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 foetus, 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 (MoBa, 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 18 (n=2990), and by lowest reported haemoglobin (Hb) in gestational weeks 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.

1. Introduction

Inadequate iron status during pregnancy may lead to unwanted effects for both the woman and the developing foetus[1, 2], including increased risk of preterm birth and low birthweight[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 foetal growth[11] and to meet the increase in maternal red blood cell 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 iron deficiency 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 iron deficiency[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 haemoglobin)[17] although iron deficiency may exist also in absence of

anaemia[18]. After revision of the Norwegian guidelines in 2018, ferritin is now again assessed for all pregnant women before gestational week 16, and moderate doses of iron supplement intake (40-60 mg/day) is 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.

2. Materials and methods

2.1 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 gestational week 18[23], and biomarkers have been measured in a subsample as part of the Norwegian Environmental Biobank (NEB)[24]. The main analysis in the current study includes n=2990 women who were pregnant in 2002-2008, with available iron status measurements from NEB (eFigure 1, Supplementary Material). 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 haemoglobin 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.

2.2 Ethics approval

The establishment and data collection in MoBa was 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).

2.3 Assessment of potential predictors from registry data and questionnaires

Definitions of all potential predictor variables are included in eTable 1, Supplementary Material. 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 (gestational week 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 food frequency questionnaire (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 (fiber, 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.

2.4 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 cutoffs 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 immuno assay (CMIA) (ARCHITECT Ferritin assay, Abbott Laboratories, Abbott Park, IL, USA). The coefficient of variation of control samples were 2.7–3.7%. Plasma transferrin was analysed by an immunoturbidimetric procedure (Architect Transferrin assay, Abbott Laboratories, Abbott Park, IL, USA). The coefficient of variation of control samples were 1.8–1.9%. As an indicator of inflammation, C-reactive protein (CRP)

was measured by the Multigent CRP Vario (CRPVa) 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 coefficient of variation of control samples were 1.5–4.2%. The laboratory participated in an external quality assessment scheme for ferritin, transferrin and CRP organized by Labquality (Finland). From a questionnaire answered around gestational week 30, participants transferred haemoglobin (Hb) measurements results from their maternity record: Lowest, highest, and latest measurement in pregnancy, with corresponding gestational weeks. In this study, we considered lowest Hb as the most clinically relevant indicator when studying low iron status.

2.5 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 regularized 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 minimized the root mean squared error of prediction by 10-fold crossvalidation. We used λ_{1se} (largest value of lambda that gives an error within 1 standard error 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 n=2990 with multiple imputation by chained equations. Variable selection by elastic net was then repeated on

each of 100 imputed datasets, 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 dependent variable and in log-binomial models with P-Fe <15 or <30 µg/L as 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 weeks, SD 1.3) to account for variation in plasma ferritin not related to iron status. Effect estimates are reported as relative difference (in %) and risk ratio (RR) with 95% confidence intervals (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 datasets. 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 plasma ferritin.

Associations were examined for non-linearity by non-parametric generalized additive models, using thin plate regression splines as smoothers (eFigure 2).

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 eTable 3, Supplemental Material. Statistical analyses were performed using R[32] and packages *mice*[33], *mgcv*[34] and *glmnet*[35].

3. Results

Median P-Fe concentration was 33 µg/L, ranging from 3.2 to 304 µg/L (interquartile range, IQR, 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 eTable 3, Supplemental Material. 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 (Figure 1). For the subset with P-Fe< 30 µg/L, Hb increased with a mean difference of 0.22 g/dL (95% CI 0.06, 0.37) per doubling in P-Fe concentration, while no clear association was seen for higher P-Fe concentrations (mean difference 0.02 (95% CI -0.09, 0.13) g/dL per doubling in P-Fe). Among those with P-Fe below 30 µg/L, 17% reported a Hb measurement lower than 10.5 g/dL. Conversely, among those with a Hb measurement below 10.5 g/dL, 55% had P-Fe below 30 g/dL.

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 mg/day (IQR 8.9-13.2), 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), e.g. women with high-dose (30-50 mg/day) supplementary iron intakes had lower median P-Fe (30 µg/L), than those taking low-dose (<=15 mg/day, 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 gestational week 20: 23 µg/L for 1-120 days of use vs. 29 µg/L for 121-210 days 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% (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 RR 2.40 1.53, 3.73] for P-Fe <15 µg/L), compared with 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 eFigure 2). The regression analysis showed lower P-Fe among underweight women compared to 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% (CI 7.4%, 32.4%) higher P-Fe, while non-oral hormonal contraceptive use was associated with a 45.8% (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/day) was associated with a 9.5% (CI 2.3%, 17.3%) increase in P-Fe compared to being in the lowest quartile (<113 g/day). 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%, CI -25.6%, -15.3% for initiation in GW 9-20, compared to 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 (eTable 4, Supplementary Material).

4. 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 cutoff for recommending supplements after gestational weeks 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 gestational week 30). Only 17% of women with P-Fe below 30 µg/L reported a Hb measurement lower than 10.5 g/dL, 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 around 11 mg/day (similar to this study), and half of the pregnant women had an iron intake below the recommendation of 15 mg/day 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 summarized 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).

Another main finding was the identification of factors associated with increased risk of iron deficiency 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 to 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 to 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 suggests that maternal iron depletion may be a potential mediator of the adverse health outcomes associated with short IPIs.

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, eFigure 2. 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, resulting in accumulation of iron both in the lung and systemically[55]. The imbalance in iron homeostasis caused by smoking has been suggested to increase oxidative stress and play a role in pathogenesis, e.g. 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 iron assessment (around gestational week 18) prior to filling in the FFQ (around gestational week 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].

Secondly, 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 generalizable to the general pregnant population in Norway. Furthermore, ethnic minorities are not well represented in MoBa. Low iron stores has 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 sociodemographic factors, medical history, lifestyle including diet, and supplement use. Moreover, coinciding CRP measurements enabled control for ongoing 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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Conflict of interest

None.

Authorship

IHC was responsible for the conception of the article, statistical analyses, and revision of the manuscript. LIV drafted the manuscript and assisted in interpretation of results. MHA and ALB assisted in conception of the article, interpretation of results and revising the manuscript. VA assisted in interpretation of results and revising the manuscript. IE was responsible for chemical analysis and assisted with interpretation of results and revising the manuscript. HMM supervised the acquisition of data and assisted with conception of the article, interpretation of results and revision of the manuscript. All authors have approved to publication of the article and accept responsibility for the content of the paper.

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Tables

Table 1. Plasma ferritin concentrations by sociodemographic and lifestyle factors

	P-Fe (µg/L)				P-Fe (µg/L) grouped, n (%)			
	n (%)	Geometric mean (SD)	Median	IQR	<15	>=15 to <30	>=30 to <70	>=70
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)[1]	32 (2.1)	32	20-53	373 (14)	779 (31)	979 (39)	386 (16)
Subset with CRP≤5 mg/L	1622 (54)[1]	32 (2.1)	31	20-53	233 (14)	529 (33)	607 (37)	253 (16)
Age								
<=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[2]								
<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 contraceptives 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)
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)

[1] Percentage of full sample (n=2990)
[2] Interpregnancy interval is shown for parous women only (n=1456, 49% of the total sample)

Table 2. Plasma ferritin concentrations by dietary intake from food and supplements

	P-Fe (µg/L)				P-Fe (µg/L) grouped, n (%)			
	n (%)	Geometric mean(SD)	Median	IQR	<15	>=15 to <30	>=30 to <70	>=70
Iron intake from diet (mg/day)								
<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/day)								
<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/day)								
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/day)								
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/day)								
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/day)								
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/day)								
<=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/day)								
<=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/day)[a]								
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 to 30	345 (11)	31 (2.0)	30.5	19-50	55 (16)	110 (32)	138 (40)	42 (12)
30 to 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[b]								
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[c]								
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)

[a] Estimated intake of iron from supplements (single and multi).
[b] Based on reported time period of single iron supplement use from 26 weeks before conception until gestational week 28.
[c] Based on reported time period and frequency of single iron supplement use from 8 weeks before conception until gestational week 20.

Table 3. Associations between plasma ferritin and selected (by elastic net regression) predictor variables, with regression coefficients (adjusted relative difference and RRs with 95% CIs) from linear and log-binomial models (n=2990).[a,b]

	Plasma ferritin (P-Fe)	P-Fe <15 vs. >= 15 µg/L		P-Fe <30 vs. >= 30 µg/L	
	Relative difference, % (95% CI)	n (%) <15 µg/L	RR (95% CI)	n (%) <30 µg/L	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					
<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[c]	-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/day)					
<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)

[a] Models are adjusted for chronic illness, reported recent infections, CRP and gestational age at the time of blood sampling in addition to mutual adjustment for all variables listed in the table.

[b] 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.

[c] For log-binomial models, <6 months was collapsed with 6-11 months due to low n.

Figure legend

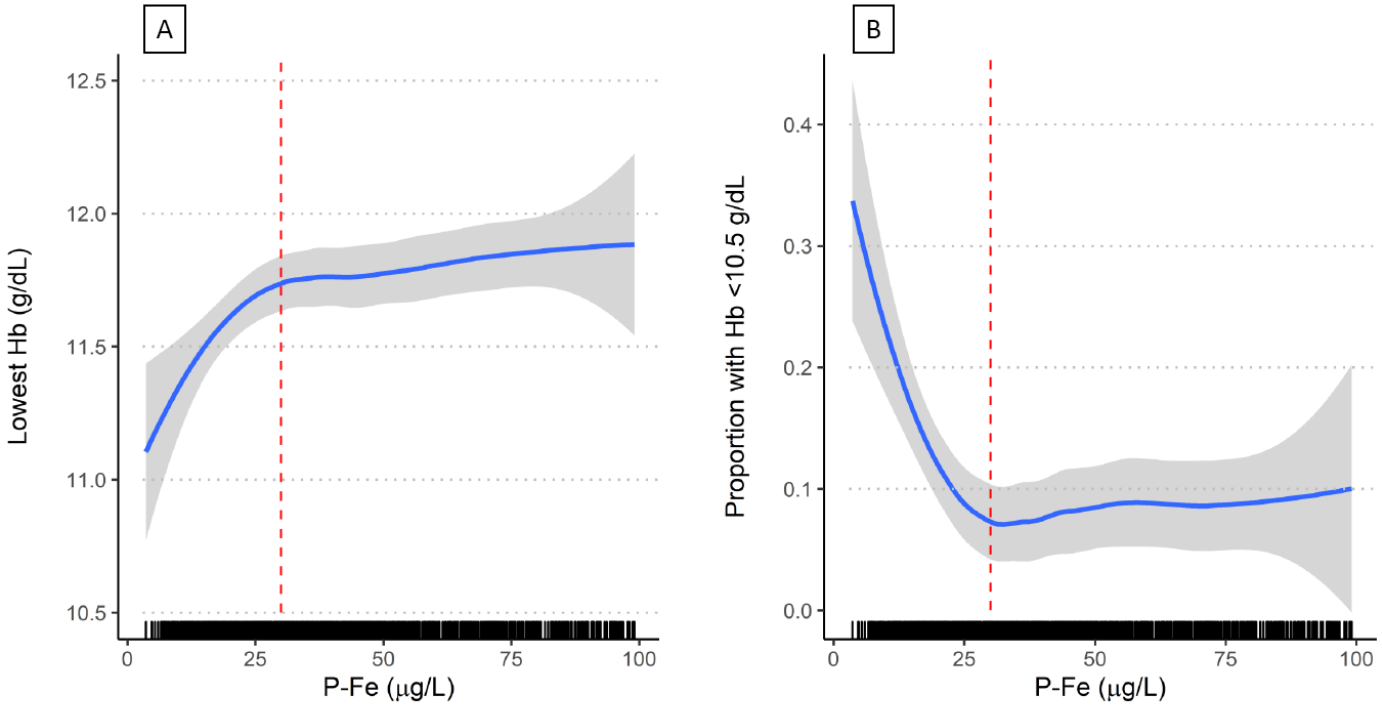

Figure 1. Crude association between ferritin (P-Fe, µg/L) measured in mid-pregnancy (mean 18.5, SD 1.2 gestational weeks) and A) lowest Hb (g/dL) during pregnancy; B) proportion with lowest Hb <10.5 g/dL (measured in mean 23.0, SD 6.2 gestational weeks), shown for a subset (n=1086) with P-Fe <100 µg/L. Red dashed vertical line indicates a P-Fe concentration of 30 µg/L. The association is estimated with 95% confidence intervals using local regression (loess) as smoother.