Depleted iron stores without anaemia early in pregnancy carries increased risk of lower birthweight even when supplemented daily with moderate iron

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BACKGROUND: Gestational iron-deficiency anaemia has adverse pregnancy outcomes. Antenatal iron supplementation can be beneficial in anaemic women, but the effects in non-anaemic women are controversial. This observational study assessed the relationship of maternal iron stores (depleted or non-depleted) at gestational Weeks 8–12 with birthweight, in non-anaemic pregnant women following the guidelines of the Ministry of Health of Spain.

METHODS: Healthy, non-anaemic pregnant women (n = 205) were studied. At the first antenatal visit, a general clinical assessment was conducted, and basal blood taken. Women were classified as having non-depleted or depleted iron stores [serum ferritin (SF), 12 mg/l]. Daily antenatal iron supplements (48 mg on average) were started at 17 (range: 16–18) weeks. Blood haemoglobin, SF and transferrin saturation (TS) were measured in each trimester.

RESULTS: Of the study sample, 20, 54 and 66% had SF, 12 mg/l in the first, second and third trimesters, respectively. The prevalence of iron-depletion (SF < 12 μg/l) and iron-deficiency (SF < 12 μg/l and TS < 16%) was greater during the entire pregnancy in women with initial iron depletion versus no depletion (81.6 and 73.7% versus 61.7 and 55.4%, respectively, in the third trimester, P < 0.05). Women with initial iron-depletion delivered babies weighing on average 192 g less than that with initial iron stores, after adjusting for confounding variables (P = 0.028).

CONCLUSIONS: Beginning pregnancy with non-depleted iron stores is beneficial for the maternal iron status during pregnancy and infant birthweight. These findings reaffirm the importance of health promotion to ensure that women have adequate iron stores prior to, or early in, pregnancy when supplemented with moderate daily iron doses.

Key words: birthweight / iron stores / pregnancy / anaemia / iron supplementation

Introduction

Many women of child-bearing age have depleted iron stores. Throughout Europe and other industrialized countries between 11 and 45% of women of fertile age have been reported to have serum ferritin (SF) concentrations ranging between 10 and 17 μg/l (Hallberg, 1995), indicating minimal or absent iron stores.

Iron requirements are higher during the second and third trimesters of pregnancy owing to growth of the foeto-placental unit and to expansion of the maternal erythrocite mass. This implies a higher risk of iron deficiency in this group (Bothwell, 2000).

Iron-deficiency anaemia during pregnancy is linked to important adverse health effects for the mother and fetus, such as increased rates of premature birth, low infant birthweight (IBW; Rasmussen,
Iron requirements during pregnancy are high and are difficult to achieve by diet alone (Bothwell, 2000). The situation is further complicated when the woman starts pregnancy with insufficient iron stores (World Health Organization, 2001). The World Health Organization (WHO) highlights the importance of starting iron supplementation early in pregnancy in order to prevent iron deficiency in mothers, and adverse effects on mother and child (World Health Organization, 2001, 2007). Starting pregnancy with good iron status can pre-empt these adverse effects.

The relationships between anaemia at the start of pregnancy and adverse effects on mother and child have received considerable attention (Scanlon et al., 2000; Ronnenberg et al., 2004). Anaemia with iron depletion early in pregnancy compared with anaemia by other causes has been associated with an increased risk of lower IBW (Scholl et al., 2004). Having identified anaemia with iron depletion as a risk factor for adverse outcomes, we wished to assess whether iron depletion in women without anaemia early in pregnancy also impairs IBW.

Hence, in the present study we investigated IBW in relation to maternal iron stores (depleted or non-depleted, based on SF levels) early in pregnancy in non-anaemic pregnant women receiving antenatal moderate iron supplementation.

Materials and Methods

This is a longitudinal study based on pregnant volunteers recruited over 3 years (2005–2008). All were attending their first visit for antenatal care (at gestational Weeks 8–12) at the Unit of Obstetrics and Gynaecology of the Hospital Universitari Sant Joan de Reus, Catalunya, Spain. The study was conducted according to the guidelines of the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee of our Hospital. Written informed consent was obtained from all subjects.

The inclusion criteria were Caucasian, healthy pregnant woman at 8–12 weeks of gestation and >18 years of age. The exclusion criteria were to have had a chronic illness or a possible inflammation (high SF levels, i.e. SF > 62 μg/l; Chen et al., 2006) and low transferring saturation (TS) levels (TS < 16%), which may affect the mother’s nutritional status, and a multiple pregnancy (twins or triplets). For the purposes of the present study, the analysis included only women without anaemia at Weeks 8–12 (haemoglobin (Hb) < 110 g/l), who received a moderate dose iron supplementation (<100 mg/day) from before Week 30 of gestation, who complied with the scheduled clinical visits, who had had blood drawn at each clinical visit and who gave birth at the same hospital where the pregnancy was being monitored.

Out of a total of 300 pregnant women recruited, 205 women (68%) also complied with all the demands of the study. Of the 95 women excluded, 3 had a possible inflammation which could alter the biochemical parameters, 7 were anaemic (see below) at early pregnancy, 52 did not take iron supplements during pregnancy or took higher doses, 6 had miscarriage and 27 were excluded for having incomplete data on the biochemical parameters.

The design of the study is shown schematically in Fig. 1. In the first visit, around the 10th week of gestation, clinical and obstetric histories of the mother were taken and venous blood was drawn for laboratory analyses, including Hb concentration. At the antenatal visit at the 15th week of gestation, the obstetrician recommended to all the women to consume daily antenatal supplements containing 40 mg of elemental iron, following the study protocol and the guidelines of the Ministry of Health of Spain (Ministerio de Sanidad y Consumo, 2006). All women were asked about the consumption of daily antenatal multivitamin/mineral preparations as well as about any prior iron supplement intake. The iron compounds in the supplements taken varied and included ferric protein succinylate, ferrous sulphate, ferriamino and ovoalbumin iron and ferrous glycine sulphate. In the follow-up visits, planned for the 24th and 34th weeks of gestation, further blood samples were taken and adherence to the iron supplementation was recorded by a trained professional who was not part of the health-care provision team, using a semi-structured interview designed for this study by the researchers. From the interviews, the monthly iron and multivitamin--mineral supplementation at the start and at follow-up were recorded, as well as the frequency (number of days per week). From the iron and multivitamin--mineral supplements, the total supplementary iron and the daily average of iron supplementation during pregnancy were calculated as:

$$ \text{Total iron supplementation (mg)} = \text{Supplement iron content} \times \text{days per week} \times \text{number of weeks}. $$

The socioeconomic status of the family was assessed by the Hollingshead index (Hollingshead, unpublished working paper, Yale University, 1975). This index enables the subjects’ social status to be estimated by assigning their occupations to nine categories (from unskilled labour to highly skilled work) and the level of education to seven categories (from non-completed primary education to completed tertiary education). The status score is calculated by multiplying the occupation scale value by a weighting of 5 and the education scale value by a weighting of 3 and then combining the two scores. In our study three socioeconomic status categories were established: low, medium and high.

We defined non-smokers as the women who had never smoked and who do not work or live in a smoking environment.

Hb concentrations were measured immediately at the clinical visits using a Coulter GENS analyzer (Coulter, Hialeah, FL, USA). Serum and plasma
were stored at −80°C in our Biobank and thawed immediately prior to batched analyses so as to reduce inter-batch variation in analysis.

SF, serum transferrin and serum iron were measured by standard methods in the Clinical Chemistry laboratories of Hospital Universitari de Sant Joan de Reus (Gómez et al., 2000).

The total iron-binding capacity (TIBC) was calculated using the measured serum transferrin value (Fairbanks and Klee, 1999) and, using this additional variable, the TS was calculated as TS = (serum iron/TIBC) × 100 (Fairbanks and Klee, 1999).

We defined TS as ‘low’ when <16%, ‘iron depletion’ when SF <12 μg/l (World Health Organization, 1993) and ‘iron deficiency’ when TS <16% and SF <12 μg/l simultaneously (World Health Organization, 2001).

The women in the study were classified into two groups according to iron stores, as ‘depleted’ (SF < 12 μg/l) or ‘non-depleted’ (SF ≥ 12 μg/l) at the start of pregnancy (Week 10 of gestation).

Anaemia was defined as Hb values <110 g/l in the first and third trimesters and <105 g/l in the second trimester (Centers for Disease Control and Prevention, 1998). Iron-deficiency anaemia was defined as anaemia and iron deficiency at the same time. Haemoconcentration was defined as Hb values >130 g/l in the second and third trimesters (Pena-Rosas and Viteri, 2009). At recruitment 7 ‘anaemic women’ (Hb <110 g/l) were excluded from the study.

IBW was measured with an electronic weighbridge SECA (Vogel & Halke GmbH & Co, Hamburg) within an accuracy of 10 g. Low birthweight was defined as a weight <2500 g and fetal macrosomia as birthweight >4000 g, according to the WHO criteria (World Health Organization, 1993). Preterm was defined as babies born before the 37th week of gestation.

Results

The women excluded from the present analysis owing to lack of blood samples at some trimester of pregnancy (n = 27) were no different statistically from the women finally included in the analysis, regarding obstetric characteristics, biochemical parameters and newborn characteristics.

Table I summarizes the main characteristics of the study participants (and their newborns) classified into two groups according to iron stores as depleted (SF < 12 μg/l) or non-depleted (SF ≥ 12 μg/l) at the start of pregnancy (Week 10 of gestation). Supplementation was continued until delivery, with an average dose of 48.3 mg/d; 78% of women were taking supplements on a daily basis, 16% on most days; 6% on an average of 2 days/week. The pregnant women took different iron supplements containing distinct iron compounds. A 72.2% (n = 148) took ferric protein succinylate, 12.7% (n = 26) ferrous sulphate, 12.2% (n = 25) ferrous glycine sulphate and the remaining 2.9% (n = 6) ferrimanol and ovoalbumin iron. There were no significant differences between the depleted and non-depleted groups. Seventy-eight per cent took folic acid supplements and 6.8% daily multivitamins before the first obstetric visit. Subsequently, only two women continued to take multivitamins until the end of gestation, and they were included in the non-depleted iron stores group. There were no significant differences between the groups with respect to weekly frequency of iron supplements or the quantity.

The IBW average was 3183 g (range: 1230–4690 g). There were 14 low birthweight babies (6.8%) and 5 babies (2.4%) were categorized as infant macrosomia.

Table II summarizes the maternal haematological and biochemical parameters of iron status at different weeks of pregnancy segregated according to iron stores (non-depleted or depleted) at 8–12 weeks of gestation. The SF levels decreased as pregnancy progressed only in the group with initial non-depleted iron stores (P < 0.05). The percentage of women with SF <12 μg/l at the end of pregnancy increased compared with the percentage of women presenting initially with depleted iron stores. In the group with initial non-depleted iron stores, the mean Hb concentrations decreased as pregnancy progressed (from 126.3 to 115.4 g/l) and, concomitantly, the percentage of women with a diagnosis of iron deficiency anaemia increased from 0 to 17.1% at Week 34.

Haemoconcentration levels increased during pregnancy but only in the group with non-depleted iron stores at 8–12 weeks.

Table III show a significant relation of iron stores (non-depleted versus depleted) with IBW assessed by two MLR models. The first model, which included the initial iron stores as the only parameter for assessing the iron status, showed that the mothers with SF <12 μg/l in the first trimester had an IBW of ~148 g (95% CI: −296, −0.5) less than newborns from mothers with initially non-depleted iron stores. This relation increased after additional adjustment for the initial Hb and TS <16% at 8–12 week of gestation, showing that the mothers with depleted iron stores (SF <12 μg/l) in the first trimester had an IBW of ~192 g (95% CI: −363, −21) less than newborns from mothers with initially non-depleted iron stores. Initial BMI and gestation length contributed positively and significantly to IBW (+30.0 and +139.8 g, respectively), while smoking habit contributed negatively and significantly to IBW (−177.2 g).
Iron stores in early pregnancy and lower birthweight

Table I  Characteristics of pregnant women and newborns according to depleted (SF <12 μg/L) or non-depleted maternal iron stores (SF ≥ 12 μg/l) measured at 8–12-week gestation.

<table>
<thead>
<tr>
<th></th>
<th>Non-depleted iron stores (n = 164)</th>
<th>Depleted iron stores (n = 41)</th>
<th>P-value*</th>
<th>Total (n = 205)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mothers</strong></td>
<td></td>
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<tr>
<td>Age of the mother (years)*</td>
<td>31.2 (30.5–31.8)</td>
<td>31.0 (29.4–32.6)</td>
<td>0.849</td>
<td>31.1 (30.5–31.7)</td>
</tr>
<tr>
<td>Socioeconomic status of the family (%)*</td>
<td>7.0 (3.1–10.9)</td>
<td>14.8 (3.9–25.7)</td>
<td>0.687</td>
<td>8.3 (4.5–12.1)</td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
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<tr>
<td>Medium</td>
<td>45.5 (37.9–53.1)</td>
<td>33.3 (18.9–47.7)</td>
<td></td>
<td>43.4 (36.6–50.2)</td>
</tr>
<tr>
<td>High</td>
<td>47.6 (40.0–55.2)</td>
<td>51.9 (36.6–67.2)</td>
<td></td>
<td>48.3 (41.5–55.1)</td>
</tr>
<tr>
<td>BMI at the first visit (kg/m²)*</td>
<td>23.1 (22.5–23.7)</td>
<td>23.6 (22.4–24.8)</td>
<td>0.476</td>
<td>23.2 (22.7–23.7)</td>
</tr>
<tr>
<td>Non-smoker(%)b</td>
<td>56.0 (48.4–63.6)</td>
<td>65.8 (51.3–80.3)</td>
<td>0.039</td>
<td>58.0 (51.2–64.8)</td>
</tr>
<tr>
<td>Primipara (%)b</td>
<td>56.2 (48.6–63.6)</td>
<td>41.4 (26.3–56.5)</td>
<td>0.132</td>
<td>51.3</td>
</tr>
<tr>
<td>Gestation length (weeks)*</td>
<td>39.1 (38.8–39.4)</td>
<td>38.7 (38.2–39.2)</td>
<td>0.012</td>
<td>39.1 (38.8–39.3)</td>
</tr>
<tr>
<td>Daily iron supplementation (mg)*</td>
<td>48.9 (45.5–52.2)</td>
<td>45.6 (37.7–53.6)</td>
<td>0.042</td>
<td>48.3 (45.2–51.4)</td>
</tr>
<tr>
<td>Starting week of supplementation (weeks)*</td>
<td>17.2 (16.1–18.2)</td>
<td>17.2 (15.0–19.3)</td>
<td>0.999</td>
<td>17.2 (16.2–18.1)</td>
</tr>
<tr>
<td><strong>Newborns</strong></td>
<td></td>
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<td></td>
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<tr>
<td>IBW (g)*</td>
<td>3208 (3137–3279)</td>
<td>3067 (2930–3205)</td>
<td>0.095</td>
<td>3183 (3119–3246)</td>
</tr>
<tr>
<td>IBW adjusted for gestation week (g)*</td>
<td>3202 (3164–3240)</td>
<td>3140 (30674–3213)</td>
<td>0.163</td>
<td>3191 (3158–3225)</td>
</tr>
<tr>
<td>Gender (% male)b</td>
<td>50.6 (42.9–58.3)</td>
<td>43.9 (28.7–59.1)</td>
<td>0.418</td>
<td>49.3 (42.5–56.1)</td>
</tr>
<tr>
<td>Preterm (%)b</td>
<td>5.3 (1.9–8.7)</td>
<td>5.4 (1.5–12.3)</td>
<td>0.816</td>
<td>5.4 (2.3–8.5)</td>
</tr>
</tbody>
</table>

*P-value using two-sample t-test for continuous variables, χ² test for categorical variables.

Regarding those parameters that assess the iron status included in the MLR, TS was correlated with SF (P < 0.001) despite measuring a different aspect of iron level but Hb was not correlated with the SF (P = 0.440).

**Discussion**

The present longitudinal study assessed, in non-anaemic pregnant women at gestational Weeks 8–12, the relation between maternal iron stores (depleted or non-depleted) in the first trimester and birthweight of children, when antenatal daily iron supplementation, at moderate doses, was taken during pregnancy.

All the women in the study were healthy (with no obstetric pathology), Caucasian, of similar socioeconomic status and smoking habits to the rest of our society (Carrillo et al., 2010; Rio et al., 2010; Pueyo et al., 2011).

As far as we are aware, there are no studies in humans on the effect of early gestational depleted iron stores without anaemia on premature birth and low birthweight. In contrast, there are several important studies correlating the presence of iron-deficiency anaemia early in pregnancy with premature delivery (Scanlon et al., 2000; Scholl, 2005). Also, there are studies that indicate the benefit of gestational iron supplementation when iron-deficiency anaemia is detected early in pregnancy (Madhavan Nair et al., 2004; Bánhidy et al., 2011). Again, as far as we are aware, the impact of moderate iron antenatal supplementation on iron depleted stores without anaemia in early pregnancy has not been explored, and controversy surrounds the impact of moderate iron supplementation in the absence of gestational anaemia (Casanueva et al., 2006; Prescrire Int, 2009 [editorial]). Current literature even suggests that high supplemental iron may have negative consequences on pregnancy outcomes (Pena-Rosas and Viteri, 2009). The women in the study received moderate dose iron supplements (mean: 48.3 mg/d, 95% CI: 45.2–54.4) and adherence to the supplementation regimen was carefully monitored by an investigator who was independent from the health-care provision personnel in the hospital. The participants were not aware of their iron status, which assured the validity of adherence, which was 92% ± 18 and was similar for both groups.

SF at gestational Weeks 8–12 was used to classify the women because it is considered to be the best biochemical parameter for monitoring a deficient iron status in pregnancy (Walsh et al., 2011). The SF measure identifies a deficient iron status earlier than other biomarkers, such as TS and Hb, identifying without error the subjects without iron stores as it does not have false negatives. However, a known limitation of SF is that it increases not only with the iron content of the organism but also with acute or chronic inflammation, malignancy or liver disease, even in women with iron deficiency (Zimmermann, 2008). As TS does not increase in the presence of inflammation (Zimmermann, 2008), some authors suggest that TS should also be measured (Rambod et al., 2008; Muñoz et al., 2011) in order to detect inconsistent values (high SF and low TS) that could hide a possible inflammation with iron deficiency. Hence, women with low TS but with elevated SF values at any time of pregnancy were excluded from the statistical analysis (n = 3; 1%) in order to pre-empt false positives of SF affecting the analyses.

In the present study, the group of women with non-depleted iron stores in early pregnancy had higher values for biochemical parameters...
of iron status than women with depleted stores in early pregnancy. However, both groups reached the end of pregnancy with a high proportion of women with biochemical iron levels below the cut-off points of SF and TS simultaneously, indicative of iron depletion and iron deficiency (Table II). However, even though Hb concentrations also decreased, the mean value remained within normal limits in both groups. The incidence of anaemia, based on current cut-off points (Centers for Disease Control and Prevention, 1998), increased but was only mild (no cases <80 g/L). These changes in biochemical and haematological parameters were similar to those observed in other studies in which supplementation with similar iron doses (Cogswell et al., 2003; Soares et al., 2010) or even five times higher doses (Romshio et al., 1983) did not pre-empt this decline. The initial iron stores positively influence the final effect on the biochemical parameters, even when the iron dose supplementation is the same. In a study in the USA, which included non-anaemic women with good iron stores (SF mean of 40 μg/l), daily iron supplementation (30 mg) for 8 weeks commencing before Week 20 of gestation did not improve biochemical levels, and iron stores became depleted (mean SF of 7.4 μg/l) by late pregnancy (Cogswell et al., 2003). However, Siega-Riz et al. (2006) found that the same level of iron supplementation (30 mg) was sufficient to avoid ending pregnancy with depleted iron stores in a greater number of women when they had high SF levels (~83 μg/l) in early pregnancy; the mean SF level

Table II Biochemical and haematological parameters of pregnant women according to their iron stores (non-depleted or depleted) as measured at 8–12-week gestation.

<table>
<thead>
<tr>
<th></th>
<th>Non-depleted iron stores (n = 164)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>SF (μg/l)§</td>
<td>At Weeks 8–12 37.2a (36.9–37.5)</td>
<td>7.5b (7.1–8.0)</td>
<td>&lt;0.001</td>
<td>28.0a (27.7–28.3)</td>
</tr>
<tr>
<td></td>
<td>At Week 24 12.7b (12.4–13.1)</td>
<td>6.5b (5.9–7.1)</td>
<td>&lt;0.001</td>
<td>11.3a (11.0–11.6)</td>
</tr>
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<td></td>
<td>At Week 34 10.8b (10.5–11.1)</td>
<td>7.9b (7.4–8.5)</td>
<td>0.005</td>
<td>10.2a (10.0–10.5)</td>
</tr>
<tr>
<td>TS (%)†</td>
<td>At Weeks 8–12 28.0b (26.5–29.5)</td>
<td>17.8b (14.5–21.1)</td>
<td>&lt;0.001</td>
<td>26.3a (24.8–27.7)</td>
</tr>
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<td></td>
<td>At Week 24 16.4b (15.3–17.6)</td>
<td>16.1b (11.5–20.6)</td>
<td>0.884</td>
<td>16.4b (15.1–17.6)</td>
</tr>
<tr>
<td></td>
<td>At Week 34 13.1b (11.9–14.3)</td>
<td>11.0b (8.7–13.2)</td>
<td>0.123</td>
<td>12.7b (11.7–13.8)</td>
</tr>
<tr>
<td>Hb (g/L)†</td>
<td>At Weeks 8–12 126.3a (125.2–127.3)</td>
<td>124.9a (122.8–127.1)</td>
<td>0.296</td>
<td>126.0a (125.1–127.0)</td>
</tr>
<tr>
<td></td>
<td>At Week 24 113.4a (112.3–114.6)</td>
<td>113.4a (110.5–116.3)</td>
<td>0.999</td>
<td>113.4a (112.3–114.5)</td>
</tr>
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<td></td>
<td>At Week 34 115.4a (114.0–116.8)</td>
<td>113.6a (110.4–116.9)</td>
<td>0.304</td>
<td>115.1a (113.8–116.3)</td>
</tr>
<tr>
<td>SF &lt;12 μg/l (%)‡</td>
<td>At Weeks 8–12 0.0a</td>
<td>100.0a</td>
<td>&lt;0.001</td>
<td>20.0b (14.5–25.5)</td>
</tr>
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<td></td>
<td>At Week 24 47.6a (40.0–55.2)</td>
<td>80.5a (68.4–92.6)</td>
<td>&lt;0.001</td>
<td>54.1a (47.3–60.9)</td>
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<td>At Week 34 61.7a (54.3–69.1)</td>
<td>81.6a (69.7–93.5)</td>
<td>0.017</td>
<td>65.8a (59.3–72.3)</td>
</tr>
<tr>
<td>Iron deficiency (%)‡</td>
<td>At Weeks 8–12 0.0a</td>
<td>55.6a (40.4–70.8)</td>
<td>&lt;0.001</td>
<td>9.5a (0.5–18.5)</td>
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<td></td>
<td>At Week 24 37.7a (30.3–45.1)</td>
<td>58.5a (43.4–73.6)</td>
<td>0.026</td>
<td>42.0a (35.2–48.8)</td>
</tr>
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<td>At Week 34 55.4a (47.8–63.0)</td>
<td>73.7a (60.2–87.2)</td>
<td>0.060</td>
<td>59.0a (52.3–65.7)</td>
</tr>
<tr>
<td>Anaemia (%)‡</td>
<td>At Weeks 8–12 0.0a</td>
<td>0.0a</td>
<td>0.0a</td>
<td>0.0a</td>
</tr>
<tr>
<td></td>
<td>At Week 24 11.6a (6.7–16.5)</td>
<td>12.2a (2.2–22.2)</td>
<td>0.871</td>
<td>11.7a (7.3–16.1)</td>
</tr>
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<td>At Week 34 25.6a (18.9–32.3)</td>
<td>31.7a (17.5–45.9)</td>
<td>0.554</td>
<td>26.8a (20.7–32.9)</td>
</tr>
<tr>
<td>Iron-deficiency anaemia (%)‡</td>
<td>At Weeks 8–12 (Hb &lt;110 g/l) 0.0a</td>
<td>0.0a</td>
<td>0.0a</td>
<td>0.0a</td>
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<tr>
<td></td>
<td>At Week 24 (Hb &lt;105 g/l) 7.3a (3.3–11.3)</td>
<td>9.8a (0.7–18.9)</td>
<td>0.845</td>
<td>7.8a (4.1–11.5)</td>
</tr>
<tr>
<td></td>
<td>At Week 34 (Hb &lt;110 g/l) 17.1a (11.3–22.9)</td>
<td>26.8a (13.2–40.4)</td>
<td>0.230</td>
<td>19.0a (13.6–24.4)</td>
</tr>
<tr>
<td>Haemoconcentration (%)‡</td>
<td>At Week 24 (Hb &gt;130 g/l) 1.8a (−0.2–3.8)</td>
<td>2.4a (−2.3–7.1)</td>
<td>0.705</td>
<td>2.0a (0.1–3.8)</td>
</tr>
<tr>
<td></td>
<td>At Week 34 (Hb &gt;130 g/l) 8.0a (3.8–12.2)</td>
<td>0.0a</td>
<td>0.132</td>
<td>6.3 (3.0–9.6)</td>
</tr>
</tbody>
</table>

SF, serum ferritin; TS, transferrin saturation Hb, haemoglobin.
Iron deficiency: SF <12 μg/l and TS <16%.
Iron-deficiency anaemia: anaemia and iron deficiency.
*P-value using two-sample t-test for continuous variables, χ²-test for categorical variables.
†Mean (95% CI); †Percentage; §Geometric mean (95% CI).
±Non-identical superscript letters indicate significant differences (P < 0.05) in means of values contained in the cells in a column.
being around 21 μg/l at the end of gestation. These studies suggest, albeit not conclusively that, in healthy pregnant women, the presence of initial iron stores benefits IBW as well as the mother, provided the gestation period ends with a good iron status. In the current study, even though the initial mean iron stores in the non-depleted group were ~40 μg/l, this was not sufficient to prevent depletion of iron stores in most women later on in pregnancy.

In our population, the 20% of non-anaemic women with depleted iron stores in early pregnancy had a more pronounced risk of becoming iron deficient during pregnancy than their counterparts with higher iron stores in early pregnancy. This difference in the birthweight was within the normal range (World Health Organization, 1993), with iron stores in early pregnancy. This difference in the birthweight was re-affirmed and even increased to 192 g (95% CI: −364, −21) when adjusting for other parameters of iron status in pregnant women (initial Hb and TS < 16%) as well as for the other confounding variables, such as age of the mother, BMI at the first visit, parity, length of pregnancy, gender of the baby, smoking habits, socioeconomic status of the family and iron supplementation.

IBW is negatively influenced by the smoking habit during pregnancy, as highlighted by Guzikowski and Pirogowicz (2008) and IBW is positively influenced by a higher maternal BMI in early pregnancy, as reported by Brynhildsen et al. (2009) and by a longer gestation period (Kramer, 1987).

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### Authors’ roles

B.R. assisted in the fieldwork, the analyses and interpretation of the data, and drafted the manuscript. N.A. assisted in the fieldwork, the analyses and interpretation of the data. F.V. contributed to the analyses and interpretation of the data, and critically revised the manuscript. C.H. assisted in the fieldwork. J.C. designed and conducted the study, and assisted in the fieldwork. V.A. designed and conducted the study, contributed to the analyses and interpretation of the data, and critically revised the final version of the manuscript. All authors have read and approved the manuscript.

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### Conflict of interest

None declared.
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