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TITLE: DIETARY IRON INTAKE DURING EARLY PREGNANCY AND BIRTH OUTCOMES IN A COHORT OF BRITISH WOMEN

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ABSTRACT

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.

INTRODUCTION

Iron deficiency during pregnancy is still common in developed countries (1-4). 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 (5-9). 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 (10-14). Iron supplements are widely recommended and used during pregnancy worldwide (15, 16). 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 (17-21). However, the evidence on what benefit iron supplements contribute to infant outcomes is still not established (22), and their routine use has its drawbacks such as gastrointestinal side effects and interactions with other micronutrients especially if taken as part of a multivitamin-mineral supplement (23-26). Iron supplements can also reduce the absorption of dietary non-haem iron (24), and can increase oxidative stress and the production of free radicals (27, 28). Therefore, they are not routinely recommended during pregnancy in the UK (29).

In the USA, dietary iron intake of 27 mg/day during pregnancy is recommended (30). In the US National Health and Nutrition Examination Survey (NHANES III), median iron intake in pregnant women was 15 mg/day (31). 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 (32). 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%) (32). The mean daily dietary intake of total iron from the 2001 National

Diet and Nutrition Survey (NDNS) in Great Britain was 10 mg for women aged 19-64 years (33). 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 (34). There is evidence from nutritional surveys in the UK and Norway that women's dietary patterns change little with pregnancy (35, 36). In the latter survey, 96% of pregnant women had an iron intake < 18 mg/day with an average iron intake of 11 mg /day (36). In order 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 iron transfer to the foetus is better in non-iron-supplemented than in supplemented women (37).

Dietary iron occurs in two forms: haem and non-haem. About 95% of iron in the average British diet is in the form of non-haem iron(38). The extent to which non-haem iron is absorbed is highly variable and depends on the individual's iron status and other dietary components. Ascorbic acid enhances non-haem iron absorption when consumed as part of a meal (39), while high calcium intakes during pregnancy might reduce non-haem iron absorption leading to iron deficiency (40). Haem 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 (39). Unlike non-haem iron, haem iron absorption is influenced little by other dietary constituents. It also enhances non-haem iron absorption from other foods consumed at the same time. Recent evidence suggests that haem and non-haem iron may have different associations withindividual health outcomes (41).

Results of studies investigating the relationship between dietary maternal iron intake during pregnancy and size at birth and/or gestational age are conflicting (9, 42-51). Many studies that 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 haem and non-haem iron. One study assessed the relationship between ascorbic acid and anaemia and well as vitamin C intake and iron status (9), however the potential interaction between iron intake and and the vitamin C intake and other micronutrients has not been explored (52). 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 (haem versus non-haem), 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 to 12 weeks 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, HIV infection, or hepatitis B infection were excluded. Eligible women were identified by screening their pre-booking 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 haemoglobin (Hb) levels and mean corpuscular volume (MCV) at 12 and 28 weeks pregnancy were available for a sub-sample of the cohort which was selected randomly from the main sample using study identification numbers. All women participating in the study gave informed written 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 weeks gestation) and third trimester (from 28 weeks gestation) and self-administered during the second trimester (13-27 weeks 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-hour dietary recall administered by a research midwife at 8-12 weeks gestation. Values for the proportion of haem iron in each type of meat were used to derive haem values for each of the food codes. These values were derived by recording the meat content of each product, together with food tables values (53), to calculate a weighted mean meat content of each food item consumed. A literature search was carried out to arrive at ‘haem factors’ for different animal products that reflect the haem iron content of these foods. Values derived from the Schricker and modified Schricker methods, and the Hornsey method were used to calculate mean values for haem iron (54, 55). These values were then used to generate total iron values for each relevant food (56). The non-haem iron values were derived as the difference between total iron from food tables (53) and calculated haem 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-hour recall. Vitamin C intake from the diet was reported in the 24-hour recall and categorized into above or equal to/below the RNI of 50 mg/d.

ASSESSMENT OF OUTCOMES

The two primary outcome measures were birthweight and preterm birth. Birthweight was measured in grams, and as expressed as customised centile using charts which take into account gestational age, maternal height, weight, ethnicity and parity, and neonatal birthweight and sex (57). Duration of gestation was calculated from the date of the last menstrual period, and confirmed by ultrasound scans dating at around 12 and 20 weeks gestation. Small for gestational age (SGA) was defined as less than the 10th centile for gestational age. Preterm birth was defined as delivery at less than 37 weeks (259 days) gestation.

ASSESSMENT OF PARTICIPANTS CHARACTERISTICS

Socioeconomic status (SES) 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 (58). 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 (ELISA) (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) (59).

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) to 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 chi-square test for categorical variables. Multiple linear regression using birthweight / customised 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).

Analysis was undertaken 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-hour recall, was analysed as a continuous variable. Intake of iron-containing supplements was analysed 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 customised 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 (haem versus non-haem). 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

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% CI: 18%, 23%). 24% of women reported iron intake ≤ the UK LRNI of 8 mg/day (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 haem iron intake was 0.6 mg/day (SD=0.8). This estimate for haem iron changed little after excluding the 114 reported vegetarian participants (with a haem iron intake of zero). Mean non-haem iron intake was 10.9 mg/day (SD=5.2) (Table I).

20% of participants (95% CI: 18%, 22%) reported taking iron-containing supplements in the recall compared to 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 > 14.8mg/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 which contains 100 mg iron per dose. Median 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 likely to have dietary iron intake ≤ 14.8 mg/day (unadjusted 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 1st trimester, OR=2.9, 95% CI: 1.9, 4.4, P<0.0001 for the 2nd trimester).

BIRTH OUTCOMES

There were 1259 babies with information on birthweight. Mean birthweight was 3439 g (SD=577 g) with 4.4% babies weighing less than 2500 g (n=55). 13% (n=166) weighed less than the 10th centile, 8% (n=99) less than the 5th centile, and 5% (n=65) less than the 3rd centile. 9% of babies (n=118) weighed more than the 90th centile. Of the 1234 pregnancies with information on gestational age, 55 (4.5%) delivered before 37 weeks gestation.

RELATIONSHIP BETWEEN BLOOD INDICES AND BIRTH OUTCOME

558 and 572 participants had information on haemoglobin (Hb) and mean corpuscular volume (MCV) at 12 and 28 weeks gestation 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 week 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 customised

birth centile or birthweight in grams and Hb/MCV at 12 or 28 weeks 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 weeks 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 customised 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 haem iron intake and customised birth centile (unadjusted change per 1 mg/day increase in haem iron intake =-1.2 centile points, 95% CI: -3.3, 0.8, P=0.2), while the relationship was statistically significant for non-haem iron (unadjusted change per 1 mg/day increase in non-haem 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 customised birth centile (unadjusted change per 10 mg/day increase in total iton 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 customised 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 = 3.7, 95% CI: 0.1, 7.3, P= 0.04), compared to -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-haem iron 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 (SGA)

Participants with dietary iron intake equal to or less than 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 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 customised 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 customised 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-haem iron intake, derived from 24-hour 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 randomised 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 (60). 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 customised birth centile which takes into account gestational age, maternal height, weight, ethnicity and parity, and neonatal birthweight and sex. However, it does not take into account paternal height which has been shown to be related to birthweight (61, 62).

Dietary iron intake was ascertained using 24-hour dietary recall recorded by a midwife-administered interview at around 12-weeks gestation. This method has been validated, and found to be comparable to other dietary assessment methods such as food frequency questionnaires and food diaries in estimating iron intake (63). However, 24-hour 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. Whilst 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 < UK LRNI (24% in our sample). However, there is evidence, when validating 24-hour recalls against other methods of dietary assessment, that recall is prone to over-reporting low intakes and under-reporting high intakes (64).

The estimation of haem iron intake may have been subject to greater error than the estimation of non-haem intake, given that it constitutes a smaller proportion of total dietary iron. The use of supplements was recorded both in the 24-hour 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 add 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-haem, rather than haem 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-haem 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 & lifestyle. This did not change the regression estimates. It could be that participants with higher intake of haem iron are more likely to have adverse birth outcomes due to lifestyle and socioeconomic factors associated with high meat intake (65), thus counteracting any positive effect for haem 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 (66).

Adjustment for total energy intake is recommended if it is a confounder of the relationship being examined (67). However, we did not adjust for it here because it did not fulfill 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 (68), 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 more than 50 mg/d is of interest as vitamin C is the best known enhancer of iron absorption (52, 68).

Effect modification was not significant for vitamin C. 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 (69). 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. Anaemic 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 trinmester and Hb at 28 weeks compared to that at 12 weeks 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 customised birth centile. Although iron intake from food alone is not significantly associated with birthweight after adjustment, intake of non-haem iron is more strongly associated with birthweight than haem 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 randomised controlled trial of high dietary iron intake combined with vitamin C at mealtimes during early pregnancy can providesome important insights. Public health messages about increasing iron intake during early pregnancy and ways to optimise iron absorption, whether from diet or supplements, need to be promoted.

AUTHOR'S ROLES

JC Cade, DC Greenwood and NAB Simpson contributed to the study design and data collection. NA Alwan performed the statistical analysis with assistance from DCG. NAA 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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Table I: Average iron intake from food and dietary supplements as reported in first trimester 24-hour dietary recall (n=1257)

	Mean	**Standard deviation**	**Median**	**Interquartile range**
Iron intake from food (mg/day)	11.5	5.3	10.5	8.1 , 13.7
Haem iron intake (mg/day)	0.6	0.8	0.3	0.1, 0.8
Non-haem 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

TABLE II: CHARACTERISTICS OF WOMEN BY DIETARY IRON INTAKE DURING THE FIRST TRIMESTER REPORTED IN A 24-HOUR DIETARY RECALL (N=1257)

	Dietary iron intake		
	>14.8 mg/day$^{\#}$ (n=257)	**≤ 14.8 mg/day** (n=1000)	**P value ***
Dietary iron intake (mg/day) (mean, 95% CI)	19.6 (15.0, 31.7)	9.4 (4.5, 13.8)	-
Age of mother (yrs) (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) **(MJ)** (mean, 95% CI)	2777(2657,2897) 11.6 (11.1, 12.1)	1958(1924,1991) 8.2 (8.1, 8.3)	<0.0001
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 more than 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

Reference nutrient intake (RNI) for iron for women aged 19-50 years in the UK
* P-value using two-sample t-test for continuous variables, chi-squared test for categorical variables
** Index of multiple deprivation

TABLE III: THE RELATIONSHIP BETWEEN DIETARY AND SUPPLEMENTAL IRON INTAKE AND MATERNAL BLOOD INDICES (HB AND MCV) DURING PREGNANCY

	Unadjusted change	95% CI	P	Adjusted change*	95% CI	P value
Dietary iron intake = < 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)**	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)**	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

*Adjusted for: maternal age, ethnicity, chronic illness, Index of multiple deprivation score, educational attainment, parity and vegetarian diet in a linear regression model

**Femtolitres

TABLE IV: THE RELATIONSHIP BETWEEN MATERNAL DIETARY IRON INTAKE (MG/DAY) DURING PREGNANCY AND CUSTOMISED SIZE AT BIRTH, LEEDS, UNITED KINGDOM, 2003-2006

Customised birth centile

(takes into account: maternal pre-pregnancy weight, height, parity, ethnicity, gestation and baby's sex)

	Unadjusted change	**95% CI**	**P**	**Adjusted change***	**95% CI**	**P-value**
Dietary iron intake †	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 †	5.3	1.9, 8.6	0.002	3.9	0.4, 7.5	0.03
Non-haem iron intake †	5.7	2.6, 8.8	<0.0001	3.4	0.0, 8.8	0.05
Non-haem iron intake in participants with vitamin C intake > 50 mg/day †	5.9	2.5, 9.3	0.001	4.4	0.7, 8.0	0.02
Haem iron intake ††	-1.2	-3.3, 0.8	0.2	-0.7	-2.8,1.4	0.6
Total iron intake *** †	4.3	2.4, 6.3	<0.0001	2.5	0.4, 4.6	0.02
Total iron intake *** in participants with vitamin C intake > 50 mg/day †	4.4	2.2, 6.5	<0.0001	3.0	0.7, 5.4	0.01

Small for gestational age (<10% centile)

	Unadjusted OR **	**95% CI**	**P**	**Adjusted OR***	**95% CI**	**P**
Dietary iron intake (≤ 14.8 mg/day)	1.6	1.0, 2.5	0.05	1.4	0.9, 2.3	0.2
Total iron intake *** (≤ 14.8 mg/day)	1.5	1.0, 2.1	0.04	1.2	0.8, 1.8	0.3

Preterm birth (<37 weeks gestation)

	Unadjusted OR **	**95% CI**	**P**	**Adjusted OR***	**95% CI**	**P**
Dietary iron intake (≤ 14.8 mg/day)	1.1	0.7, 2.3	0.7	1.0	0.5, 2.3	0.8
Total iron intake *** (≤ 14.8 mg/day)	1.5	0.8, 2.7	0.2	1.3	0.7, 2.5	0.4

*Adjusted 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
**Odds ratio with dietary iron intake > 14.8 mg/day as the reference group
†Percentage point change in customised centile per 10 mg/day increase in iron intake
†† Percentage point change in customised centile per 1 mg/day increase in haem iron intake
*** From food and supplements excluding therapeutic iron supplement takers (≥ 65mg/dose)