Iron status in mid-pregnancy and associations with interpregnancy interval, hormonal contraceptives, dietary factors and supplement use

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Abstract

Adequate iron supply in pregnancy is important for both the woman and the fetus, but iron status is often assessed late in first trimester, if assessed at all. Therefore, identification of factors associated with iron status is important to target vulnerable groups with increased risk of deficiency. Our objectives were to (1) describe iron status in mid-pregnancy and (2) identify sociodemographic and lifestyle predictors of pregnancy iron status. This cross-sectional study uses data from The Norwegian Mother, Father and Child Cohort Study (collected 2002–2008) and The Medical Birth Registry of Norway. Iron status was measured as non-fasting plasma ferritin (P-Fe) and transferrin in gestational week (GW) 18 (*n* 2990), and by lowest reported Hb in GW 0–30 (*n* 39 322). We explored predictors of iron status with elastic net, linear and log-binomial regression models. Median P-Fe was 33 µg/l, and 14 % had depleted iron stores (P-Fe <15 µg/l). P-Fe below 30 µg/l was associated with reduced Hb. We identified eleven predictors, with interpregnancy interval (IPI) and parity among the most important. Depleted iron stores was more common among women with IPI < 6 months (56 %) and 6–11 months (33 %) than among those with IPI 24–59 months (19 %) and among nulliparous women (5 %). Positively associated factors with iron status included hormonal contraceptives, age, BMI, smoking, meat consumption and multi-supplement use. Our results highlight the importance of ferritin measurements in women of childbearing age, especially among women not using hormonal contraceptives and women with previous and recent childbirths.

Key words: **Iron deficiency: Ferritin: Transferrin: Pregnancy: Interpregnancy interval**

Inadequate iron status during pregnancy may lead to unwanted effects for both the woman and the developing fetus[1,2], including increased risk of preterm birth and low birth weight[3–5], as well as adverse effects on child neurodevelopment[6,7]. According to WHO, iron deficiency (ID) contributes to about half of all anaemia cases globally, which affects about 25–35 % of women of reproductive age[8]. Although supplementation initiated in pregnancy can correct a maternal deficiency, it is not necessarily sufficient to reverse or prevent adverse effects on child health[9,10].

Women of childbearing age may be at risk of ID resulting from inadequate dietary iron intake, blood loss from menstruation and after childbirth due to depletion of maternal reserves[2]. In pregnancy, iron demands increase progressively to support placental and fetal growth[11] and to meet the increase in maternal erythrocyte count[12]. It has been suggested that a serum ferritin concentration of at least 70 µg/l is required at the time of conception to avoid developing ID or ID anaemia during a normal pregnancy[13]. The depletion of maternal iron stores during pregnancy and lactation can therefore have consequences for a subsequent pregnancy if maternal reserves are not sufficiently replaced during the interpregnancy period[2].

Iron supplementation has for many decades been universally recommended for all pregnant women in many countries[14], but not all[15]. In Norway, iron supplementation has historically been recommended at moderate doses for women with ID[16]. However, assessment of iron status (ferritin) was not included in the antenatal guidelines between 2005 and 2018. In this period, iron supplements were recommended based on anaemia screening (low Hb)[17], although ID may also exist in the absence of anaemia[18]. After revision of the Norwegian guidelines in

2018, ferritin is now again assessed for all pregnant women before gestational week (GW) 16 and moderate doses of iron supplement intake (40–60 mg/d) are indicated at ferritin < 70 μg/l[16].

Given the relatively high prevalence of ID in the Norwegian population[19], efforts should be made to secure an adequate iron status in women not only in the last half of pregnancy but also prior to conception.[20] Therefore, identification of factors associated with iron status is important to target vulnerable groups with increased risk of ID. The aims of this study were therefore, in a group of 2990 pregnant women, (1) to describe iron status in mid-pregnancy and (2) to identify sociodemographic and lifestyle predictors of pregnancy iron status.

Materials and methods

Study population

This study is based on The Norwegian Mother, Father and Child Cohort study (MoBa, www.fhi.no/moba), a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health[21]. MoBa participants were recruited from all over Norway during 1999–2008, and the participation rate was 40·6 %. MoBa data also include information from The Medical Birth Registry of Norway, which comprises information about pregnancy, delivery and health of the mother and the neonate for all births in Norway[22]. In MoBa, blood samples were collected in GW 18[23] and biomarkers have been measured in a subsample as part of the Norwegian Environmental Biobank[24]. The main analysis in the current study includes 2990 women who were pregnant in 2002–2008, with available iron status measurements from Norwegian Environmental Biobank (see online Supplementary material, Supplementary Fig. S1). In a secondary analysis, we included all participants in MoBa with singleton pregnancies, available birth records from the Medical Birth Registry and available self-registered pregnancy Hb measurement and determinant variables in MoBa (n 39 322). This study is based on version 11 of the quality-assured MoBa data files released for research in 2018.

Ethics approval

The establishment and data collection in MoBa were previously based on a license from the Norwegian Data Protection Agency and approval from The Regional Committee for Medical Research Ethics, and it is now based on regulations related to the Norwegian Health Registry Act. The current study has been approved by The Regional Committee for Medical Research Ethics South East Norway (2015/2393).

Assessment of potential predictors from registry data and questionnaires

Definitions of all potential predictor variables are included in online Supplementary Table S1. Information about age, participation year, parity and time since previous pregnancy (for multiparae women) were obtained from the MoBa linkage to Medical Birth Registry of Norway[22]. Interpregnancy interval (IPI) was calculated as time from date of birth of the previous child to date of conception of the current pregnancy, rounded down to whole months. From the first questionnaire in MoBa (GW 15), we collected information on medical history, hormonal contraceptives use, regularity of menstrual cycle, sociodemographic factors and lifestyle. Chronic disease was defined as any self-reported asthma, diabetes, inflammatory bowel disease, rheumatic disease, epilepsy, multiple sclerosis or cancer, before or during pregnancy.

Diet and dietary supplement use were assessed by a semi-quantitative FFQ answered in mid-pregnancy. The FFQ was designed to capture dietary habits and use of supplements during the first half of pregnancy and has been described previously[25,26]. We converted food frequencies to food and nutrient intakes based on standard Norwegian portion sizes and using FoodCalc[27] and the Norwegian food composition table. We aimed to include food groups (milk, meat, tea, coffee) and food components (fibre, vitamin C) which are relevant for iron status, according to the literature. The nutrient intake from supplements was estimated using a database with nutrient content of more than 1000 different supplement brands collected from suppliers[28]. Participating women recorded the frequency and quantity, as well as the name and manufacturer of supplement(s) used.

Assessment of iron status and biomarkers from blood samples

Biochemical analyses were performed at the Finnish Institute for Health and Welfare (THL) in Helsinki, Finland. Non-fasting plasma ferritin (P-Fe) indicates the size of iron stores in the absence of concurrent infection[29]. Concentrations <15 μg/l are generally considered to be indicative of depleted iron stores for individuals above 5 years of age[29]; however, no cut-offs for ID are established for pregnancy[15]. In this study, we defined depleted iron stores as P-Fe concentrations <15 μg/l and low iron stores as P-Fe <30 μg/l. P-Fe was analysed by a chemiluminescent microparticle immunoassay (ARCHITECT Ferritin assay; Abbott Laboratories). The CV of control samples was 2·7–3·7 %. Plasma transferrin was analysed by an immunoturbidimetric procedure (Architect Transferrin assay; Abbott Laboratories). The CV of control samples was 1·8–1·9 %. As an indicator of inflammation, C-reactive protein (CRP) was measured by the Multigent CRP Vario assay, which is suitable for measuring CRP at variable assay ranges, including the low range requiring high sensitivity. The quantification limit was 0·10 mg/l. The CV of control samples was 1·5–4·2 %. The laboratory participated in an external quality assessment scheme for ferritin, transferrin and CRP was organised by Labquality (Finland). From a questionnaire answered around GW 30, participants transferred Hb measurements results from their maternity record: lowest, highest and latest measurement in pregnancy, with corresponding GW. In this study, we considered lowest Hb as the most clinically relevant indicator when studying low iron status.

Statistical analyses

We used a three-step exploratory approach to identify main predictors of iron status. First, we report descriptive statistics of iron status and prevalence of iron depletion across potentially relevant predictors from literature.

Second, we used elastic net regression to select variables associated with iron status, with natural log-transformed (ln-) P-Fe as the dependent variable. Elastic net is a regularised regression method and a useful variable selection strategy in case of multicollinearity between predictor variables[30]. To determine the penalty parameter (α) and the amount of penalisation (λ), we minimised the root-mean-squared error of prediction by 10-fold cross-validation. We used λ_{1se} (largest value of lambda that gives an error within 1 SE of the minimum), which gives a more parsimonious model than λ_{min} (gives the minimum mean cross-validated error). Before running elastic net regression, we imputed missing values in independent variables up to the full sample of 2990 with multiple imputation by chained equations. Variable selection by elastic net was then repeated on each of 100 imputed data sets, and variables that were selected in more than half of the models were included in further analysis[31].

In the third step, the variables selected by elastic net regression were included as independent variables in a linear model with ln P-Fe as a dependent variable and in log-binomial models with P-Fe <15 or <30 µg/l as a dependent variable. Continuous independent variables were scaled. All models were adjusted for chronic illness, recent cold, CRP and gestational age at the time of blood sampling (mean 18·5 (SD 1·3) weeks) to account for variation in P-Fe not related to iron status. Effect estimates are reported as relative differences (in %) and risk ratios with 95 % CI. All predictors were included in the regression model and therefore mutually adjusted for each other. Linear and log-binomial models were run on pooled imputed data sets. This third step was repeated in the large study sample (*n* 39 322) with lowest Hb value in pregnancy as dependent variable in a linear model, to investigate associations between lowest Hb and the main predictor variables selected by elastic net regression with P-Fe.

Associations were examined for non-linearity by non-parametric generalised additive models, using thin plate regression splines as smoothers (see online Supplementary material, Supplementary Fig. S2).

In a secondary analysis, we used plasma transferrin as an alternative measure of iron status and repeated the variable selection by elastic net regression, followed by linear regression models with transferrin as the dependent variable. The variables selected by the elastic net regression to predict transferrin were similar to the variables selected for ferritin; however, age and education were not among selected predictors for transferrin. The transferrin results are presented in online Supplementary Table S3. Statistical analyses were performed using R[32] and packages *mice*[33], *mgcv*[34] and *glmnet*[35].

Results

Median P-Fe concentration was 33 µg/l, ranging from 3·2 to 304 µg/l (interquartile range 20–56 µg/l). In total, 84 % had a P-Fe concentration below 70 µg/l, 44 % below 30 µg/l, 14 % below 15 µg/l (Table 1) and 9 % had P-Fe below 12 µg/l. P-Fe concentrations and use of single iron supplement across the study participation years are shown in online Supplementary Table S3. P-Fe was associated with reported lowest Hb measurement, and the reduction in Hb was evident at P-Fe concentrations lower than 30 µg/l (Fig. 1). For the subset with P-Fe< 30 µg/l, Hb increased with a mean difference of 2·8 (95 % CI 1·1, 4·5) g/l per doubling in P-Fe concentration, while no clear association was seen for higher P-Fe concentrations (mean difference 0·6 (95 % CI −0·4, 1·6) g/l per doubling in P-Fe). Among those with P-Fe below 30 µg/l, 17 % reported an Hb measurement lower than 105 g/l. Conversely, among those with an Hb measurement below 105 g/l, 55 % had P-Fe below 30 µg/l.

Geometric mean and median P-Fe concentrations suggested a crude positive association with pre-pregnancy BMI (Table 1). P-Fe was lower among non-smokers and non-consumers of alcohol during pregnancy. Median concentrations decreased with increasing parity (40 µg/l for primiparae women to 15 µg/l for women with ≥4 children) and with shorter IPI (31 µg/l for ≥60 months to 14 µg/l for <6 months). Users of hormonal contraceptives, either non-oral or oral, had higher median P-Fe than non-users, and P-Fe increased with longer duration of oral contraceptives use. Moreover, women reporting anaemia before pregnancy (3 %) had lower P-Fe concentrations (median 23 µg/l) than those not reporting anaemia (33 µg/l).

Median intake of iron from the diet (excluding supplements) was 10·8 (interquartile range 8·9–13·2) mg/d, and P-Fe tended to increase with meat intake (Table 2). P-Fe concentrations were lower among consumers of milk and slightly lower for consumers of coffee. Median values of P-Fe did not substantially differ across categories of black tea, herbal tea, vitamin C or fibre intake.

Use of iron-containing supplements during the first half of pregnancy was reported by 52 %, and 59 % reported to have used iron supplements between 29 weeks before conception and 28 weeks of gestation. P-Fe was lower for those with iron supplement intake (Table 2), for example, women with high-dose (30–50 mg/d) supplementary iron intakes had lower median P-Fe (30 µg/l), than those taking low dose (≤15 mg/d, 34 µg/l) and those with no iron supplement intake (35 µg/l). The negative association between iron supplement use and P-Fe appeared to be most profound among women who initiated iron supplement use after becoming pregnant. Moreover, P-Fe increased with longer duration of single iron supplement use in the period 8 weeks before conception to GW 20: 23 µg/l for 1–120 d of use *v.* 29 µg/l for 121–210 d of single iron supplement use. Regarding multi-supplements, women with supplemental iron intake only from multi-supplements (i.e. non-users of single supplements) had higher P-Fe than others. Also, users of multi-supplements *without* iron had higher P-Fe than users of iron-containing multi-supplements and those not using multi-supplements at all, Table 2.

Eleven variables were selected by the elastic net regression model and subsequently included in linear and log-binomial models while mutually adjusting for each other (Table 3). Parity and IPI were strongly associated with P-Fe; for parous women, an IPI < 6 months was associated with a −50·5 (95 % CI −64·6, −31·0) % reduction in P-Fe compared with 24–59 months. Further, an IPI < 12 months was associated with higher risk of depleted iron stores (adjusted risk ratio 2·40 (95 % CI 1·53, 3·73) for P-Fe < 15 µg/l), compared with

Table 1. Plasma ferritin (P-Fe) concentrations by sociodemographic and lifestyle factors
(Numbers and percentages; mean values and standard deviations; medians and interquartile ranges (IQR))

| | P-Fe (µg/l) | | | | | | P-Fe (µg/l) grouped | | | | | | | |
| | | | | | | | <15 | | ≥15 to <30 | | ≥30 to <70 | | ≥70 | |
	n	%	Geometric mean	SD	Median	IQR	n	%	n	%	n	%	n	%
All	2990	100	33	2·1	33	20–56	431	14	897	30	1166	40	496	16
Subset with CRP ≤ 10 mg/l	2517 (86)*		32	2·1	32	20–53	373	14	779	31	979	39	386	16
Subset with CRP ≤ 5 mg/l	1622 (54)*		32	2·1	31	20–53	233	14	529	33	607	37	253	16
Age (years)														
≤25	383	13	33	2·1	36	22–53	55	14	100	26	166	43	62	16
26–30	1222	41	35	2·0	34	21–58	142	12	368	30	502	41	210	17
31–35	1056	35	32	2·1	32	19–53	176	17	323	31	384	36	173	16
>35	329	11	30	2·2	30	18–52	58	18	106	32	114	35	51	16
Education														
<12 years	134	4	28	2·1	28	17–47	31	23	37	28	49	37	17	13
Upper secondary	749	25	33	2·1	34	20–56	114	15	208	28	294	39	133	18
Bachelor	1371	46	34	2·0	34	20–56	178	13	420	31	560	41	213	16
Master	673	23	33	2·1	32	20–56	96	14	213	32	248	37	116	17
Missing	63	2	36	2·3	30	19–72	12	19	19	30	15	24	17	27
Pre-pregnancy BMI (kg/m^2)														
<18·5	95	3	23	1·9	23	15–36	25	26	38	40	25	26	7	7
18·5–24·9	1918	64	32	2·1	32	20–53	285	15	592	31	756	39	285	15
25–29·9	689	23	37	2·1	38	23–63	84	12	174	25	286	42	145	21
≥30	230	8	37	2·2	38	21–68	27	12	65	28	84	37	54	23
Missing	58	2	26	2·0	26	17–44	10	17	28	48	15	26	5	9
Parity														
Primipara	1535	51	40	2·0	40	25–65	120	8	406	26	661	43	348	23
1 child	992	33	28	2·0	28	16–44	206	21	333	34	353	36	100	10
2 children	379	13	28	2·1	28	17–48	78	21	126	33	130	34	45	12
3 children	65	2	24	1·9	24	15–35	17	26	26	40	19	29	3	5
≥4 children	19	1	17	1·8	15	12–24	10	53	6	32	3	16	0	0
Interpregnancy interval†														
<6 months	16	1	14	2·1	14	8–23	9	56	4	25	3	19	0	0
6–11 months	109	8	21	1·9	21	13–32	36	33	41	38	28	26	4	4
12–17 months	225	16	24	1·9	25	16–37	50	22	84	37	83	37	8	4
18–23 months	210	14	26	1·9	25	16–38	47	22	78	37	68	32	17	8
24–59 months	630	43	29	2·0	30	17–50	117	19	204	32	237	38	72	11
≥60 months	230	16	32	2·2	31	18–56	40	17	71	31	79	34	40	17
Missing	35	2	39	2·5	34	22–71	12	33	9	25	7	19	8	22
Smoking during pregnancy														
No	2756	92	33	2·1	33	20–55	403	15	837	30	1071	39	445	16
Yes	174	6	39	2·2	41	23–67	19	11	47	27	66	38	42	24
Missing	60	2	35	2·1	37	25–60	9	15	13	22	29	48	9	15
Alcohol during pregnancy														
No	2649	89	33	2·1	33	20–55	387	15	805	30	1028	39	429	16
<2 units/month	287	10	36	2·0	36	22–59	36	13	82	29	116	40	53	18
≥2 units/month	54	2	40	2·4	43	25–71	8	15	10	19	22	41	14	26
Non-oral hormonal contraceptives (IUD)														
No	2680	90	33	2·1	33	20–55	391	15	809	30	1055	39	425	16
Yes	129	4	41	2·2	42	25–73	14	11	30	23	51	40	34	26
Missing	181	6	33	2·2	31	19–54	26	14	58	32	60	33	37	20
Oral hormonal contraceptive use														
Never	323	11	26	2·1	25	16–41	74	23	115	36	103	32	31	10
Recent use (≤12 months)	1293	43	35	2·1	35	22–57	147	11	390	30	525	41	231	18
Past use (>12 months)	1058	35	33	2·1	34	20–56	156	15	305	29	424	40	173	16
Missing	316	11	33	2·2	32	18–56	54	17	87	28	114	36	61	19

Table 1. (*Continued*)

| | P-Fe (µg/l) | | | | | | P-Fe (µg/l) grouped | | | | | | | |
| | n | % | Geometric mean | SD | Median | IQR | <15 | | ≥15 to <30 | | ≥30 to <70 | | ≥70 | |
							n	%	n	%	n	%	n	%
Oral hormonal contraceptives, duration of use														
Never	323	11	26	2·1	25	16–41	74	23	115	36	103	32	31	10
<1 years	212	7	29	2·0	27	17–45	40	19	75	35	72	34	25	12
1–3 years	516	17	32	2·0	32	21–50	73	14	159	31	210	41	74	14
4–6 years	654	22	33	2·1	33	20–56	93	14	197	30	257	39	107	16
7–9 years	600	20	35	2·0	38	22–58	71	12	167	28	254	42	108	18
≥10 years	490	16	42	2·1	43	25–67	39	8	134	27	197	40	120	24
Missing	195	7	30	2·1	31	17–54	41	21	50	26	73	37	31	16
Regular menstruation cycle														
No	660	22	33	2·1	34	19–55	106	16	175	27	271	41	108	16
Yes	2316	77	33	2·1	33	20–56	323	14	716	31	890	38	387	17
Missing	14	1	27	2·2	23	15–47	2	14	6	43	5	36	1	7
Anaemia before pregnancy														
No	2886	97	34	2·1	33	20–56	402	14	863	30	1142	40	479	17
Yes	104	3	25	2·4	23	14–48	29	28	34	33	24	23	17	16

CRP, C-reactive protein; IUD, intrauterine device.
* Percentage of full sample (n 2990).
† Interpregnancy interval is shown for parous women only (n 1456, 49 % of the total sample).

24–59 months. Notably, P-Fe was no longer negatively associated with age in the regression analysis, rather, regression analysis controlling for other variables showed increased P-Fe with increasing age (Table 3 and see online Supplementary material, Supplementary Fig. S2). The regression analysis showed lower P-Fe among underweight women compared with normal weight. Also, overweight and obesity were associated with higher P-Fe compared with normal weight. Further, smoking during pregnancy and use of hormonal contraceptives were also selected as predictors of P-Fe; smokers had 19·2 (95 % CI 7·4, 32·4) % higher P-Fe, while non-oral hormonal contraceptive use was associated with a 45·8 (95 % CI 29·6, 64·0) % increase in P-Fe.

Dietary variables were also associated with P-Fe in the regression analysis. A meat intake in the highest quartile (>156 g/d) was associated with a 9·5 (95 % CI 2·3, 17·3) % increase in P-Fe compared with being in the lowest quartile (<113 g/d). Initiation of iron-containing supplement in the period before pregnancy or during pregnancy was associated with lower P-Fe compared with no use, and the negative association between supplement use and P-Fe was stronger when the use was initiated after becoming pregnant (–20·6 (95 % CI −25·6, −15·3) % for initiation in GW 9–20, compared with no use). The opposite trend was seen for those with supplementary iron intake from multi-supplements only, which was associated with 20·3 % increased P-Fe concentrations.

The alternative model, using lowest Hb as an outcome, agreed with the P-Fe results for education, pre-pregnancy BMI, use of hormonal contraceptives, meat intake and duration and use of iron-containing supplements, but did not show the same strong association with IPI and parity. Associations were of opposite directions for age and smoking, which were positively associated with P-Fe, but negatively associated with Hb (see online Supplementary material, Supplementary Table S4).

Discussion

A main finding of this study was that a substantial number of women had low iron stores in mid-pregnancy: 14 % had P-Fe below 15 µg/l and 44 % below 30 µg/l. Further, 84 % had P-Fe below 70 µg/l, which is the cut-off for recommending supplements after GW 18–20 in the updated Norwegian antenatal guidelines[16]. Our results suggested that a P-Fe concentration below approximately 30 µg/l was associated with reduced Hb in pregnancy (as reported in GW 30). Only 17 % of women with P-Fe below 30 µg/l reported an Hb measurement lower than 105 g/l, suggesting that Hb measurements may not be a sensitive indicator of low iron status in pregnancy. In a larger study in MoBa[28], median intake of iron from diet was about 11 mg/d (similar to this study) and half of the pregnant women had an iron intake below the recommendation of 15 mg/d for women[36]. Median ferritin concentrations and prevalence of ID in this group of pregnant women were within the same range as in European women of reproductive age, as summarised by Milman *et al.*[15]. Data from >15 European countries showed an average serum ferritin concentration at 26–38 µg/l, and about 40–55 % had low or depleted iron stores (P-Fe < 30 µg/l).
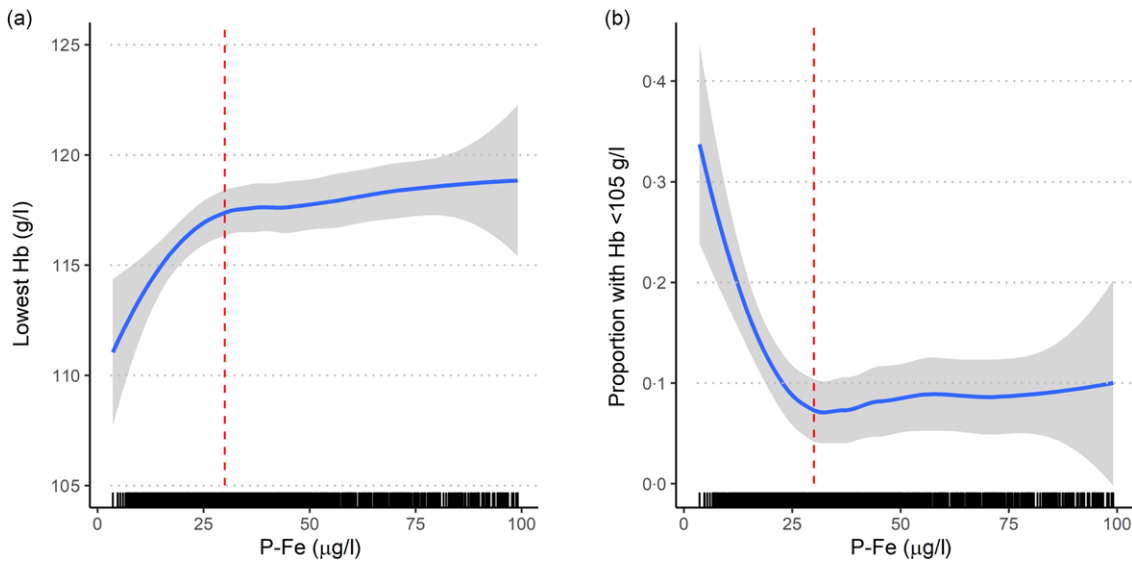
Fig. 1. Crude association between ferritin (P-Fe, μg/l) measured in mid-pregnancy (mean 18·5 (sd 1·2) gestational weeks) and (a) lowest Hb (g/l) during pregnancy; (b) proportion with lowest Hb < 105 g/l (measured in mean 23·0 (sd 6·2) gestational weeks), shown for a subset (n 1086) with P-Fe < 100 μg/l. Red dashed vertical line indicates a P-Fe concentration of 30 μg/l. The association is estimated with 95 % CI using local regression (loess) as smoother.

Another main finding was the identification of factors associated with increased risk of ID among pregnant women. Using an exploratory approach, we identified eleven sociodemographic, reproductive and lifestyle variables as predictors of low iron stores, including short IPI, increasing parity and low BMI. Moreover, prolonged pre-pregnancy use of hormonal contraceptives, particularly non-oral, was associated with higher iron status, together with increasing age and high meat intake. Early initiation of an iron-containing supplement before or early in pregnancy was associated with higher P-Fe compared with initiation after pregnancy was known (GW 9–20). Women who were taking supplementary iron from multi-supplements only (i.e. not from prescribed single high-dose supplements) had higher P-Fe compared with others.

In contrast, users of high-dose iron supplements had lower median P-Fe than non-users in this group of women; however, among those who did take single iron, prolonged use was associated with increasing P-Fe. This finding may reflect that single iron supplements were used mainly by women with known ID, according to prevailing guidelines in the study period. Also, high-dose iron supplements may potentially decrease iron absorption through increased hepcidin[37]. The increase in P-Fe with iron-containing multi-supplement use and prolonged use of high-dose iron supplement suggests a beneficial effect of supplements on iron status, although the direction of causality could not be assessed in this study.

We found a positive association with average meat consumption as reported by the FFQ, and meat consumption was among the selected predictors. Average intakes of other specific foods or beverages were not selected as important predictors. However, median P-Fe was slightly lower among those with high average intake of milk, black tea, coffee and fibre, and slightly higher among those with high vitamin C intake. These foods and beverages are known in the literature to affect the bioavailability of iron in the diet when consumed in the same meal[36,38,39].

We found that short IPI was associated with lower ferritin concentrations and increased risk of small or depleted iron stores, suggesting insufficient repletion of iron stores after a previous pregnancy. Our findings thus support the recommendation from WHO of at least 24 months between pregnancies in order to reduce risk of adverse maternal, perinatal and infant outcomes[40,41]; however, a reduction in iron stores was found for all multiparae women compared with primiparae. Indeed, short IPI has been linked to adverse maternal or child outcomes[40,42,43]. Micronutrient depletion of both iron and folic acid has been suggested to play a role[44], as these stores often remain low for several months after delivery[45]. Our results suggest that maternal iron depletion may be a potential mediator of the adverse health outcomes associated with short IPI.

The positive association between use of hormonal contraceptives and iron status may be explained by the reduced menstrual flow quantity caused by modern low-dose hormonal contraceptives[46,47]. Oral hormonal contraceptive use has been shown to increase serum ferritin levels especially in women with low iron stores (<10 μg/l)[46].

Pre-pregnancy BMI was positively associated with P-Fe for underweight, normal-weight and overweight women, but the direction of the association was unclear for obese women, online Supplementary Fig. S2. Low iron status has been related to low BMI[48], but more often with high BMI[49,50], although with inconsistent evidence when assessed as serum ferritin[51]. The low-grade inflammation related to obesity has been shown to increase secretion of hepcidin, which in turn decreases iron absorption and thus leads to low iron status[52,53].

Smokers tend to have higher ferritin levels than non-smokers[54], which we also observed in this study. There is substantial evidence that cigarette smoking leads to iron dysregulation,

Table 2. Plasma ferritin (P-Fe) concentrations by dietary intake from food and supplements
(Numbers and percentages; mean values and standard deviations; medians and interquartile ranges (IQR))

							P-Fe (µg/l) grouped							
	P-Fe (µg/l)						<15		≥15 to <30		≥30 to <70		≥70	
	n	%	Geometric mean	SD	Median	IQR	*n*	%	*n*	%	*n*	%	*n*	%
Iron intake from diet (mg/d)														
<8·9	747	25	33	2·1	32·9	20–54	108	14	225	30	290	39	124	17
9·0–10·8	747	25	33	2·1	33·6	21–56	107	14	220	29	303	41	117	16
10·9–13·1	747	25	33	2·1	32·0	20–56	103	14	242	32	276	37	126	17
≥13·2	748	25	33	2·1	33·4	20–56	113	15	210	28	297	40	128	17
Meat intake (g/d)														
<113	463	25	33	2·0	32·0	19–50	105	14	242	33	279	38	106	14
113–134	475	25	33	2·0	31·2	21–51	109	15	227	30	295	39	116	16
135–154	475	25	32	2·1	31·2	20–53	114	15	237	32	274	37	122	16
>154	475	25	37	2·0	37·8	24–62	94	13	188	25	315	42	151	20
Milk (g/d)														
No	122	4	38	2·2	38·1	23–70	14	11	32	26	46	38	30	25
≤200	1033	35	34	2·1	33·5	21–54	131	13	312	30	422	41	168	16
201–500	1264	42	33	2·1	32·4	20–57	201	16	363	29	486	38	214	17
>500	571	19	32	2·1	31·4	19–53	85	15	190	33	212	37	84	15
Tea, black (g/d)														
No	599	20	34	2·1	35·4	20–58	82	14	160	27	249	42	108	18
≤100	1169	39	33	2·1	32·5	20–57	172	15	351	30	455	39	191	16
>100	1222	41	33	2·1	32·2	20–53	177	14	386	32	462	38	197	16
Tea, herbal (g/d)														
No	1592	53	33	2·2	32·6	19–56	262	16	453	28	605	38	272	17
≤100	935	31	34	2·0	33·3	21–56	110	12	295	32	383	41	147	16
>100	463	15	34	2·0	33·6	20–55	59	13	149	32	178	38	77	17
Coffee (g/d)														
No	1076	36	34	2·1	34·1	20–57	159	15	297	28	423	39	197	18
≤100	1056	35	34	2·0	33·4	21–56	143	14	321	30	422	40	170	16
>100	858	29	32	2·1	30·9	19–53	129	15	279	33	321	37	129	15
Total vitamin C intake (mg/d)														
≤141	998	33	33	2·1	32·9	20–54	149	15	302	30	384	38	163	16
142–218	997	33	33	2·1	33·0	21–56	151	15	289	29	390	39	167	17
>218	994	33	34	2·1	33·4	20–56	131	13	306	31	392	39	165	17
Fibre (g/d)														
≤25·7	996	33	34	2·1	34·2	20–57	139	14	292	29	388	39	177	18
25·8–33·4	996	33	33	2·1	33·0	20–57	145	15	300	30	386	39	165	17
≥33·5	997	33	32	2·1	32·0	20–53	147	15	305	31	392	39	153	15
Iron intake from supplements (mg/d)*														
No iron from supplements	1442	48	35	2·1	35·1	21–58	201	14	403	28	580	40	258	18
≤15	886	30	34	2·1	34·1	21–55	120	14	259	29	351	40	156	18
15–30	345	11	31	2·0	30·5	19–50	55	16	110	32	138	40	42	12
30–50	105	4	33	2·1	29·6	21–50	9	9	44	42	22	31	19	18
>50	212	7	27	2·0	25·1	16–43	46	22	81	38	64	30	21	10
Iron from supplements, initiation†														
No reported use	1209	40	35	2·1	35·6	21–59	165	14	332	27	488	40	224	19
26–9 weeks before conception	364	12	35	2·1	34·6	21–60	46	13	105	29	147	40	66	18
8–0 weeks before conception	153	5	31	1·9	29·8	20–46	21	14	56	37	58	38	18	12
GW 0–4	201	7	36	2·0	37·0	24–56	19	9	56	28	93	46	33	16
GW 5–8	218	8	30	2·0	29·0	20–46	32	15	82	38	79	36	25	11
GW 9–12	131	4	29	2·1	28·2	17–47	29	22	39	30	44	34	19	15
GW 13–16	320	11	29	2·2	28·1	16–50	68	21	104	33	103	32	45	14
GW 17–20	70	2	27	1·8	27·2	16–41	9	13	27	39	29	41	5	7
Missing	324	11	34	2·1	34·2	20–56	42	13	96	30	125	39	61	19
Iron supplement, number of days used‡														
Not reported	2607	87	35	2·1	35·9	21–58	344	13	751	29	1044	40	468	18
1–120	262	9	24	2·0	22·9	15–37	70	27	99	38	72	27	21	8
121–210	121	4	29	1·8	29·3	19–44	17	14	47	39	50	41	7	6
Multi-supplement														
No use	467	16	32	2·2	31·9	19–54	85	18	130	28	179	38	73	16
Yes, multi-supplement with iron	1507	50	32	2·0	31·8	20–52	215	14	482	32	576	38	234	16
Yes, multi-supplement without iron	1016	34	36	2·1	36·1	21–60	131	13	285	28	411	40	189	19
Iron from multi-supplement only														
No	2110	71	32	2·1	31·4	19–53	343	16	654	31	800	38	313	15
Yes	880	29	37	2·0	36·2	23–63	88	10	243	28	366	42	183	21

GW, gestational week.
* Estimated intake of iron from supplements (single and multi).
† Based on reported time period of single iron supplement use from 26 weeks before conception until GW 28.
‡ Based on reported time period and frequency of single iron supplement use from 8 weeks before conception until GW 20.

Table 3. Associations between plasma ferritin (P-Fe) and selected (by elastic net regression) predictor variables, with regression coefficients (adjusted relative difference and risk ratios (RR) with 95 % confidence intervals) from linear and log-binomial models*†
(Numbers and percentages; risk ratios and 95 % confidence intervals, n 2990)

| | P-Fe | | P-Fe < 15 v. ≥15 µg/l | | | | P-Fe < 30 v. ≥30 µg/l | | | |
| | Relative difference | | <15 µg/l | | | | <30 µg/l | | | |
	%	95 % CI	n	%	RR	95 % CI	n	%	RR	95 % CI
Age (1 SD, 4·2 years)	2·1	−0·8, 5·1	431	14	0·97	0·85, 1·10	1328	44	0·99	0·90, 1·09
Education										
<12 years	−15·8	−25·4, −5·1	32	23	2·03	1·25, 3·23	69	50	1·36	0·92, 2·02
Upper secondary	−0·5	−6·4, 5·7	117	15	1·17	0·88, 1·54	331	43	0·96	0·78, 1·17
Bachelor	0·0	Reference	181	13	1·00	Reference	609	44	1·00	Reference
Master	−2·3	−8·2, 4·0	101	15	1·14	0·85, 1·51	319	46	1·06	0·86, 1·29
Pre-pregnancy BMI (kg/m^2)										
<18·5	−23·8	−33·6, −12·4	25	26	2·00	1·17, 3·32	63	66	2·30	1·45, 3·69
18·5–24·9	0·0	Reference	285	15	1·00	Reference	880	46	1·00	Reference
25–29·9	7·0	1·0, 13·4	91	12	0·85	0·64, 1·11	286	39	0·84	0·69, 1·01
≥30	7·5	−2·0, 17·9	30	12	0·85	0·54, 1·31	99	41	0·96	0·70, 1·30
Interpregnancy interval and parity										
<6 months	−50·5	−64·6, −31·0	9	56			13	81		
6–11 months‡	−23·7	−33·4, −12·5	37	33	2·40	1·53, 3·73	79	71	2·26	1·46, 3·57
12–17 months	−12·1	−20·6, −2·6	53	23	1·23	0·83, 1·80	138	60	1·24	0·90, 1·72
18–23 months	−10·6	−19·3, −0·9	48	22	1·28	0·86, 1·88	129	59	1·41	1·02, 1·97
24–59 months	0·0	Reference	121	19	1·00	Reference	329	51	1·00	Reference
≥60 months	5·6	−4·7, 17·0	42	18	1·00	0·65, 1·52	113	48	0·94	0·68, 1·30
Primiparae	40·9	31·8, 50·7	121	8	0·35	0·26, 0·48	527	34	0·44	0·35, 0·55
Smoking										
No	0·0	Reference	412	15	1·00	Reference	1262	45	1·00	Reference
Sometimes or daily	19·2	7·4, 32·4	19	11	0·61	0·34, 1·01	66	38	0·73	0·51, 1·03
Non-oral hormonal contraceptives										
No	0·0	Reference	416	15	1·00	Reference	1281	45	1·00	Reference
Yes	45·8	29·6, 64·0	15	11	0·46	0·25, 0·80	47	34	0·41	0·28, 0·60
Oral hormonal contraceptives, duration of use										
No use	0·0	Reference	87	23	1·00	Reference	215	57	1·00	Reference
<1 years	12·8	1·0, 26·1	44	19	0·72	0·46, 1·12	126	55	0·82	0·57, 1·17
1–3 years	16·5	6·6, 27·3	80	14	0·64	0·44, 0·92	245	44	0·64	0·48, 0·85
4–6 years	14·6	5·2, 24·9	101	15	0·73	0·52, 1·04	309	45	0·72	0·54, 0·94
7–9 years	21·2	11·0, 32·2	76	12	0·58	0·40, 0·83	252	40	0·58	0·43, 0·77
≥10 years	38·2	26·0, 51·6	43	8	0·42	0·27, 0·64	181	36	0·50	0·37, 0·68
Meat intake (g/d)										
<113	0·0	Reference	105	14	1·00	Reference	347	47	1·00	Reference
113–134	2·6	−4·1, 9·8	109	15	1·02	0·75, 1·38	336	45	0·89	0·72, 1·11
135–156	1·4	−5·3, 8·6	114	15	1·07	0·79, 1·46	351	47	0·98	0·79, 1·22
>156	9·5	2·3, 17·3	94	13	0·89	0·65, 1·23	282	38	0·68	0·55, 0·86
Iron from supplements, time of initiation										
No reported use	0·0	Reference	170	13	1·00	Reference	516	41	1·00	Reference
26–9 weeks before	−8·7	−15·9, −0·9	47	12	1·12	0·75, 1·64	159	40	1·29	0·99, 1·69
8–0 weeks before	−14·8	−23·9, −4·6	21	13	1·18	0·68, 1·97	78	47	1·67	1·16, 2·41
GW 0–8	−19·2	−25·2, −12·8	62	13	1·37	0·96, 1·95	218	45	1·82	1·42, 2·34
GW 9–20	−20·6	−25·6, −15·3	131	19	1·70	1·29, 2·25	357	52	1·91	1·55, 2·36
Supplementary iron from multi-supplements only										
No	0·0	Reference	343	16	1·00	Reference	997	47	1·00	Reference
Yes	20·3	13·2, 27·9	88	10	0·57	0·42, 0·75	331	38	0·57	0·47, 0·70

GW, gestational week.
* Models are adjusted for chronic illness, reported recent infections, C-reactive protein and gestational age at the time of blood sampling in addition to mutual adjustment for all variables listed in the table.
† The following variables were included in the elastic net regression, but not selected: Intake of coffee, herbal tea, black tea, milk, fibre, vitamin C intake, total intake of iron, duration of single iron supplement use, cumulative use of single iron supplement (frequency × duration), use of iron-containing multi-supplements, regularity of menstruation cycle, recent use of oral contraceptives (last 12 months, yes/no) and previous smoking.
‡ For log-binomial models, <6 months was collapsed with 6–11 months due to low n.

resulting in accumulation of iron both in the lung and systemically[55]. The imbalance in iron homoeostasis caused by smoking has been suggested to increase oxidative stress and play a role in pathogenesis, for example, of respiratory diseases[54,56].

Ferritin has limitations as indicator of iron status, especially during pregnancy due to physiological haemodilution, which also introduces additional inter-individual variation. Moreover,

the normal decrease in iron status throughout pregnancy is accompanied with increased intestinal iron absorption[57]. As women with depleted reserves have higher iron absorption than those with adequate iron status[57,58], this may introduce bias when studying dietary intake as a predictor. However, the increase in iron demands is largest in the second half of pregnancy[13], and we assume that the distribution of P-Fe in week 18 is representative of that earlier in

pregnancy. Although most women in this study donated blood for ferritin assessment (around GW 18) prior to filling in the FFQ (around GW 22), studies show that dietary patterns are fairly consistent between the first and second trimester[59,60]. Therefore, we consider this to have minimal influence on the findings. Although we adjusted ferritin for CRP and included transferrin as a second iron status indicator in a sensitivity analysis, additional indicators of iron status, such as transferrin saturation, would have strengthened our study[29].

Second, this study was observational with limitations to external validity. Predictors of iron status vary between populations[61], and important predictors in Norwegian pregnant women will likely differ from those in universally supplemented populations. Also, iron status was measured in a sample of women who had completed all the first six questionnaires in MoBa, possibly introducing selection bias to our study. Still, we expect that important predictors of iron status found in this study are generalisable to the general pregnant population in Norway. Furthermore, ethnic minorities are not well represented in MoBa. Low iron stores have been shown to be more common among pregnant women in certain minority groups in Norway[62]. We had no information of recent blood donations prior to pregnancy, which reduce iron stores[48,63].

A third limitation of this study relates to the estimation of iron intake from food and supplements based on questionnaires, which are, as all dietary assessments, prone to bias due to misreporting. Dietary iron intake is strongly correlated with energy intake (Pearson correlation coefficient, $r = 0.8$ in this study), and the estimated iron intake in this study will thus be biased by under- or overreporting in the FFQ[26]. Also, we had no information on meal composition, only on frequency of food consumption, which limits the assessment of dietary intakes as predictors of iron status.

Two main strengths of this study were (i) the large number of women with available ferritin measurements in mid-pregnancy (n 2990) and (ii) the extensive data collection in MoBa, which allows studying a wide range of variables related to socio-demographic factors, medical history, lifestyle including diet and supplement use. Moreover, coinciding CRP measurements enabled control for on-going inflammation in the analysis.

Conclusions

Mid-pregnancy P-Fe in this study suggested that a considerable group of Norwegian women may have low or depleted iron stores. The potential health consequences for mother and child of low ferritin, also at stages where Hb is within a range considered normal for pregnancy, should be elucidated in further research. Main predictors of P-Fe status were related to reproductive factors as IPI, parity and use of hormonal contraceptives in the past. Lifestyle factors, including diet, were of less importance. The presence of depleted iron stores in mid-pregnancy in an assumed well-nourished population like the Norwegian underlines the importance of ferritin measurements in women of childbearing age, and particularly in women with previous and recent childbirths, and among those not using hormonal contraceptives.

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Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114521000295

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