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ORIGINAL ARTICLE

Should we lower the dose of iron when treating anaemia in pregnancy? A randomized dose–response trial

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Background/Objectives: To compare the efficacy and side effects of low-dose vs high-dose iron supplements to correct anaemia in pregnancy.
Subjects/Methods: One hundred and eighty women with anaemia (haemoglobin $<110\,g\,l^{-1}$) in mid-pregnancy. The women were randomly allocated to 20; 40 or 80 mg of iron daily for 8 weeks from mid-pregnancy.
Results: One hundred and seventy-nine (99%) women completed the trial. At the end of treatment, there was a clear dose–response of increasing mean haemoglobin concentration with iron dose ($111\pm13\,g\,l^{-1}$ at 20 mg per day, $114\pm11\,g\,l^{-1}$ at 40 mg per day and $119\pm12\,g\,l^{-1}$ at 80 mg per day, $P=0.006$). However, the incidence of anaemia did not differ statistically between groups. Compared with women in the 80 mg iron group, the odds ratio of anaemia was 1.9 (95% CI: 0.8, 4.3, $P=0.130$) and 1.1 (95% CI: 0.5, 2.6, $P=0.827$), respectively, for women in the 20 mg iron group and the 40 mg iron group. The incidence of gastrointestinal side effects was significantly lower for women in the 20 mg iron group compared with women in the 80 mg iron group; the odds ratio was 0.4 (95% CI: 0.2, 0.8, $P=0.014$) for nausea, 0.3 (95% CI: 0.2, 0.7, $P=0.005$) for stomach pain and 0.4 (95% CI: 0.2, 0.9, $P=0.023$) for vomiting.
Conclusions: Low-dose iron supplements may be effective at treating anaemia in pregnancy with less gastrointestinal side effects compared with high-dose supplements.
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Keywords: anaemia; pregnancy; iron supplements; randomized controlled trial; industrialized country

Introduction

Anaemia is a common problem for pregnant women and has been linked with adverse pregnancy outcomes such as preterm birth and low birth weight, as well as maternal mortality and morbidity (WHO, 2001). The most common cause of anaemia in pregnancy is iron deficiency (ID) (WHO, 2001) and is often treated with iron tablets. The Cochrane systematic review that investigated treatment options for iron deficiency anaemia (IDA) in pregnancy (Cuervo and Mahomed, 2003) highlighted the lack of quality trials specifically designed to estimate the best dose of iron to treat anaemia and concluded that treatments for anaemia in pregnancy are currently based on expert opinions rather than quality randomized controlled trial.

Currently, the most common practice in Australia and other industrialized countries is to treat anaemia with iron tablets containing at least 80 mg elemental iron as ferrous sulphate, which may cause gastrointestinal side effects (Hallberg *et al*., 1966; Reddaiah *et al*., 1989), impair mineral absorption (Solomons, 1986) and increase the risk of haemoconcentration (Pena-Rosas and Viteri, 2006), a

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condition that is associated with adverse pregnancy outcomes. Although different forms of iron may result in fewer or no gastrointestinal side effects, the Recommended Upper Limit of iron for pregnant women in Australia is 45 mg per day based on minimizing gastrointestinal side effects (DHA and NHMRC, 2006). Evidence from prophylactic trials in non-anaemic pregnant women in industrialized countries demonstrated that low-dose iron given at 18–30 mg per day was as effective as high-dose iron supplements in the prevention of anaemia and IDA throughout pregnancy (Eskeland *et al*., 1997; Makrides *et al*., 2003; Lee *et al*., 2005) without gastrointestinal side effects (Makrides *et al*., 2003). However, there is a lack of evidence on the alternatives to high-dose iron supplements to treat anaemia in pregnancy. Because iron absorption is influenced by iron stores (The British Nutrition Foundation, 1995) and iron dose (Hahn *et al*., 1951; The British Nutrition Foundation, 1995), the amount absorbed increases with lower iron stores and lower iron doses. Therefore, the difference in the actual amounts of iron absorbed from high-dose vs low-dose iron supplements may be insignificant and low-dose iron supplements may be adequate to treat anaemia in pregnancy. The aim of this study was to determine the efficacy and side effects of low- vs high-dose iron supplements to correct anaemia in pregnant women.

Subjects and methods

Participants

Pregnant women attending antenatal care at the Children, Youth and Women's Health Service (CYWHS), Adelaide, are routinely screened for anaemia (defined as haemoglobin (Hb) $< 110\,g\,l^{-1}$) (WHO, 2001) in mid-pregnancy as part of standard obstetric care. Women diagnosed with anaemia at the routine mid-pregnancy blood test were approached to take part in our study. They were eligible if between 24 and 32 weeks gestation and had a singleton pregnancy. Women were excluded if they were taking iron or vitamin and mineral supplements containing iron, had further investigations which suggested that ID was not the cause of anaemia, had a history of thalassaemia or drug and alcohol abuse and had diabetes requiring insulin or a known fetal abnormality. All participants were recruited from the antenatal clinics between January 2004 and July 2005. Informed written consent was obtained from all participants. The study was approved by the CYWHS Human Research Ethics Committee and was registered with the Australian Clinical Trials Registry (ACTR) (Reference no. 12606000357550).

Intervention

Women were randomly allocated to one of the three intervention groups: ferrous sulphate containing 20, 40 or 80 mg of elemental iron per tablet, which did not contain other micronutrients. Women were asked to take one tablet daily between meals from enrolment for 8 weeks or until birth, whichever occurred sooner. All women received standard obstetric care and were advised to take additional iron supplements only if their clinicians considered it necessary. For example, if women displayed increasing symptoms of anaemia and a blood test suggested worsening of anaemia. The 20 and the 80 mg dose of iron are referred to as low and high dose, respectively, throughout the report.

The doses of iron used in the intervention were based on the finding of Ekstrom *et al*. (2002) that the major determinant of Hb response in pregnant women supplemented with iron was the total iron load. A total dose of 1200 mg of iron was required to achieve the greatest effect on improving Hb concentration (Ekstrom *et al*., 2002). This is equivalent to 20 mg iron daily for 8 weeks duration. Furthermore, saturation of the Hb response was achieved with 2400 mg of total iron (Ekstrom *et al*., 2002), which is equivalent to 40 mg iron per day for 8 weeks duration. The 80 mg iron per day is the most common approach in Australia.

Randomization and blinding

A computer-generated randomization schedule was generated by an independent consultant with stratification for Hb (Hb $\geqslant 100$ or $< 100\,g\,l^{-1}$) to ensure that there were equal proportions of women with more severe anaemia in all groups. The iron tablets were packed in bottles with child-proof lids, which were labelled with a unique study number according to the randomization schedule by the Pharmacy Department at the CYWHS. The tablets were identical in size, colour and shape and were manufactured and donated by Sole Pattinson Manufacturing, Kingsgrove, NSW, Australia. Trial participants and the research team were unaware of the treatment group assignment. The trial was unblinded after the analysis of the primary outcomes.

Outcomes and assessments

The primary outcomes of the study were Hb levels and incidence of anaemia at the end of the intervention, and gastro-intestinal side effects during treatment. Secondary outcomes included incidence of ID, IDA and haemoconcentration at the end of treatment as well as pregnancy outcomes.

A 5-ml blood sample was taken from the women by venipuncture at the end of treatment to assess their iron status, including Hb, serum ferritin, serum iron, mean cell volume, serum transferrin and transferrin saturation. The tests were conducted by the diagnostic laboratory at the CYWHS according to standard methods. Details of the methods were reported in our previous trial of prophylactic iron supplements in pregnancy (Makrides *et al*., 2003). ID was defined as ferritin $< 12\,\mu g\,l^{-1}$ and IDA was defined as Hb $< 110\,g\,l^{-1}$ and ferritin $< 12\,\mu g\,l^{-1}$ (WHO, 2001). Anaemia was defined as Hb $< 110\,g\,l^{-1}$ (WHO, 2001), moderate anaemia was

defined as Hb $< 100\,g\,l^{-1}$ and haemoconcentration was defined as Hb $>130\,g\,l^{-1}$ (Pena-Rosas and Viteri, 2006).

Common gastro-intestinal side effects often associated with iron supplements including nausea, heartburn, abdominal discomfort, vomiting and constipation, were assessed using a structured questionnaire (Makrides *et al*., 2003) at 2, 4 and 6 weeks of treatment via telephone calls and at 8 weeks clinical visit via interview. Women were asked if they experienced any symptoms of potential side effects during the 2 weeks immediately preceding each assessment. For each side effect considered, women who reported symptoms during any of the assessment time points were classified as having that side effect, while those who reported no symptoms at all the four assessments were classified as having no side effect. Missing data at only one of the four assessment time points were treated as no symptoms. No woman had missing data on more than one assessment and overall only 3% of data points were missing (Figure 1).

The following information was collected from each woman's medical records: mode of delivery, gestational age at birth, birth weight, birth length, birth head circumference, placental weight, neonatal complications including fetal distress, the need for assisted ventilation and admission to the neonatal intensive care unit and pregnancy complications including antepartum haemorrhage requiring hospital admission and postpartum haemorrhage requiring transfusion.

Potential confounders were assessed. Hb level at baseline was the result of the mid-pregnancy screening test obtained from the women's medical records. No blood test was conducted at enrolment because there were only a few days between the screening test and trial entry. Iron intake from foods was assessed at baseline using a validated iron checklist (Zhou *et al*., 2005). Education level, smoking during pregnancy, number of previous births and history of anaemia were collected at enrolment via structured interview. Compliance was assessed by monitoring the number of tablets not taken during the previous 2 weeks at each study telephone call. Women were supplied with excess tablets and they were asked to return any unused tablets at the end of the intervention. This strategy was used to measure compliance.

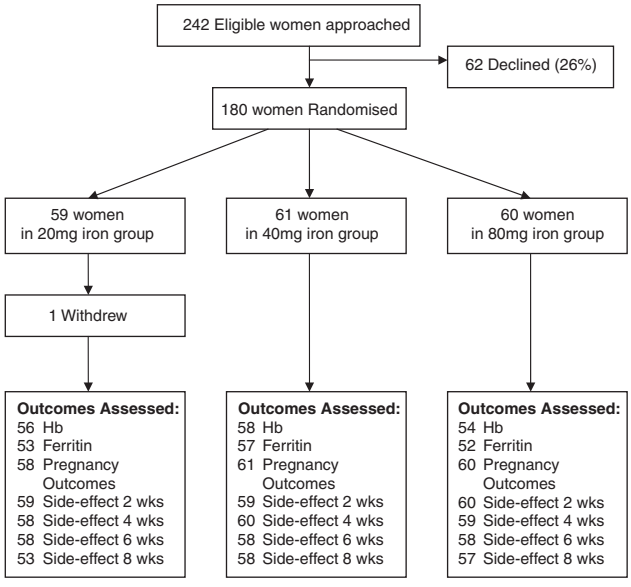

Figure 1 Participant flow chart.

Statistical analysis

Statistical analyses were performed using SPSS for WINDOWS (version 10.0, SPSS Inc., Chicago, IL, USA). Primary analyses were based on the intention-to-treat principle. Subgroup analyses among women with baseline Hb $\geqslant 100\,g\,l^{-1}$ were conducted to assess the efficacy of the three doses of iron for treating less severe or borderline anaemia. Mean difference between treatment groups was assessed by analysis of variance with a Bonferroni adjustment to the significance for continuous outcome variables and odds ratio between treatment groups was estimated using logistic regression for dichotomous outcome variables. Linear regression analysis was also conducted as a secondary analysis to compare the change in Hb and change in ferritin concentration by dose of iron. Ferritin, iron, transferrin and TFS data were not normally distributed and were log-transformed for statistical analyses. Statistical significance was set at $P<0.05$ for all analyses.

Results

Baseline characteristics of participants and compliance

A total of 242 eligible women were approached and 180 (74%) consented to take part (Figure 1) indicating strong external validity. All women completed the study except one because of transfer to another hospital for antenatal care and birth. At trial entry, the baseline characteristics of women listed in Table 1 were not different between groups. At entry to the trial, the mean Hb level of the women was $104\pm4\,g\,l^{-1}$. A majority of women were Caucasian (86%) and 53% were nulliparous. At the end of treatment, blood samples for Hb analysis were available for 168/180 (93%) of women (Figure 1).

Compliance with taking trial tablets was not different between groups based on tablet back-count (82% for the 20 mg, 81% for the 40 mg and 76% for the 80 mg group, $P=0.382$). There was a strong correlation between the compliance data based on the tablet back-count and those based on the total number of days women reported not taking allocated tablets during the regular monitoring telephone calls ($r=0.813$, $P<0.0001$).

Iron status at the end of intervention

At the end of the treatment, there was a clear dose–response of increasing Hb concentration with iron dose ($111\pm13\,g\,l^{-1}$ at 20 mg per day, $114\pm11\,g\,l^{-1}$ at 40 mg per day and

Table 1 Trial entry characteristics of participating women[a]

	20 mg (n = *59*)	*40 mg* (n = *61*)	*80 mg* (n = *60*)
Age (years)	28.4 ± 6.0	29.5 ± 6.4	27.5 ± 6.3
Race			
Caucasian	48/59 (81)	53/61 (87)	53/60 (88)
Aboriginal	2/59 (3)	3/61 (5)	3/60 (5)
Asian	5/59 (9)	1/61 (2)	3/60 (5)
Others	4/59 (7)	4/61 (6)	1/60 (2)
Number of previous birth			
1	30/59 (51)	32/61 (52)	33/60 (55)
2	16/59 (27)	11/61 (18)	13/60 (22)
⩾3	13/59 (22)	18/61 (30)	14/60 (23)
Highest education			
<year 12	24/59 (41)	25/61 (41)	25/60 (42)
Year 12 or trade	18/59 (31)	22/61 (36)	16/60 (27)
Diploma or degree	17/59 (29)	14/61 (23)	19/60 (32)
Smoked in pregnancy	13/59 (22)	12/61 (20)	10/60 (17)
Iron intake from food[b] (mg per day)	11.8 (8.4, 16.6)	12.7 (10.3, 19.5)	11.6 (8.5, 16.5)
Haemoglobin ($g\,l^{-1}$)	104 ± 5	104 ± 4	104 ± 5
Mean cell volume (fl)	87.8 ± 6.1	86.9 ± 5.9	86.5 ± 5.8
Moderate anaemia[c]	11/59 (19)	11/61 (18)	10/60 (17)
Gestational age (weeks)	29.4 ± 1.0	29.2 ± 1.0	29.3 ± 1.1

[a]Data are mean ± s.d. or *n*/*N* (%) unless otherwise specified.
[b]Data are median (interquartile range).
[c]Moderate anaemia defined as Hb $<100\,g\,l^{-1}$.

$119 \pm 12\,g\,l^{-1}$ at 80 mg per day, $P = 0.006$). The regression coefficient ± s.e. was $0.12 \pm 0.04\,g\,l^{-1}$ ($P = 0.001$) for Hb concentration and $1.0 \pm 1.0\,\mu g\,l^{-1}$ ($P < 0.001$) for ferritin concentration at the end of treatment, which suggested that for every unit increase in iron dose (mg), the Hb increased by $0.12\,g\,l^{-1}$ and ferritin increased by $1.0\,\mu g\,l^{-1}$. Fewer women in the 80 mg iron group had ID compared with women in the 40 mg iron group and 20 mg iron group, and IDA compared with women in the 20 mg iron group (Table 2). Also, there was a trend towards higher incidence of anaemia in women in the 20 mg iron group compared with women in the 80 mg iron group (38 vs 24%, $P = 0.128$). The incidence of moderate anaemia or moderate IDA did not differ between groups (Table 2). Compared with women in the 80 mg iron group, the odds ratio of anaemia and moderate anaemia was 1.9 ($P = 0.130$) and 1.6 ($P = 0.418$), respectively, for women in the 20 mg iron group; and 1.1 ($P = 0.827$) and 0.9 ($P = 0.906$), respectively, for women in the 40 mg iron group. The proportion of women who had Hb concentration greater than $130\,g\,l^{-1}$, a marker of haemoconcentration, was 2/56 or 4%, 4/58 or 7% and 8/54 or 13% for the 20, 40 and 80 mg group, respectively, $P = 0.175$. The odds ratio was 0.2 ($P = 0.058$) for women in the 20 mg iron group and 0.4 ($P = 0.185$) for women in the 40 mg iron group compared with women in the 80 mg iron group (Table 2). The mean change in Hb concentration per gram of iron received was $0.4 \pm 0.6\,g\,l^{-1}$ for the 20 mg group, $0.3 \pm 0.2\,g\,l^{-1}$ for the 40 mg group and $0.2 \pm 0.1\,g\,l^{-1}$ for the 80 mg group ($P = 0.03$). No women in the study reported taking additional iron supplements during the intervention.

Subgroup analysis of women with less severe or borderline anaemia at enrolment (baseline Hb ⩾100 & < $110\,g\,l^{-1}$) showed a similar trend to the intention-to-treat analysis, but the only statistically significant difference in iron status between groups at the end of treatment was Hb concentration. *Post-hoc* analysis showed that the difference lie between the 20 and the 80 mg groups ($111 \pm 13\,g\,l^{-1}$ at 20 mg per day vs $119 \pm 13\,g\,l^{-1}$ at 80 mg per day, mean difference: −6, 95% CI: −12, −1, $P = 0.025$).

Gastrointestinal side effects during the intervention

The incidences of nausea, stomach pain and vomiting were significantly lower for women in the 20 mg iron group compared with women in the 80 mg iron group; the odds ratio was 0.4 ($P = 0.014$) for nausea, 0.3 ($P = 0.005$) for stomach pain and 0.4 ($P = 0.023$) for vomiting (Table 3). Fewer women in the 20 mg iron group (27/59 or 46%) reported feeling unwell compared to women in the 40 mg (40/61 or 66%) or the 80 mg (37/60 or 62%) iron groups (Table 3). We found no differences in incidence of constipation between groups as indicated by hard stool or bowel movement less than three times per week (Table 3).

Pregnancy outcomes and neonatal complications at birth

With our limited sample size, our results showed that pregnancy outcomes between treatment groups were not different in terms of mode of birth, gestational age at birth, birth weight and preterm birth or other pregnancy outcomes

Table 2 Iron status of women at the end of intervention[a]

Continuous outcome	*Group allocation*			*Mean difference (95% CI) between groups*[b]		
	20 mg	*40 mg*	*80 mg*	*20 vs 80 mg*	*40 vs 80 mg*	*20 vs 40 mg*
Hb ($g\,l^{-1}$)	111±13 (56)a	114±11 (58)a,b	119±12 (54)c	−7.4 (−12 to −3.0)	−4.4 (−8.8 to 0.0)	−3.0 (−7.5 to 1.4)
Mean cell volume (fl)	87±7 (56)	87±7 (56)	87±7 (52)	−0.9 (−3.5 to 1.8)	−0.2 (−2.7 to 2.4)	−0.7 (−3.3 to 1.9)
Ferritin ($\mu g\,l^{-1}$)[c]	9.8±1.8 (53)a,b	9.1±1.8 (57)a	12.7±2.2 (52)b	−1.3 (−1.7 to 1.0)	−1.4 (−1.8 to −1.1)	1.1 (−1.2 to 1.4)
Iron ($\mu mol\,l^{-1}$)[c]	10.9±1.9 (51)	11.8±1.7 (57)	14.0±1.8 (52)	−1.3 (−1.6 to 1.0)	−1.2 (−1.5 to 1.1)	−1.1 (−1.4 to 1.2)
Transferrin ($g\,l^{-1}$)[c]	3.8±1.2 (53)	3.7±1.1 (56)	3.7±1.2 (51)	1.0 (−1.0 to 1.1)	1.0 (−1.1 to 1.1)	1.0 (−1.0 to 1.1)
Transferrin saturation[c]	11.3±1.9 (51)	12.4±1.8 (56)	14.9±1.9 (51)	−1.3 (−1.7 to −1.0)	−1.2 (−1.5 to 1.7)	−1.1 (−1.4 to 1.2)
	Group allocation			*Odds ratio (95% CI) between groups*[b]		
Dichotomous outcome	*20 mg*	*40 mg*	*80 mg*	*20 vs 80 mg*	*40 vs 80 mg*	*20 vs 40 mg*
Anaemia (Hb $<110\,g\,l^{-1}$)	21/56 (38)	15/58 (26)	13/54 (24)	1.9 (0.8, 4.3)	1.1 (0.5, 2.6)	1.7 (0.8, 3.8)
Moderate anaemia (Hb $<100\,g\,l^{-1}$)	8/56 (14)	5/58 (9)	5/54 (9)	1.6 (0.5, 5.3)	0.9 (0.3, 3.4)	1.8 (0.5, 5.8)
ID[d]	34/53 (64)a	37/57 (65)a	22/52 (42)b	2.4 (1.1, 5.3)	2.5 (1.1, 5.5)	1.0 (0.4, 2.1)
IDA[e]	16/53 (30)a	13/57 (22)a,b	6/53 (11)b	3.3 (1.2, 9.2)	2.3 (0.8, 6.5)	1.5 (0.6, 3.4)
Moderate IDA[f]	5/54 (9)	3/57 (5)	3/53 (6)	1.7 (0.4, 7.5)	0.9 (0.2, 4.7)	1.9 (0.4, 8.2)
Haemoconcentration (Hb $>130\,g\,l^{-1}$)	2/56 (4)	4/58 (7)	8/54 (15)	0.2 (0.0, 1.0)	0.4 (0.1, 1.5)	0.5 (0.1, 2.8)

Abbreviations: CI, confidence interval; Hb, haemoglobin.
[a]Data are mean±s.d. (*n*) for continuous outcomes or *n*/*N* (%) for dichotomous outcomes.
[b]The reference group was the second group listed in comparison.
[c]Data are geometric mean±s.d.
[d]ID, iron deficiency defined as ferritin $<12\,\mu g\,l^{-1}$.
[e]IDA, iron deficiency anaemia defined as Hb $<110\,g\,l^{-1}$ & ferritin $<12\,\mu g\,l^{-1}$.
[f]Moderate IDA was defined as Hb $<100\,g\,l^{-1}$ & ferritin $<12\,\mu g\,l^{-1}$.
Different letters denote significant differences ($P<0.05$).

Table 3 Proportion of women reporting gastro-intestinal side effects during the trial[a]

Symptoms	*Group allocation*			*Odds ratio (95% CI) between groups*[b]		
	20 mg	*40 mg*	*80 mg*	*20 vs 80 mg*	*40 vs 80 mg*	*20 vs 40 mg*
Nausea	36/59 (61)	43/61 (71)	49/60 (82)	0.4 (0.2, 0.8)	0.5 (0.2, 1.3)	0.7 (0.3, 1.4)
Heartburn	40/59 (68)	46/61 (75)	43/60 (72)	0.8 (0.4, 1.8)	1.2 (0.5, 2.7)	0.7 (0.3, 1.5)
Stomach pain	18/59 (31)	33/61 (54)	34/60 (57)	0.3 (0.2, 0.7)	0.9 (0.4, 1.8)	0.4 (0.2, 0.8)
Vomiting	13/59 (22)	19/61 (31)	25/60 (42)	0.4 (0.2, 0.9)	0.6 (0.3, 1.3)	0.6 (0.3, 1.4)
Bowel habit: <3 per week	8/59 (14)	8/61 (13)	9/60 (15)	0.9 (0.3, 2.5)	0.9 (0.3, 2.4)	1.0 (0.4, 3.0)
Hard stool	19/59 (32)	18/61 (30)	23/60 (38)	0.8 (0.4, 1.6)	0.7 (0.3, 1.4)	1.1 (0.5, 2.5)
Feeling unwell	27/59 (46)	40/61 (66)	37/60 (62)	0.5 (0.3, 1.1)	1.2 (0.6, 2.5)	0.4 (0.2, 0.9)

[a]Data are *n*/*N* (%) except odds ratio.
[b]The reference group was the second group listed in comparison.

listed in Table 4. Only one woman in the 20 mg iron group had an antepartum haemorrhage and three women had a postpartum haemorrhage requiring blood transfusion, of which one was in the 40 mg iron group and the other two were in the 80 mg iron group. No infant required admission to neonatal intensive care unit or assisted ventilation.

Discussion

The efficacy of low-dose iron supplements in our trial was demonstrated by the fact that the mean Hb concentrations at the end of intervention were within the normal reference range for women in all three treatment groups. As predicted by Ekstrom *et al*. (2002), the greatest mean change in Hb concentration (Hb response) per gram of iron received was in the 20 mg per day group. Although women in the 80 mg iron group had a higher mean Hb concentration and a lower incidence of ID and IDA at the end of the treatment compared with women in the 20 mg iron group, they had a higher incidence of gastrointestinal side effects, whereas the incidence of moderate anaemia and moderate IDA was not different between groups, which raises the issue of biochemical benefit vs patient comfort.

Table 4 Pregnancy outcomes of women in the trial[a]

	Group allocation			P
	20 mg (n = *58*)	*40 mg* (n = *61*)	*80 mg* (n = *60*)	
Mode of delivery				0.941
Normal vaginal	37/58 (64)	41/61 (67)	36/60 (60)	
Instrumental vaginal	7/58 (12)	6/61 (10)	7/60 (12)	
Caesarean	14/58 (24)	14/61 (23)	17/60 (28)	
Blood loss at birth (ml)	366 ± 228	385 ± 477	451 ± 331	0.423
Gestational age at birth (week)	39.4 ± 1.4	39.6 ± 1.2	39.3 ± 1.7	0.468
Male sex	20/58 (35)	25/61 (41)	27/60 (45)	0.502
Birth weight (g)	3464 ± 444	3496 ± 523	3503 ± 522	0.906
Birth length (cm)	50.0 ± 2.2	50.0 ± 2.2	50.0 ± 2.4	0.990
Birth head circumference (cm)	34.6 ± 1.4	34.6 ± 1.5	34.5 ± 1.5	0.820
Placental weight (g)	646 ± 132	658 ± 141	670 ± 146	0.686
Preterm birth[b]	2/58 (3)	3/61 (5)	5/60 (8)	0.493
Small for gestational age[c]	2/58 (3)	6/61 (10)	2/60 (3)	0.205
Fetal distress	5/58 (9)	7/61 (12)	9/60 (15)	0.559

[a]Data are mean ± s.d. or *n* (%) unless otherwise specified.
[b]Defined as gestational age at birth < 37 weeks.
[c]Defined as birth weight < 10th percentile for gestational age.

The clinical benefits of Hb concentrations above the cutoff for anaemia are unclear. Several large population studies indicate that the Hb levels associated with optimal pregnancy outcomes range from 96–105 g l^{-1} (Steer *et al*., 1995) to 100–120 g l^{-1} (Garn *et al*., 1981). Such Hb concentrations are mostly below the current cutoff for anaemia (WHO, 2001). These observations have led to increasing debate on whether the current Hb cutoff for defining anaemia in pregnancy is too conservative and whether diagnostic criteria based on functional outcomes may be more appropriate.

Although our study may not have adequate statistical power to detect small differences in pregnancy outcomes if they exist, our findings of no differences in pregnancy outcomes between groups are consistent with the literature (Sood *et al*., 1975; Milman *et al*., 2005). Systematic reviews of randomized controlled trials have also suggested that high-dose iron supplements in pregnancy are effective in preventing ID and IDA at birth, but they have no detectable benefits on clinical outcome measures such as preterm birth and low birth weight (Mahomed, 2000; Pena-Rosas and Viteri, 2006), at least in industrialized countries where anaemia and ID are relatively mild.

It is well documented that the relationship between Hb concentration in pregnancy and pregnancy outcomes is U-shaped (Garn *et al*., 1981; Murphy *et al*., 1986; Steer *et al*., 1995; Zhou *et al*., 1998), with Hb at both low and high ends being associated with adverse pregnancy outcomes. However, the majority of iron supplementation trials in pregnancy have focused on the efficacy of iron supplements in improving iron status, but few have assessed potential side effects beyond gastrointestinal symptoms. Whether higher Hb levels with iron treatment have any potential adverse effect has been overlooked. We recently reported that low-dose iron supplements in non-anaemic pregnant women resulted in higher mean Hb (127 vs 120 g l^{-1}) at birth compared with placebo supplements (Makrides *et al*., 2003), but supplementation was associated with a higher risk of abnormal childhood behaviour (Zhou *et al*., 2006). A recent study by Ziaei *et al*. (2007) also showed that iron supplements at 50 mg per day in non-anaemic pregnant women were associated with higher risk of small-for-gestational-age infants and pregnancy hypertension. Iron supplements in pregnancy may also increase the risk of haemoconcentration (Letsky, 1991; Enkin *et al*., 1995; Pena-Rosas and Viteri, 2006), a condition that is associated with adverse pregnancy outcomes (Enkin *et al*., 1995). Our study suggested that women in the 80 mg iron group may be at higher risk of haemoconcentration as indicated by an Hb level greater than 130 g l^{-1}, a cutoff which has been used in other studies (Pena-Rosas *et al*., 2004; Pena-Rosas and Viteri, 2006). Whether a diagnosis of haemoconcentration can be reliably based on Hb concentration is unknown at present because it may lack specificity for women with a higher iron status.

We identified two relevant studies that compared the effectiveness of low-dose with high-dose iron supplements to treat anaemia in pregnancy (Sood *et al*., 1975; Milman *et al*., 2005). One of the study is the WHO-sponsored Collaborative Studies on Nutritional Anaemia in India (Sood *et al*., 1975), which compared the efficacy of 30, 60, 120 or 240 mg of iron supplements from mid-pregnancy. Although women supplemented with 120 or 240 mg iron appeared to have a lower incidence of anaemia (56%) compared with women supplemented with 30 (70%) or 60 mg (73%), there was no statistical comparison between groups (Sood *et al*., 1975). The only analysis reported was the difference between pre- and post-treatment within each group (Sood *et al*., 1975). Furthermore, the study was conducted in a population

where anaemia in pregnancy is more severe and other causes of anaemia like hookworm infection are common (Sood *et al*., 1975). The other study is a randomized dose–response trial of iron supplements in pregnancy in Denmark (Milman *et al*., 2005). The higher incidence of ID and IDA at 39 weeks of gestation in women supplemented with 20 mg iron daily compared with those received 80 mg iron daily in the Danish study (Milman *et al*., 2005) is consistent with our findings. However, it is difficult to compare our studies directly because of the different criteria used in defining anaemia. In addition, Milman's study had high attrition rate (30%) and post-randomization exclusions as well as low participation (427/3217, 13% of eligible women) (Milman *et al*., 2005). Ninety-nine per cent of women in our study completed the trial and 74% of eligible women participated highlighting the internal and external validity of our study.

In summary, although high-dose iron supplements are more effective in improving iron status of pregnant women than low-dose supplements, the lack of difference in the incidence of anaemia, the higher incidence of gastrointestinal side effects, the potential risk of haemoconcentration and the lack of benefits in clinical outcome measures suggest that there is a need to re-evaluate the risk and benefit of the high-dose iron approach. Further research is needed to determine the range of Hb levels in pregnancy associated with optimal pregnancy outcomes, and to re-evaluate the criteria for diagnosis of anaemia in pregnancy based on functional outcomes.

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References

Cuervo LG, Mahomed K (2003). Treatments for iron deficiency anaemia in pregnancy. *Cochrane Database Syst Rev* Issue 2, CD 003094.

Department of Health and Aging (DHA) & National Health and Medical Research Council (NHMRC) (2006). *Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes*. Commonwealth of Australia: Canberra.

Ekstrom EC, Hyder SM, Chowdhury AM, Chowdhury SA, Lonnerdal B, Habicht JP *et al*. (2002). Efficacy and trial effectiveness of weekly and daily iron supplementation among pregnant women in rural Bangladesh: disentangling the issues. *Am J Clin Nutr* **76**, 1392–1400.

Enkin M, Keirse M, Halmers I (1995). *A Guide to Effective Care in Pregnancy and Childbirth*, 2nd edn. Oxford University Press: New York.

Eskeland B, Malterud K, Ulvik RJ, Hunskaar S (1997). Iron supplementation in pregnancy: is less enough? A randomized, placebo controlled trial of low dose iron supplementation with and without heme iron. *Acta Obstet Gynecol Scand* **76**, 822–828.

Garn SM, Ridella SA, Petzold AS, Falkner F (1981). Maternal hematologic levels and pregnancy outcomes. *Semin Perinatol* **5**, 155–162.

Hahn PF, Carothers EL, Darby WJ, Martin M, Sheppard CW, Tenn N *et al*. (1951). Iron metabolism in human pregnancy as studied with the radioactive isotope, FE^{59*}. *Am J Obstet Gynecol* **61**, 477–486.

Hallberg L, Ryttinger L, Solvell L (1966). Side-effects of oral iron therapy. a double-blind study of different iron compounds in tablet form. *Acta Med Scand Suppl* **459**, 3–10.

Lee JI, Lee JA, Lim HS (2005). Effect of time of initiation and dose of prenatal iron and folic acid supplementation on iron and folate nutriture of Korean women during pregnancy. *Am J Clin Nutr* **82**, 843–849.

Letsky E (1991). The Haematological system. In: Hytten F, Chamberlain G (eds). *Clinical Physiology in Obstetrics*. Blackwell Scientific Publications: Oxford, UK. pp 39–82.

Mahomed K (2000). Iron supplementation in pregnancy. *Cochrane Database Syst Rev* Issue 2, CD 000117.

Makrides M, Crowther CA, Gibson RA, Gibson RS, Skeaff CM (2003). Efficacy and tolerability of low-dose iron supplements during pregnancy: a randomized controlled trial. *Am J Clin Nutr* **78**, 145–153.

Milman N, Bergholt T, Eriksen L, Byg KE, Graudal N, Pedersen P *et al*. (2005). Iron prophylaxis during pregnancy—how much iron is needed? A randomized dose–response study of 20–80 mg ferrous iron daily in pregnant women. *Acta Obstet Gynecol Scand* **84**, 238–247.

Murphy JF, O'Riordan J, Newcombe RG, Coles EC, Pearson JF (1986). Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. *Lancet* **1**, 992–995.

Pena-Rosas J, Viteri F (2006). Effects of routine oral iron supplementation with or without folic acid for women during pregnancy. *Cochrane Database Syst Rev* Issue 3, CD004736.

Pena-Rosas JP, Nesheim MC, Garcia-Casal MN, Crompton DW, Sanjur D, Viteri FE *et al*. (2004). Intermittent iron supplementation regimens are able to maintain safe maternal hemoglobin concentrations during pregnancy in Venezuela. *J Nutr* **134**, 1099–1104.

Reddaiah VP, Raj PP, Ramachandran K, Nath LM, Sood SK, Madan N *et al*. (1989). Supplementary iron dose in pregnancy anemia prophylaxis. *Indian J Pediatr* **56**, 109–114.

Solomons NW (1986). Competitive interaction of iron and zinc in the diet: consequences for human nutrition. *J Nutr* **116**, 927–935.

Sood SK, Ramachandran K, Mathur M, Gupta K, Ramalingaswamy V, Swarnabai C *et al*. (1975). W.H.O. sponsored collaborative studies on nutritional anaemia in India. 1. The effects of supplemental oral iron administration to pregnant women. *Q J Med* **44**, 241–258.

Steer P, Alam MA, Wadsworth J, Welch A (1995). Relation between maternal haemoglobin concentration and birth weight in different ethnic groups. *BMJ* **310**, 489–491.

The British Nutrition Foundation (1995). Iron absorption. In: *Iron Nutritional and Physiological Significance, the Report of the British Nutrition Foundation's Task Force*. Chapman & Hall: London. pp 3–12.

WHO (2001). *Iron Deficiency Anaemia: Assessment, Prevention and Control—a Guide for Programme Managers*. WHO: Geneva.

Zhou LM, Yang WW, Hua JZ, Deng CQ, Tao X, Stoltzfus RJ (1998). Relation of hemoglobin measured at different times in pregnancy to preterm birth and low birth weight in Shanghai, China. *Am J Epidemiol* **148**, 998–1006.

Zhou SJ, Gibson RA, Crowther CA, Baghurst P, Makrides M (2006). Effect of iron supplementation during pregnancy on the intelligence quotient and behavior of children at 4 y of age: long-term follow-up of a randomized controlled trial. *Am J Clin Nutr* **83**, 1112–1117.

Zhou SJ, Schilling MJ, Makrides M (2005). Evaluation of an iron specific checklist for the assessment of dietary iron intake in pregnant and postpartum women. *Nutrition* **21**, 908–913.

Ziaei S, Norrozi M, Faghihzadeh S, Jafarbegloo E (2007). A randomised placebo-controlled trial to determine the effect of iron supplementation on pregnancy outcome in pregnant women with haemoglobin $\geqslant$13.2 g/dl. *BJOG* **114**, 684–688.