

Dietary iron intake during early pregnancy and birth outcomes in a cohort of British women

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BACKGROUND: Iron deficiency during pregnancy is associated with adverse birth outcomes, particularly, if present during early gestation. Iron supplements are widely recommended during pregnancy, but evidence of their benefit in relation to infant outcomes is not established. This study was performed in the UK, where iron supplements are not routinely recommended during pregnancy, to investigate the association between iron intake in pregnancy and size at birth.

METHODS: From a prospective cohort of 1274 pregnant women aged 18–45 years, dietary intake was reported in a 24-h recall administered by a research midwife at 12-week gestation. Dietary supplement intake was ascertained using dietary recall and three questionnaires in the first, second and third trimesters.

RESULTS: Of the cohort of pregnant women, 80% reported dietary iron intake below the UK Reference Nutrient Intake of 14.8 mg/day. Those reported taking iron-containing supplements in the first, second and third trimesters were 24, 15 and 8%, respectively. Women with dietary iron intake >14.8 mg/day were more likely to be older, have a higher socioeconomic profile and take supplements during the first trimester. Vegetarians were less likely to have low dietary iron intake [odds ratio = 0.5, 95% confidence interval (CI): 0.4, 0.8] and more likely to take supplements during the first and second trimesters. Total iron intake, but not iron intake from food only, was associated with birthweight centile (adjusted change = 2.5 centiles/10 mg increase in iron, 95% CI: 0.4, 4.6). This association was stronger in the high vitamin C intake group, but effect modification was not significant.

CONCLUSION: There was a positive relationship between total iron intake, from food and supplements, in early pregnancy and birthweight. Iron intake, both from diet and supplements, during the first trimester of pregnancy was higher in vegetarians and women with a better socioeconomic profile.

Key words: birthweight / pregnancy / preterm birth / iron / diet

Introduction

Iron deficiency during pregnancy is still common in developed countries (Beard, 1994; Milman *et al.*, 1998; Robinson *et al.*, 1998; Bergmann *et al.*, 2002). It is associated with adverse birth outcomes such as small for gestational age (SGA), preterm birth and delayed offspring neurological development, particularly if present during the first half of pregnancy (Zhou *et al.*, 1998; Scholl, 2005; Rao and Georgieff, 2007; Beard, 2008; Baker *et al.*, 2009). There is evidence from animal studies that low iron intake during pregnancy adversely affects the offspring's blood pressure, obesity levels and other cardiovascular

outcomes in the long term (Gambling *et al.*, 2003; Lisle *et al.*, 2003; Gambling *et al.*, 2004; Zhang *et al.*, 2005; Andersen *et al.*, 2006). Iron supplements are widely recommended and used during pregnancy worldwide (Centers for Disease Control and Prevention, 1998; World Health Organization, 2006). There are far more studies examining the effect of iron supplements during pregnancy than those measuring total dietary iron intake in the mother and investigating its association with birth outcomes (Preziosi *et al.*, 1997; Christian *et al.*, 2003; Cogswell *et al.*, 2003; Vaidya *et al.*, 2008). However, the evidence on what benefit iron supplements contribute to infant outcomes is still not established (Pena-Rosas and Viteri,

2009), and their routine use has drawbacks such as gastrointestinal side effects and interactions with other micronutrients especially if taken as part of a multivitamin-mineral supplement (Rossander-Hulten et al., 1991; O'Brien et al., 1999, 2000; Roughead and Hunt, 2000). Iron supplements can also reduce the absorption of dietary non-heme iron (Roughead and Hunt, 2000) and can increase oxidative stress and the production of free radicals (Casanueva and Viteri, 2003; Scholl, 2005). Therefore, they are not routinely recommended during pregnancy in the UK (NICE, 2008).

In the USA, a dietary iron intake of 27 mg/day during pregnancy is recommended (IOM, 2001). In the US National Health and Nutrition Examination Survey III, median iron intake in pregnant women was 15 mg/day (National Center for Health Statistics, 1994). In the UK, the Reference Nutrient Intake (RNI) for women aged 19–50 years is 14.8 mg/day, and Lower Reference Nutrient Intake (LRNI) is 8 mg/day, with no specific recommended increment during pregnancy (Department of Health, 1991). The RNI is the amount of a nutrient that is enough to ensure that the needs of 97.5% of the population are being met. LRNI is the amount adequate for only the small number of people who have low requirements (2.5%) (Department of Health, 1991). The mean daily dietary intake of total iron from the 2001 National Diet and Nutrition Survey in Great Britain was 10 mg for women aged 19–64 years (FSA, 2003). Around 25% of women aged 19–64 years, 41% of women aged <34 years and 53% of women receiving income-benefits had daily dietary iron intakes less than the LRNI. Such low levels of iron intake were also seen in other European countries such as Denmark (Andersen et al., 1995). There is evidence from nutritional surveys in the UK and Norway that women's dietary patterns change little with pregnancy (Trygg et al., 1995; Crozier et al., 2009). In the latter survey, 96% of pregnant women had an iron intake <18 mg/day with an average iron intake of 11 mg/day (Trygg et al., 1995). To meet the iron demand in pregnancy, women would need to make considerable changes in their dietary pattern, which some argue to be unrealistic, hence the recommendation of iron supplements. However, it has been shown that transfer of iron to the fetus is better in non-iron supplemented than in supplemented women (O'Brien et al., 2003).

Dietary iron occurs in two forms: heme and non-heme. About 95% of iron in the average British diet is in the form of non-heme iron (Food Standards Agency, 2003). The extent to which non-heme iron is absorbed is highly variable and depends on the individual's iron status and other dietary components. Ascorbic acid enhances non-heme iron absorption when consumed as part of a meal (Skikne and Baynes, 1994), while high calcium intakes during pregnancy might reduce non-heme iron absorption, leading to iron deficiency (Robinson et al., 1998). Heme iron comes mainly from meat. It has a higher bioavailability and is well absorbed. Its absorption is further facilitated by organic compounds present in meat called meat factors (Skikne and Baynes, 1994). Unlike that of the non-heme iron, heme iron absorption is influenced little by other dietary constituents. It also enhances non-heme iron absorption from other foods consumed at the same time. Recent evidence suggests that heme and non-heme iron may have different associations with individual health outcomes (Tzoulaki et al., 2008).

Results of studies investigating the relationship between dietary maternal iron intake during pregnancy and size at birth and/or gestational age are conflicting (Doyle et al., 1990; Haste et al., 1991; Scholl

et al., 1992; Scholl and Hediger, 1994; Godfrey et al., 1996; Mathews et al., 1999, 2004; Mitchell et al., 2004; Lagiou et al., 2005; Al-Shoshan, 2007; Baker et al., 2009). Many studies that have assessed total iron intake did not model the relationships separately for iron from food and that from dietary supplements. Neither did they consider the potential differential effects of heme and non-heme iron. One study assessed the relationship between ascorbic acid and anemia and well as vitamin C intake and iron status (Baker et al., 2009); however, the potential interaction between iron intake and the vitamin C intake and other micronutrients has not been explored (Gibney et al., 2004). The aims of this study were to investigate the association between maternal iron intake during early pregnancy and both birthweight and gestational age, to assess whether any relationships differ by source of iron (food versus dietary supplements) or by type of iron (heme versus non-heme), and to explore the role of vitamin C intake as an effect modifier.

Materials and Methods

Study design and participants

The Caffeine and Reproductive Health (CARE) study is a prospective birth cohort in which low-risk pregnant women aged 18–45 years with singleton pregnancies were prospectively recruited at 8–12-week gestation from the Leeds Teaching Hospitals maternity units between 2003 and 2006. This was part of a multicentre study into maternal diet and birth outcomes. Women with concurrent medical disorders, psychiatric illness, human immunodeficiency virus infection or hepatitis B infection were excluded. Eligible women were identified by screening their pre-booked maternity notes. They were then sent detailed information about the study and were asked to return a reply slip to state whether they were willing to take part. Those who agreed to participate were then interviewed. This interview was conducted either at the hospital, the participant's general practice, or her home by a research midwife. Demographic details were obtained using a self-reported questionnaire. Information was obtained from the hospital maternity records on antenatal pregnancy complications and delivery details (gestational age at delivery, birthweight and sex of the baby). Data on hemoglobin (Hb) levels and mean corpuscular volume (MCV) at 12 and 28 weeks pregnancy were available for a subsample of the cohort that was selected randomly from the main sample using study identification numbers. All women participating in the study gave written informed consent, and the study was approved by the Leeds West Local Research Ethics Committee (reference number 03/054).

Assessment of diet and supplement use

Supplement use was ascertained throughout pregnancy using questionnaires in the first, second and third trimesters. The questionnaires were interviewer administered during the first (up to 12-week gestation) and third trimester (from 28-week gestation) and self-administered during the second trimester (13–27-week gestation). The respondents were asked to report the type/brand, frequency and the amount of all the dietary supplements they were using during each trimester.

Dietary and supplement intake was reported through a 24-h dietary recall administered by a research midwife at 8–12-week gestation. Values for the proportion of heme iron in each type of meat were used to derive heme values for each of the food codes. These values were derived by recording the meat content of each product, together with food tables values (McCance, 1990), to calculate a weighted mean meat content of each food item consumed. A literature search was carried

out to arrive at 'heme factors' for different animal products that reflect the heme iron content of these foods. Values derived from the Schricker and modified Schricker methods, and the Hornsey method was used to calculate mean values for heme iron (Hornsey, 1956; Schricker and Stouffer, 1982). These values were then used to generate total iron values for each relevant food (O'Hara, 2004). The non-heme iron values were derived as the difference between total iron from food tables (McCance, 1990) and calculated heme values. Total iron was derived from adding dietary intake and supplement intake as reported in the recall. Iron content of each supplement reported was added to the dietary intake multiplied by total number of supplement tablets/capsules taken during the 24-h recall. Vitamin C intake from the diet was reported in the 24-h recall and categorized into above or equal to/below the RNI of 50 mg/day.

Assessment of outcomes

The two primary outcome measures were birthweight and preterm birth. Birthweight was measured in grams, and expressed as customized centile using charts that take into account gestational age, maternal height, weight, ethnicity and parity, and neonatal birthweight and sex (Gardosi, 2004). Duration of gestation was calculated from the date of the last menstrual period, and confirmed by ultrasound scans dating at around 12 and 20-week gestation. SGA was defined as less than the 10th centile for gestational age. Preterm birth was defined as delivery at <37-week (259 days) gestation.

Assessment of participants' characteristics

Socioeconomic status was assessed using the Index of Multiple Deprivation (IMD) score. The IMD 2007 combines a number of indicators (chosen to cover a range of economic, social and housing issues) into a single deprivation score for each small area in England. This allows each area to be ranked relative to one another according to their level of deprivation (Department for Communities and Local Government, 2009). IMD, however, is an area (not an individual) deprivation measure.

Mothers' educational level, smoking status, alcohol intake, parity, ethnicity, pre-pregnancy weight, past history of miscarriage, long-term chronic illness and vegetarian diet were self-reported in a first-trimester questionnaire. Salivary cotinine levels were measured using an enzyme-linked immunosorbent assay (Cozart Bioscience, Oxfordshire, UK). Participants were classified, on the basis of these cotinine concentrations, as active smokers (>5 ng/ml), passive/occasional smokers (1–5 ng/ml) or non-smokers (<1 ng/ml; CARE Study Group, 2008).

Statistical power calculations

Comparing birthweights between mothers with dietary iron intake of >14.8 mg/day (the recommended UK RNI for women of childbearing age) with those with ≤14.8 mg/day during the first trimester of pregnancy, using the ratios of the low-intake to the high-intake group and the standard deviation for birthweight identified in this study (SD = 577 g), we had 85% power to detect a difference of 120 g in birthweight between the two groups for $P < 0.05$ and a two-sided test.

Statistical methods

Univariable comparisons were made using Student's *t*-test for continuous variables and χ^2 test for categorical variables. Multiple linear regression using birthweight/customized birth centile as continuous outcomes, and unconditional logistic regression with preterm birth and SGA as binary outcomes were performed using STATA version 11 (College Station, TX, 2009).

Analyses were conducted using dietary iron intake as a continuous variable and a binary variable using the UK RNI cut-off of 14.8 mg/day. Total iron from diet and supplements, assessed by the 24-h recall, was analyzed as a continuous variable. Intake of iron-containing supplements was analyzed as a binary variable. Maternal height, weight, ethnicity, parity, neonatal gestation at delivery and baby's sex were taken into account in the definition for customized birth centile, and were adjusted for in the model for birthweight. Statistical adjustment was also made for maternal age, salivary cotinine levels and alcohol consumption. Sensitivity analyses for the linear model were performed by excluding vegetarians from the model, and adding an interaction term for daily vitamin C intake in the model. Subgroup analysis using the multiple linear model was performed using type of dietary iron (heme versus non-heme). Multiple linear regression was also used to explore the association between iron intake and Hb and MCV levels at 12 and 28 weeks of pregnancy.

Results

Iron intake

A total of 1257 women had dietary recall information in the first trimester. The mean dietary iron intake from food was 11.5 mg/day (SD = 5.3), with only 20% ($n = 257$) of women reporting intake >14.8 mg/day [95% confidence interval (CI): 18, 23%]. Women who reported iron intake less than or equal to the UK LRNI of 8 mg/day were 24% (95% CI: 22%, 27%). Only 4% reported a dietary iron intake of more than the US recommended intake during pregnancy of 27 mg/day (95% CI: 3, 5%). Mean heme iron intake was 0.6 mg/day (SD = 0.8). This estimate for heme iron changed little after excluding the 114 reported vegetarian participants (with a heme iron intake of zero). Mean non-heme iron intake was 10.9 mg/day (SD = 5.2; Table I).

In the recall, 20% of participants (95% CI: 18%, 22%) reported taking iron-containing supplements compared with 24% (95% CI: 22%, 26%) in the first trimester questionnaire (Kappa agreement = 0.85). 15% (95% CI: 13%, 18%) and 8% (95% CI: 7%, 10%) reported taking iron-containing supplements in the second and third trimester questionnaires, respectively. Mean total iron intake from diet and supplements, as recorded in the recall, was 16.5 mg/day (SD = 21.1). 34% (95% CI: 32%, 37%) of women had an iron intake >14.8 mg/day from diet and supplements. Only 11 participants reported taking iron-only preparations in the recall, which were assumed to be the conventional therapeutic preparation with a dose of 65 mg iron/tablet, and 5 reported taking a preparation of iron and folic acid that contains 100 mg iron per dose. Mean total iron excluding these 16 participants was 14.3 mg/day (SD = 8.4). Only 8, 21 and 29 participants reported taking iron-only supplements in the first, second and third trimester questionnaires, respectively.

Characteristics of women with high versus low iron intake groups

Women with dietary iron intake >14.8 mg/day were more likely to be older, report a higher total energy intake (Kcal/day), have a university degree, be vegetarian and take daily supplements during the first trimester, including iron-containing supplements. They were less likely to be smokers, live in an area with the worst IMD quartile or have a long-term illness (Table II). Vegetarian participants were less

Table I Average iron intake from food and dietary supplements as reported in first trimester 24-h dietary recall ($n = 1257$).

	Mean	Standard deviation	Median	Inter-quartile range
Iron intake from food (mg/day)	11.5	5.3	10.5	8.1, 13.7
Heme iron intake (mg/day)	0.6	0.8	0.3	0.1, 0.8
Non-heme iron intake (mg/day)	10.9	5.2	10	7.6, 13.0
Total iron from food and supplements (mg/day)	16.5	21.1	11.8	8.6, 19.1
Total iron from food and supplements excluding therapeutic iron preparations (≥ 65 mg/dose) (mg/day)	14.3	8.4	11.6	8.5, 18.6

likely to have dietary iron intake ≤ 14.8 mg/day [unadjusted odds ratio (OR) = 0.5, 95% CI: 0.4, 0.8, $P = 0.004$]. Vegetarians were also more likely to take iron-containing supplements during the first and second trimester (OR = 2.9, 95% CI: 2.0, 4.3, $P < 0.0001$ for the first trimester; OR = 2.9, 95% CI: 1.9, 4.4, $P < 0.0001$ for the second trimester).

Birth outcomes

There were 1259 babies with information on birthweight. Mean birthweight was 3439 g (SD = 577 g) with 4.4% of babies weighing < 2500 g ($n = 55$). Totally, 13% ($n = 166$) weighed less than the 10th centile, 8% ($n = 99$) less than the fifth centile and 5% ($n = 65$) less than the third centile. Babies weighing more than the 90th centile were 9% ($n = 118$). Of the 1234 pregnancies with information on gestational age, 55 (4.5%) delivered before 37-week gestation.

Relationship between blood indices and birth outcome

The number of participants who had information on Hb and MCV at 12 and 28-week gestation were 558 and 572, respectively. Mean Hb was 12.7 g/dl (SD = 0.9 g/dl) at 12 weeks and 11.5 g/dl (SD = 1 g/dl) at 28 weeks. The proportion of participants with Hb < 11 g/dl was 3% at 12 weeks and 23% at 28 weeks. Mean MCV was 90 fl (SD = 5.0 fl) at 12 weeks and 89 fl (SD = 5.5 fl) at 28 weeks. There was no relationship between customized birth centile or birthweight in grams and Hb/MCV at 12 or 28-week pregnancy in this study. Hb at 28 weeks was associated with SGA (unadjusted OR per g/dl increase in Hb = 1.4, 95% CI: 1.1, 1.8, $P = 0.02$; OR adjusted for maternal age, salivary cotinine levels and alcohol intake = 1.4, 95% CI: 1, 1.8, $P = 0.03$). Adjusting for dietary iron intake did not alter this relationship.

Relationship between blood indices and dietary intake

There was no relationship between Hb/MCV at 12 or 28-week pregnancy with dietary iron intake in the first trimester. However, there was a positive relationship between taking iron-containing supplements as reported in the first trimester questionnaire and Hb at 12 and 28 weeks, and MCV at 28 weeks. The relationship remained significant for Hb at 12 and 28 weeks after adjusting for maternal age, ethnicity, parity, educational attainment, vegetarian diet and

IMD score in multiple linear regression model. Taking iron-containing supplements in the second trimester was also positively associated with Hb at 28 weeks (Table III).

Relationship between iron intake and birthweight

Dietary iron intake from food was significantly related to birthweight measured on the customized birth centile (unadjusted change per 10 mg/day increase in dietary iron intake during the first trimester = 5.2 centile points, 95% CI: 2.2, 8.2, $P = 0.001$). Adjusting for maternal age, salivary cotinine levels and alcohol intake attenuated this relationship (adjusted change = 3.1 centile points, 95% CI: -0.2, 6.3, $P = 0.07$; Table IV). The estimate changed little when excluding vegetarians, or including calcium or zinc intake as interaction terms with iron intake (data not shown). Considering birthweight in grams as an outcome, the unadjusted change per 10 mg/day increase in dietary iron intake was 70 g (95% CI: 10, 130, $P = 0.02$). When adjusting for maternal age, cotinine levels, alcohol intake, maternal weight, height, parity, ethnicity, gestational age and baby's sex, the change was 34 g (95% CI: -13, 80, $P = 0.2$).

There was no relationship between heme iron intake and customized birth centile (unadjusted change per 1 mg/day increase in heme iron intake = -1.2 centile points, 95% CI: -3.3, 0.8, $P = 0.2$), while the relationship was statistically significant for non-heme iron (unadjusted change per 1 mg/day increase in non-heme iron intake = 0.6, 95% CI: 0.3, 0.9, $P < 0.0001$; adjusted change = 0.3, 95% CI: 0, 0.9, $P = 0.05$). There was a positive relationship between total iron intake, from food and supplements, with customized birth centile (unadjusted change per 10 mg/day increase in total iron intake = 4.3, 95% CI: 2.4, 6.3, $P < 0.0001$, adjusted change = 2.5, 95% CI 0.4, 4.6, $P = 0.02$; Table IV).

Role of vitamin C intake

The relationship between dietary iron intake from food and customized birth centile was significant in participants with vitamin C intake above 50 mg/day (adjusted change per 10 mg/day increase in dietary iron intake = 3.9, 95% CI: 0.4, 7.5, $P = 0.03$), compared with -1.9 (95% CI: -11.1, 7.5, $P = 0.7$, $n = 253$) for those with vitamin C intake ≤ 50 mg/day. However, the interaction between iron and vitamin C intakes on the outcome was not significant ($P = 0.3$). Similar relationships were observed for non-heme iron

Table II Characteristics of women by dietary iron intake during the first trimester reported in a 24-h dietary recall (n = 1257).

	Dietary iron intake		P-value*
	>14.8 mg/day ^a (n = 257)	≤14.8 mg/day (n = 1000)	
Dietary iron intake (mg/day), mean (95% CI)	19.6 (15.0, 31.7)	9.4 (4.5, 13.8)	–
Age of mother (years), mean (95% CI)	31 (30, 31)	30 (29, 30)	0.004
Pre-pregnancy weight (kg), mean (95% CI)	66 (64, 68)	68 (67, 68)	0.1
Total energy intake (kcal), mean (95% CI)	2777 (2657, 2897)	1958 (1924, 1991)	<0.0001
(MJ), mean (95% CI)	11.6 (11.1, 12.1)	8.2 (8.1, 8.3)	
Active smoker at 12 weeks (%; 95% CI)	8 (5, 12)	20 (17, 23)	<0.0001
IMD most deprived quartile (%; 95% CI)	25 (20, 31)	32 (29, 35)	0.03
Caucasian (%; 95% CI)	91 (87, 95)	94 (92, 95)	0.2
Higher education (%; 95% CI)	52 (49, 58)	35 (32, 39)	<0.0001
Vegetarian (ovo-lacto) (%; 95% CI)	13 (10, 18)	8 (6, 10)	0.004
Primigravida (%; 95% CI)	47 (41, 54)	46 (43, 49)	0.7
History of long-term illness (%; 95% CI)	9 (6, 13)	14 (12, 16)	0.04
Average alcohol consumption >0.5 units/day throughout pregnancy (%; 95% CI)	30 (24, 36)	26 (23, 29)	0.2
Past history of miscarriage (%; 95% CI)	20 (16, 26)	25 (22, 27)	0.08
Report taking any form of daily supplements in the first trimester questionnaire (%; 95% CI)	87 (82, 91)	81 (78, 83)	0.01
Report taking daily iron-containing supplements in the first trimester questionnaire (%; 95% CI)	29 (23, 35)	23 (20, 25)	0.04

IMD, index of multiple deprivation.

*P-value using two-sample t-test for continuous variables, χ^2 -test for categorical variables.^aReference nutrient intake (RNI) for iron for women aged 19–50 years in the UK.

and total iron intake from diet and supplements using an interaction term between iron intake and vitamin C intake in the models (Table IV).

Relationship between iron intake and small for gestational age

Participants with dietary iron intake ≤14.8 mg/day were 1.6 times more likely to have a SGA baby (95% CI: 1.0, 2.5, $P=0.05$). However, the adjusted relationship was not significant (1.4, 95% CI: 0.9, 2.3, $P=0.2$). This pattern is similar for total iron intake from diet and supplements (Table IV).

Relationship between iron intake and preterm birth

There was no relationship between iron intake from diet only, or from diet and supplements, as recorded in the recall diary in the first trimester, and preterm birth (Table IV).

Relationship between intake of iron-containing supplements and birth outcomes

There was no association between daily intake of iron-containing supplements in the first and second trimester and customized birth centile. There was an inverse association between taking

iron-containing supplements in the third trimester (73% of which as part of multivitamin-mineral preparations) and customized birth centile adjusted for salivary cotinine levels, alcohol intake and maternal age (adjusted difference = −10.7, 95% CI: −16.7, −4.8, $P<0.0001$).

Discussion

This study shows a positive relationship between both total iron intake (from food and supplements) and non-heme iron intake, derived from 24-h dietary recall in the first trimester of pregnancy, and birthweight. There was no association between iron intake and preterm birth.

Strengths and limitations of the study

This was a large prospective cohort study. Although a randomized controlled trial is the gold standard study design to investigate causality, this design would be difficult to execute especially when the exposure is dietary intake. The response rate to take part in the study was 20% out of all the women who were invited, and the percentage of low birthweight babies (<2500 g) in this study (4.4%) was less than the National (7.2%) and the Yorkshire & Humber region average (7.8%) for 2007 (Office for National Statistics, 2007). This raises the possibility that women who are more likely to have low birthweight babies were less likely to participate in this study. We have used customized birth centile that takes into account gestational age, maternal height, weight, ethnicity and parity and neonatal

Table III The relationship between dietary and supplemental iron intake and maternal blood indices (Hb and MCV) during pregnancy.

	Unadjusted change	95% CI	P-value	Adjusted change ^a	95% CI	P-value
Dietary iron intake \leq 14.8 mg/day in the first trimester						
Hb at 12 weeks (g/dl)	0.1	-0.1, 0.3	0.2	0.09	-0.1, 0.3	0.4
Hb at 28 weeks (g/dl)	-0.1	-0.3, 0.1	0.3	-0.1	-0.3, 0.1	0.4
MCV at 12 weeks (fl ^b)	0.2	-0.1, 1.2	0.7	0.3	-0.7, 1.3	0.6
MCV at 28 weeks (fl)	-0.9	-2.0, 0.2	0.1	-0.8	-1.9, 0.3	0.2
Daily intake of iron-containing supplements in the first trimester						
Hb at 12 weeks (g/dl)	0.3	0.1, 0.4	0.005	0.2	0.05, 0.4	0.01
Hb at 28 weeks (g/dl)	0.4	0.2, 0.6	<0.0001	0.3	0.2, 0.5	<0.0001
MCV at 12 weeks (fl ^b)	0.6	-0.4, 1.5	0.2	0.1	-0.8, 1.1	0.8
MCV at 28 weeks (fl)	1.3	0.4, 2.4	0.008	0.8	-0.2, 1.8	0.1
Daily intake of iron-containing supplements in the second trimester						
Hb at 28 weeks (g/dl)	0.3	0.1, 0.6	0.002	0.2	0.0, 0.5	0.05
MCV at 28 weeks (fl)	1.5	0.4, 2.8	0.01	0.7	-0.05, 2.0	0.3

^aAdjusted for: maternal age, ethnicity, chronic illness, Index of Multiple Deprivation score, educational attainment, parity and vegetarian diet in a linear regression model.

^bFemtolitres.

Table IV The relationship between maternal dietary iron intake during pregnancy and customized size at birth, Leeds, UK, 2003–2006.

Customized birth centile (takes into account: maternal pre-pregnancy weight, height, parity, ethnicity, gestation and baby's sex)						
	Unadjusted change	95% CI	P-value	Adjusted change ^a	95% CI	P-value
Dietary iron intake ^b	5.2	2.2, 8.2	0.001	3.1	-0.2, 6.3	0.07
Dietary iron intake in participants with vitamin C intake >50 mg/day ^b	5.3	1.9, 8.6	0.002	3.9	0.4, 7.5	0.03
Non-heme iron intake ^b	5.7	2.6, 8.8	<0.0001	3.4	0.0, 8.8	0.05
Non-heme iron intake in participants with vitamin C intake >50 mg/day ^b	5.9	2.5, 9.3	0.001	4.4	0.7, 8.0	0.02
Heme iron intake ^c	-1.2	-3.3, 0.8	0.2	-0.7	-2.8, 1.4	0.6
Total iron intake ^{d,b}	4.3	2.4, 6.3	<0.0001	2.5	0.4, 4.6	0.02
Total iron intake ^d in participants with vitamin C intake >50 mg/day ^b	4.4	2.2, 6.5	<0.0001	3.0	0.7, 5.4	0.01
Small for gestational age (<10% centile)						
	Unadjusted OR ^e	95% CI	P-value	Adjusted OR ^a	95% CI	P-value
Dietary iron intake (\leq 14.8 mg/day)	1.6	1.0, 2.5	0.05	1.4	0.9, 2.3	0.2
Total iron intake ^d (\leq 14.8 mg/day)	1.5	1.0, 2.1	0.04	1.2	0.8, 1.8	0.3
Preterm birth (<37-week gestation)						
	Unadjusted OR ^e	95% CI	P-value	Adjusted OR ^a	95% CI	P-value
Dietary iron intake (\leq 14.8 mg/day)	1.1	0.7, 2.3	0.7	1.0	0.5, 2.3	0.8
Total iron intake ^d (\leq 14.8 mg/day)	1.5	0.8, 2.7	0.2	1.3	0.7, 2.5	0.4

^aAdjusted for maternal age, salivary cotinine levels and alcohol intake in a multiple linear regression model, with an interaction term between iron and vitamin C intakes where the estimates are reported in the table to be for iron intake in the group with vitamin C intake >50 mg/day.

^bPercentage point change in customized centile per 10 mg/day increase in iron intake.

^cPercentage point change in customized centile per 1 mg/day increase in heme iron intake.

^dFrom food and supplements excluding therapeutic iron supplement takers (\geq 65 mg/dose).

^eOdds ratio with dietary iron intake >14.8 mg/day as the reference group.

birthweight and sex. However, it does not take into account paternal height, which has been shown to be related to birthweight (Morrison *et al.*, 1991; Nahum and Stanislaw, 2003).

Dietary iron intake was ascertained using 24-h dietary recall recorded by a midwife-administered interview at around 12-week gestation. This method has been validated, and found to be comparable with other dietary assessment methods such as food frequency questionnaires and food diaries in estimating iron intake (Bingham *et al.*, 1997). However, 24-h recall has its limitations such as failure to recall diet accurately and the chance of consuming non-typical diet during the day prior to the assessment. While the study has a large sample size and hence good probable estimates of mean daily intake, these estimates may be more widely dispersed than in reality due to the use of this dietary assessment method. It, therefore, may over-estimate the proportion of mothers with extremely high or low iron intakes—for example, the proportion with daily iron intake less than UK LRNI (24% in our sample). However, there is evidence, when validating 24-h recalls against other methods of dietary assessment, that recall is prone to over-reporting low intakes and under-reporting high intakes (Gersovitz *et al.*, 1978).

The estimation of heme iron intake may have been subject to greater error than the estimation of non-heme intake, given that it constitutes a smaller proportion of total dietary iron. The use of supplements was recorded both in the 24-h recall and the interviewer-administered and self-reported questionnaires. The extent of agreement was high between the two methods in this study for reporting iron-containing supplements intake; however, there is potential for measurement error using both methods. It is unlikely that women with adverse outcomes would have reported their supplement-use pattern or dietary intake differently to other women since it is a prospective study, therefore reducing the chance of differential bias. We decided to use the supplements reported in the recall, rather than the questionnaire, to add to the dietary iron to derive the total iron intake variable as they were both reported in the same recall.

Interpretation of findings

We found that non-heme (rather than heme) iron was positively related to size at birth. This raises the possibility that the observed relationship is due to residual confounding by an unmeasured factor associated with both non-heme iron intake and size at birth. We, therefore, carried out a sensitivity analysis by excluding vegetarians, as vegetarian status may be associated with a generally healthier diet and lifestyle. This did not change the regression estimates. It could be that participants with a higher intake of heme iron are more likely to have adverse birth outcomes due to lifestyle and socioeconomic factors associated with high meat intake (Hulshof *et al.*, 2003), thus counteracting any positive effect for heme iron. However, adjusting for educational status and IMD group did not change the results (data not shown). Findings from the Motherwell cohort study suggest that a diet high in low-quality meat might itself reduce fetal growth, perhaps through stimulating a stress response in the mother (Herrick *et al.*, 2003).

Adjustment for total energy intake is recommended if it is a confounder of the relationship being examined (Willett *et al.*, 1997). However, we did not adjust for it here because it did not fulfil the definition of a 'true' confounder. Confounding can result if total energy

intake is associated with both the exposure of interest and the main outcome (Pearl, 2000), which is not the case in this study as total energy intake was not associated with birthweight (data not shown).

Although effect modification was not significant for vitamin C, the stronger association between iron intake and birthweight in participants whose vitamin C intake was >50 mg/day is of interest as vitamin C is the best known enhancer of iron absorption (Pearl, 2000; Gibney *et al.*, 2004). We used a cut-off of the pregnancy RNI of 50 mg/day for vitamin C, but the threshold where daily vitamin C intake starts to have an effect on iron absorption *in vivo* is not exactly known.

Hb and MCV were used as proxies for iron status to assess the extent of agreement with iron intake levels. However, there are major limitations for the use of Hb and MCV levels as indicators of iron status as they do not represent specific or sensitive measures of body iron stores (Milman, 2006). We found no association between dietary iron intake and Hb or MCV levels. This is not a surprising finding as these blood indices are only affected when iron deficiency is pronounced. It is difficult to determine the direction of the relationship between iron-containing supplements and Hb. Anemic participants are more likely to take iron-containing supplements. This is supported by the stronger positive relationship between taking iron-containing supplements in the first trimester and Hb at 28 weeks compared with that at 12-week gestation.

Conclusion and implications for research and practice

This study confirms a positive association between total iron intake, from food and supplements, in the first trimester of pregnancy, and customized birth centile. Although iron intake from food alone is not significantly associated with birthweight after adjustment, intake of non-heme iron is more strongly associated with birthweight than heme iron. Further research is needed to explore the role of vitamin C intake in the relationship between dietary and supplementary iron intake and birth outcomes. A randomized controlled trial of high dietary iron intake combined with vitamin C at mealtimes during early pregnancy could provide some important insights. Public health messages about increasing iron intake during early pregnancy and ways to optimize iron absorption, whether from diet or supplements, need to be promoted.

Authors' roles

J.E.C., D.C.G. and N.A.B.S. contributed to the study design and data collection. N.A.A. performed the statistical analysis, with assistance from D.C.G., N.A.A. wrote the first draft of the paper. All authors participated in the reporting stage and have seen and approved the final draft of the paper.

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