neonatal complications. *J Nutr* 2011;141:2172–9.

39 Furness DL, Yasin N, Dekker GA, Thompson SD, Roberts CT. Maternal red blood cell folate concentration at 10–12 weeks gestation and pregnancy outcome. *J Matern Fetal Neonat Med* 2012;25:1423–7.

40 Bakker R, Timmermans S, Steegers EAP, Hofman A, Jaddoe VWV. Folic acid supplements modify the adverse effects of maternal smoking on fetal growth and neonatal complications. *J Nutr* 2011;141:1.

41 Wen SW, Rennicks CJ, White R, Walker M. Effect of folic acid supplementation in pregnancy on preeclampsia– Folic acid clinical trial (FACT). *Pregnancy Hypertension* 2012;2:198.

42 Figueras F, Figuieras J, Meler E, Eixarch E, Coll O, Gratacos E, et al. Customised birthweight standards accurately predict perinatal morbidity. *Arch Dis Child Fetal Neonat Ed* 2007;92:277–80.

43 Timmermans S, Jaddoe V, Silva LM, Hofman A, Raat H, Steegers-Theunissen RPM, et al. Folic acid is positively associated with uteroplacental vascular resistance: The Generation R Study. *Nutr Metab Cardiovasc Dis* 2011;21:54–61.

