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Title of the article;

Serum ferritin, soluble transferrin receptor and total body iron for the detection of iron deficiency in early pregnancy. A multi-ethnic population-based study with low use of iron supplements.

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Abbreviations list

Adjusted; adj

C-reactive protein; CRP

Coefficient of variation; CV

Confidence intervals; CV

Electro-chemiluminescence immunoassay; ECLIA

Glycosylated hemoglobin; HbA1c

Hemoglobin; Hb

Intrauterine device; IUD

Iron deficiency; ID

R-square; R^2

Standard deviation: SD

Soluble transferrin receptor; sTfR

Statistics and data; Stata

Statistical Package for the Social Sciences; SPSS

Abstract

Background: Which blood-based indicator best reflects the iron status in pregnant women is unclear. Better assessments of iron status in today's multi-ethnic populations are needed to optimize treatment and clinical recommendations.

Objective: To determine the prevalence of anemia (hemoglobin <11.0 g/dL in first and <10.5 g/dL in second trimester) and iron deficiency (ID) by the iron indicators serum ferritin <15 µg/L, serum soluble transferrin receptor (sTfR) >4.4 mg/L and calculated total body iron <0 mg/kg, and their associations with ethnicity.

Design: Population-based cross-sectional study from primary antenatal care of 792 healthy women in early pregnancy in Oslo, Norway. We categorized the women into six ethnic groups; Western European, South Asian, Middle Eastern, Sub-Saharan African, East Asian, and Eastern European.

Results: Anemia was found in 5.9% (Western Europeans: 1.8%, non-Western: 0 – 14% (*P<0.05*)). ID from ferritin was found in 33% (Western Europeans: 15%, non-Western: 27 – 55% (*P<0.05*)). ID from sTfR was found in 6.5% (Western Europeans: 0.3%, non-Western: 0 – 20%, *P<0.01*). Calculated total body iron indicated ID in 11% (Western Europeans: 0.6%, non-Western: 7.0 – 28%, *P<0.01*). The prevalence of ID was significantly higher by all measures in South Asian, Sub-Saharan African and Middle Eastern than in Western European women, and the ethnic differences persisted after adjusting for confounders. South Asians, Sub-Saharan Africans, and Middle Easterners had lower iron levels by all measures for all hemoglobin intervals. Anemia related to ID varied from 35% (sTfR), 46% (total body iron) to 72% (ferritin) dependent on the iron indicator used.

Conclusion: Women at highest risk of ID and anemia were of South Asian, Middle Eastern and Sub-Saharan African origin. The prevalence of ID differed considerably depending on the iron indicator used.

INTRODUCTION

The WHO report on anemia (1) states that the prevalence in pregnant women it is about 38 % globally and 25 % in Europe, further that approximately half of anemia cases are caused by iron deficiency (ID), although this proportion differs by population. Both gestational anemia and ID are far more common in low-income countries, and in women who have migrated from low- and middle-income countries to high-income countries (2, 3) compared to women in high-income countries.

During pregnancy, iron requirements increase due to the expansion of the red blood cell mass and the transfer of increasing amounts of iron to the placental structures and the growing fetus (4-6). Maternal anemia is associated with preterm birth, small-for-gestational-age birth, low birthweight and stillbirth (5, 6). There is less evidence for associations between maternal iron status and adverse birth outcomes, although a review found that both ID and high iron status were associated with low birthweight and preterm delivery (6). Maternal ID may lead to poorer neonatal iron stores, and ID in infancy and childhood is associated with reduced mental and motor development, fatigue and reduced immune function (5-8).

To meet the increased iron requirements, most low-income countries follow recommendations endorsed by WHO on 60 mg iron daily to all pregnant women during 6 months of pregnancy (7), however, the recommendations in high-income countries, and even within Scandinavian countries differ (9-13).

As in clinical practice, most studies use serum ferritin as indicator on ID in pregnancy. However, the validity of this measure can be questioned as inflammation, infection, and the physiological hemodilution in pregnancy influence the ferritin concentration (7, 14). Serum soluble transferrin receptor (sTfR) is not influenced by pregnancy-related changes (15), but a standardized assay and a definite cut-off value for diagnostic use are still lacking. Total body

iron, based on both serum ferritin and sTfR concentrations, has been introduced as an indicator that might better reflect iron status in pregnancy (15-18).

Little is known about the prevalence of anemia and ID in pregnant women with different ethnicities living in Europe. Guidelines that reflect today's multi-ethnic populations are needed, as well as a standardized definition of ID in pregnant women. Therefore, the aims of this study were to describe the prevalence of anemia and ID by different indicators in early pregnancy, and associations with ethnicity.

SUBJECTS AND METHODS

Study population and sample selection

Data from the STORK Groruddalen Cohort study are from the pregnant multiethnic population in three administrative districts in Oslo, collected at Child Health Clinics for primary antenatal care in the period 2008 – 2010. The study design has been described in detail elsewhere (19). In short, information material and questionnaires were translated into Arabic, English, Sorani, Somali, Tamil, Turkish, Urdu and Vietnamese and quality checked by bilingual health professionals. Women were eligible if they (I) lived in the district, (II) planned to give birth at one of the two study hospitals, (III) were in <20 gestational week calculated from self-reported first day on their last menstrual period, (IV) were not suffering from diseases necessitating intensive hospital follow-up during pregnancy, (V) could communicate in Norwegian or any of the specified languages and (VI) were able to provide written consent. Maternal data were collected through interviews by authorized study personnel, assisted by professional interpreters when needed (19). Clinical measurements and blood samples were collected according to the study protocol, and covered a wide range of demographic and health issues. In total, 823 pregnant healthy women from 65 countries were included, and the participating women were found representative for the main ethnic groups of pregnant women attending the Child Health Clinics (19). Ethical approval was obtained by The Regional Ethics Committee, and written consent was received from all participants.

At the time our study was conducted, pregnant women in Norway were offered screening for anemia during pregnancy, but not for iron status. Anemic women were offered iron supplementation in adequate doses (9). In this study we present cross-sectional data from early pregnancy in the Stork Groruddalen Cohort study. We excluded pregnant women with missing serum ferritin or hemoglobin measurements (n=31). Our final sample included 792 pregnant women. See **Supplemental Figure 1** under “Online Supporting Material” for the

flow chart of participant recruitment. Missing value of serum sTfR (n=11) where equally distributed in the ethnic groups and resulted in a subsample of 781 women.

Laboratory analysis and variable definitions

Measures of iron deficiency and anemia

For this study, serum ferritin (μg/L) and hemoglobin (g/dL) were analyzed consecutively at the Department of Multidisciplinary Laboratory Medicine and Medical Biochemistry in Akershus University Hospital, Oslo, Norway. Serum ferritin was measured using an electro-chemiluminescence immunoassay (ECLIA) method (Unicel DxI 800 from Beckman Coulter) with an inter-assay coefficient of variation (CV) of <7 %. Hemoglobin was measured using a SLS method (XE 5000 from Sysmex) with an inter-assay CV of 0.7 %.

After storage at −80°C, serum samples were thawed and high sensitivity C-reactive protein (CRP) and serum sTfR analyzed at the Department of Medical Biochemistry at Oslo University Hospital, Oslo, Norway in 2016. CRP was measured using a particle-enhanced turbidimetric immunoassay (CRP Vario from Sentinel on Vitros 5.1 FS) with an inter-assay CV of <5 %. We measured serum sTfR by using ELISA (Modular P800 from Roche) with an inter-assay CV of <5 %.

We calculated total body iron according to Cook et al (16) on the basis of the ratio of sTfR concentration to ferritin concentration: $-[\log_{10}(\text{sTfR} \times 1000 \div \text{ferritin}) - 2.8229] \div 0.1207$). Positive values of total body iron represent storage iron and negative values indicate a deficient iron supply to peripheral tissues (15, 16, 20). This model is based on sTfR concentration by Flowers assay. To convert our Roche sTfR concentration to Flowers sTfR concentrations, we used the previously described conversion equation (21) Flowers sTfR = 1.5 x Roche sTfR + 0.35 mg/L.

Anemia was defined as hemoglobin concentrations <11.0 g/dL in the first trimester and hemoglobin concentrations <10.5 g/dL in the second trimester (1, 22). In addition, three established definitions for ID were used: ferritin concentration <15 μg/l (14), sTfR concentration >4.4 mg/L according to the manufacturer's guidelines, and total body iron <0 mg/kg (16-18). Hemoglobinopathy was either self-reported, identified from the HPLC (Tosoh G8, Tosoh Corporation) analysis of HbA1c (glycosylated hemoglobin) or from a combination of microcytic anemia and high ferritin.

Other variables

Ethnic groups were defined as each participant's country of birth or the participant's mother's country of birth if the participant's mother was born outside of Europe or North America. The Western European group comprised participants born in Norway (93%), Sweden, Denmark, other Western European countries and North America. The non-Western groups were categorized as South Asians (primarily from Pakistan and Sri Lanka), Middle Easterners (mainly from Iraq, Morocco and Turkey), East Asians (primarily from Vietnam and The Philippines), Sub-Saharan Africans (primarily from Somalia) and Eastern Europeans (primarily from Poland, Kosovo and Russia). Parity was dichotomized into no children (nulliparous) and one or more children (parity ≥1). Education level was dichotomized into <10 years and ≥10 years. All participants were asked about their intake of iron supplements during the past two weeks and intake of iron supplements was dichotomized into "yes" and "no".

All participants were asked of conditions increasing the risk of anemia or iron status from their medical history, use of medication and contraception use prior to pregnancy. This variable was categorized into 3 groups: (I) no chronic illness/medication, (II) chronic illness and/or medication associated with normochromic anemia (rheumatism or kidney disease,

regular medication where anemia is listed as a possible side effect in their profile) and (III) chronic illness/medication associated with hypochromic anemia (gastrointestinal disease or use of copper intrauterine device (IUD) before the current pregnancy).

Pre-pregnancy BMI (in kg/m^2) was calculated from self-reported weight before pregnancy and height measured at inclusion. Smoking was dichotomized into regular-smoking and non-smoking/not regular smoking three months before pregnancy. Other variables of interest were age and gestational week at inclusion, calculated from self-reported first day on their last menstrual period.

Data from a food frequency questionnaire, developed to capture dietary components in a multi-ethnic sample, was collected at the participants second visit (gestational week 28) and self-reported dietary patterns were extracted through cluster analysis using the Ward's method (23). We further dichotomized these four clusters into "healthy" and "unhealthy". The healthy diet contained a more frequent intake of meat, vegetables, wholegrain bread with pate and meat spread.

Statistical analyses

Descriptive statistics were presented as frequencies with proportions for categorical variables and mean with standard deviations (SD) and medians with 25th and 75th percentile for continuous variables. The distribution of sTfR, total body iron and hemoglobin were approximately normal. We calculated percentages of abnormal values for ferritin (<15 μg/l), sTfR (>4.4 mg/L), total body iron (<0 mg/kg) and hemoglobin (<11.0 g/dL in the first and <10.5 g/dL in the second trimester) for the total sample, and for each ethnic group. The differences in prevalence between Western Europeans and each non-Western group were tested by chi-square tests. In a sensitivity analysis, women with elevated CRP concentration were excluded to explore the possible effect of inflammation. We chose trimester-specific

cutoffs for elevated CRP, as reported in a study that assessed reference values for CRP in pregnancy (24); (I) >12 mg/L in gestational week 8-16, (II) >14 mg/L in gestational week 17-24, (III) >20 mg/L in gestational week 24-27, and (IV) >37 mg/L in gestational week 28-31).

To examine associations between ethnic groups and ID and anemia, we performed linear regression analyses with sTfR, total body iron, and hemoglobin as the outcome variables, and logistic regression analyses with ferritin <15 µg/L as the outcome variable. Ethnic origin was the variable of greatest interest, while gestational week, age, parity, education, iron supplements, chronic illness/medication affecting iron status and anemia, pre-pregnant BMI, smoking and dietary pattern were considered as possible confounders. Factors with a p-value <0.2 in the univariate analysis were included into the multiple regression analyses. Further, stepwise backward selection was performed by deleting the least significant variable, one at a time, until all included variables were statistically significant. Interactions with ethnicity were examined graphically and by entering cross-product terms one-by-one. No significant interactions were observed. Results from linear regressions are presented as β-coefficients and results from logistic regression as odds ratios, both with accompanied 95 % confidence intervals (CV). Model fit is presented by adjusted R^2 or Nagelkerke R^2, as appropriate. *P*-values <0.05 were considered statistically significant. SPSS (Statistical Package for the Social Sciences; Version 24, IBM SPSS statistics, NY, USA) and Stata (Statistics and data; Version 15.0, Stata Corp LLC, Collage Station, Texas, USA) software were used for statistical analysis.

198 **RESULTS**

199 A total of 792 women, 59 % of other ethnic origin than Western European, were included
200 (**Table 1**). The mean ± SD age was 29.8 ± 4.8 years, 46 % were nulliparous, pre-pregnant
201 BMI was 24.5 ± 4.8 kg/m^2 and 11 % smoked regularly 3 months before conception. At the
202 time of examination, mean ± SD gestational week was 15.4 ± 3.5. The non-Western women
203 were younger, had higher parity, and lower education than Western European women, and
204 86 % of the non-Western women reported an unhealthy dietary pattern. In total, 18 % of the
205 women used iron supplements (14 % of Western Europeans and 21% of non-Western). There
206 were no significant differences between the study sample and the 31 excluded women for
207 ethnicity, age, gestational week, parity, pre-pregnant BMI and education (data not shown).
208 The mean ± SD hemoglobin concentration was 12.5 ± 1.0 g/dL (**Table 2**) and the
209 prevalence of anemia (abnormal value of hemoglobin; hemoglobin <11.0 g/dL in the first and
210 <10.5 g/dL in the second trimester) was 5.9 % in the whole sample. Women with Sub-
211 Saharan African, South Asian, Middle Eastern and East Asian ethnic origin had significant
212 higher prevalence of anemia (abnormal value of hemoglobin, $P < 0.01$ - 0.05) compared to
213 Western Europeans. In total, 65 women had low red blood cell mean corpuscular volume
214 values (<80 fL), of whom 63 were of non-Western origin (data not shown) and three had
215 hemoglobinopathy (Table 2).
216 The prevalence of ID (abnormal value of serum ferritin, sTfR and total body iron;
217 Table 2) differed by iron indicator, and was significantly higher by ferritin than by sTfR and
218 total body iron (chi-square, $P < 0.01$). Further, the prevalence of ID by total body iron was
219 significantly higher than that from sTfR (chi-square, $P < 0.01$). Of the women with ID from
220 total body iron, 98 % were also iron deficient from ferritin, and 52 % were iron deficient by
221 sTfR. Further, in women with normal total body iron, 25 % were iron deficient from ferritin
222 and 1% from sTfR. Of the anemic women in the total sample, 72 % had ID defined by

ferritin, however, only 35 % and 46 % from sTfR and total body iron (data not shown). The prevalence of ID by all iron indicators differed between ethnic groups, and was consistently higher in women of South Asian, Middle Eastern, and Sub-Saharan African origin compared to Western European women (Table 2). To study the distribution of total body iron, we categorized the values into nine equally wide groups, presented as grouped midpoint. **Figure 1** illustrates that the total body iron concentration shifted to the left for all non-Western groups compared to Western Europeans.

Further, we categorized hemoglobin concentration into four groups, presented as grouped midpoint, to explore the distribution of the three different iron indicators within the four hemoglobin intervals by ethnicity. **Figure 2** shows that by increasing hemoglobin concentration, women of South Asian, Middle Eastern, and Sub-Saharan African origin had consistently lower mean iron stores than Western European women by all measures.

In unadjusted regression analyses, the hemoglobin concentration was lower, and the iron status was poorer by all three iron indicator (i.e. serum sTfR was higher, total body iron was lower, and the risk for ID by ferritin was higher) in women of South-Asian, Middle Eastern and Sub-Saharan African origin, compared to Western European women (**Table 3 and table 4**). These ethnic differences persisted after adjustment for possible confounders (gestational week, age, parity, education, pre-pregnant BMI, iron supplements, chronic illness and medication affecting iron status and anemia, smoking and dietary pattern). In addition, women of Eastern European origin had lower total body iron and increased risk for ID from ferritin, compared to Western European women. High gestational week was associated with low hemoglobin and poor iron status by all three iron indicators (i.e. serum sTfR was higher, total body iron was lower, and the risk for ID by ferritin was higher), whereas multiparity (≥1) and low education (<10 years) were associated with poorer iron status (i.e. serum sTfR was higher, total body iron was lower, and the risk for ID by ferritin was higher) only. In addition,

low pre-pregnant BMI, low age and use of iron supplements were independently associated with low total body iron. Lastly, low pre-pregnant BMI and use of iron supplements were also independently associated with low hemoglobin, as well as chronic illness and medication affecting iron status and hypochromic anemia. The ethnic groups with highest prevalence of anemia and iron deficiency also had the highest prevalence of an unhealthy dietary pattern (Table 1), but our dietary pattern variable was not independently associated with any iron indicator in the multiple regression analyses. The sensitivity analysis indicated that 11 % (2 – 13 % within the different ethnic groups) of the women had elevated CRP concentrations. Excluding these women with possible inflammation from the analyses, resulted in modest changes in the prevalence of ID (**Supplemental Table 1** under "Online Supporting Material").

DISCUSSION

To the best of our knowledge, this is one of very few population-based studies from Europe assessing anemia and ID in a multiethnic sample of healthy pregnant women, and the only study comparing three indicators of ID. Of note, only 18% of women in the sample used iron supplements in early pregnancy, and the prevalence of anemia in Western Europeans was low. The prevalence of ID varied substantially depending on the iron indicator used. The highest prevalence of ID was found for ferritin, and the lowest for sTfR. Women from South Asia, Middle East, and Sub-Saharan Africa were more prone to anemia and ID by all iron indicators, and these ethnic differences persisted after adjustments for confounders. Further, the iron level remained lower for these ethnic minority groups regardless of the hemoglobin concentration interval. In Western European women, the prevalence of ID from sTfR and total body iron was low.

In our study, the overall prevalence of anemia (5.9 %) in early pregnancy is comparable to other studies from Europe (3, 25-27) and the United States (18, 28), although some epidemiological European studies in pregnant women report a prevalence of anemia up to 30% (29). The low prevalence of anemia in Western Europeans (1.8%) in our study is in accordance to the prevalence in Caucasians in other studies from Europe and the United States (3, 18, 28). The prevalence of anemia in non-Western women (0 – 14 %) in our study is comparable to reports on non-Caucasians from the United States (4 – 18 %) (18, 28), but lower than in a Danish study (3) and considerably lower than the prevalence of anemia in their countries of origin (1).

Women who has migrated to Western Europe from low – and middle income countries are generally more iron deficient (3, 26, 27, 30) than Western European women. The overall prevalence of ID from ferritin in early pregnancy in our study was relatively high (33%) compared to other epidemiologic studies performed in Europe (6 – 23 %) (31) as well

as in two population-based studies from the United States (NHANES) (7 %) (18) and Australia (20 %) (32). However, in Western Europeans, our findings were similar to the prevalence in Caucasians in a British study and in NHANES (20 – 23 %) (18, 26), but for non-Western the prevalence was higher (45%) compared to results from NHANES (23 – 39 %) (18), although the composition of ethnic groups differed.

The overall prevalence of ID by sTfR (6.5%) and total body iron (11%) in our study was similar to findings in first trimester in NHANES (18), but lower than in an Australian study (32). However, in contrast to findings in NHANES, where 12 – 13 % of the Caucasians were iron deficient by sTfR and total body iron, less than 1% of Western Europeans in our study were iron deficient by these indicators. The prevalence of ID in non-Westerners in our study was similar to the findings of non-Caucasians in NHANES (18).

Generally, comparisons between studies are hampered by different sampling methods, assays or cut-off values. Without information from the "gold standard" method, iron staining of bone marrow during, the 'true' prevalence of ID is unknown, and we cannot calculate the sensitivity or specificity for the proxy indicators. In the first study of total body iron, the change in iron parameters after repeated phlebotomy was reported (20). In 2003, a stronger correlation was found for total body iron than for sTfR or serum ferritin between a given iron dose and the increase in the iron indicators in anemic pregnant women (16). The large difference in the prevalence of ID between the iron indicators in our study contrasts somewhat with findings from the United States and Australia (18, 32). Interestingly, a study from Belgium found even lower prevalence of ID by ferritin and sTfR, and higher median total body iron in early pregnancy than in our study, but the use of iron supplements in the study population was higher than in our (27). The low prevalence on ID from both sTfR and total body iron in the Western Europeans might indicate that these measures better reflect the low prevalence of anemia in these groups than serum ferritin.

Although we lack detailed information about dietary iron intake in our study, about 90% in the ethnic minority groups with highest prevalence of ID had an unhealthy dietary pattern. Results from other Norwegian studies, indicated that the diet of pregnant women of South Asian origin was richer on phytates, inhibiting the iron absorption (2), but even in ethnic Norwegian pregnant women the estimated daily intake of iron was less than recommended for pregnant women (33).

Regarding iron loss, 10% in our sample reported a chronic disease or used relevant medication that could induce anemia or ID, with no difference between ethnic groups. We can, however, not rule out that iron loss might be aggravated in some ethnic minority women due to intestinal - or other infections. However, when it comes to increased iron requirements, the higher parity of non-Western women might contribute to their higher prevalence of iron deficiency and anemia (14, 34).

In total, we found indications of hemoglobinopathy in only three women. This is an underestimate when we compare our findings to a British study (26) , although our results are in line with the results in a study from Oslo University Hospital where all hemoglobin samples with mean corpuscular volume less than 70 fL were tested for hemoglobinopathies (35).

Strengths of the present study include its population-based sample, and several adaptations to reduce barriers for inclusion of ethnic minorities even illiterate women (19). We collected a broad, high quality data set, which enabled us to explore relations between several indicators of iron status, and we adjusted for a range of possible confounders. As the majority of the women in our study did not use iron supplements, our study adds important knowledge on the iron status in early pregnancy and helps to detect high risk women. We also added sensitivity analyses where women with elevated CRP and possible inflammation that could influence our results were excluded.

However, there are also limitations to report. First, it was not feasible in a primary care setting of pregnant women, to use iron staining of bone marrow to measure ID. Second, as women were recruited consecutively as they attended Child Health Clinics, the gestational week differed somewhat, indicating that the degree of hemodilution affecting ferritin concentrations varied, and we therefor adjusted for gestational week (36). Further, the collected food frequency data was used to identify dietary patterns, not directly the iron intake (23). In addition, the usefulness of serum sTfR as a marker of ID is limited by lack of standardized commercial immunoassays for sTfR. Lastly, as few women had anemia, these estimates are less precise.

As clinical implications of our research, we suggest enhanced awareness of the high prevalence of anemia and ID in pregnant women of South Asian, Middle Eastern and Sub-Saharan African origin. Our findings also support that these women should be offered screening for ID in pregnancy even with normal hemoglobin concentrations, in line with some clinical guidelines (11, 12), but not all (13). Lastly, good-quality studies exploring the optimal level of iron indicators in relation to important clinical outcomes, as well as a threshold defining ID by different measures in pregnancy are required.

To conclude, we present the first population based study from Europe on ID from three different blood-based indicators in a multi-ethnic sample of healthy, pregnant women where few women used iron supplements. The prevalence of anemia was low in the WHO context (1), and the ID prevalence differed substantially by indicator. Clinicians should be aware of the substantially higher prevalence of anemia and ID by all three iron indicators in pregnant women with origin from South Asia, Middle East and Sub-Saharan Africa compared to women with origin from Western Europe and lower iron levels by all measures for all hemoglobin intervals.

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The authors' responsibilities were as follows: MNA had full access to all data in this study and is responsible for the integrity of data and accuracy of the data analysis; MNA, ÅRE, JPB, and AKJ contributed to the study concept and design, to analysis, tables and interpretation of data, and critical revision of the manuscript. RSF guided the statistical analysis, revised the tables and the manuscript. None of the authors have conflicts of interest to report.

References

1. The global prevalence of anaemia in 2011, WHO, 2015.
2. Brunvand L, Henriksen C, Larsson M, Sandberg A-S. Iron deficiency among pregnant Pakistanis in Norway and the content of phytic acid in their diet. Acta obstetricia et gynecologica Scandinavica 1995;74(7):520-5.
3. Nybo M, Friis-Hansen L, Felding P, Milman N. Higher prevalence of anemia among pregnant immigrant women compared to pregnant ethnic Danish women. Annals of hematology 2007;86(9):647-51. Doi: 10.1007/s00277-007-0305-7.
4. Bothwell TH. Iron requirements in pregnancy and strategies to meet them. Am J Clin Nutr 2000;72(1 Suppl):257s-64s. Doi: 10.1093/ajcn/72.1.257S
5. Scholl TO. Iron status during pregnancy: setting the stage for mother and infant. The American journal of clinical nutrition 2005;81(5):1218S-22S.
6. Dewey KG, Oaks BM. U-shaped curve for risk associated with maternal hemoglobin, iron status, or iron supplementation. Am J Clin Nutr 2017. doi: 10.3945/ajcn.117.156075.
7. Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers, WHO, 2001.
8. Allen LH. Anemia and iron deficiency: effects on pregnancy outcome. The American journal of clinical nutrition 2000;71(5):1280s-4s. Doi: 10.1093/ajcn/71.5.1280s.
9. National professional guideline for antenatal care (IS-1179), Norwegian Directorate of Health, 2005.
10. Ekstroem E-C. Supplementation for Nutritional Anemias Edtion. 1st. ed. In: Ramakrishnan ed. Nutritional Anemias, CRC Press 2001.
11. Danish Health Authority. Recommendation for pregnant women. Dietary supplements. Version current 31 August 2018. Internet: https://www.sst.dk/da/sundhed-og-livsstil/graviditet-og-foedsel/information-til-gravide/folsyre,-jern,-d-vitamin-og-eventuelt-kalk (accessed June 2012).
12. National Institute for Health and Care Excellence (NICE). Clinical Guideline. Screening for haematological conditions. Version current 31 August 2018. Internet: https://www.nice.org.uk/guidance/cg62/chapter/1-Guidance#screening-for-haematological-conditions (accessed January 2017).
13. German Federal Government. Guidelines on medical care during pregnancy and after delivery. Version current 31 August 2018. Internet: https://www.g-ba.de/downloads/62-492-1223/Mu-RL_2016-04-21_iK-2016-07-20.pdf (accessed July 2016).
14. World Health Organization, Centers for Disease Control and Prevention. Assessing the Iron Status of Populations. 2nd. ed. 2007
15. Carriaga MT, Skikne BS, Finley B, Cutler B, Cook JD. Serum transferrin receptor for the detection of iron deficiency in pregnancy. Am J Clin Nutr 1991;54(6):1077-81.
16. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood 2003;101(9):3359-64. Doi: 10.1182/blood-2002-10-3071.
17. Milman N, Byg KE, Bergholt T, Eriksen L, Hvas AM. Body iron and individual iron prophylaxis in pregnancy--should the iron dose be adjusted according to serum ferritin? Annals of hematology 2006;85(9):567-73. Doi: 10.1007/s00277-006-0141-1.
18. Mei Z, Cogswell ME, Looker AC, Pfeiffer CM, Cusick SE, Lacher DA, Grummer-Strawn LM. Assessment of iron status in US pregnant women from the National Health and Nutrition Examination Survey (NHANES), 1999-2006. Am J Clin Nutr 2011;93(6):1312-20. Doi: 10.3945/ajcn.110.007195.
19. Jenum AK, Sletner L, Voldner N, Vangen S, Mørkrid K, Andersen LF, Nakstad B, Skrivarhaug T, Rognerud-Jensen O-H, Roald B. The STORK Groruddalen research programme: a population-based cohort study of gestational diabetes, physical activity, and obesity in pregnancy in a multiethnic population. Rationale, methods, study population, and participation rates. Scandinavian journal of public health 2010;38(5 suppl):60-70.
20. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. Blood 1990;75(9):1870-6.
21. Flowers CH, Skikne BS, Covell AM, Cook JD. The clinical measurement of serum transferrin receptor. The Journal of laboratory and clinical medicine 1989;114(4):368-77.
22. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. 2011.

23. Sommer C, Sletner L, Jenum AK, Mørkrid K, Andersen LF, Birkeland KI, Mosdøl A. Ethnic differences in maternal dietary patterns are largely explained by socio-economic score and integration score: a population-based study. Food & nutrition research 2013;57.
24. Larsson A, Palm M, Hansson LO, Basu S, Axelsson O. Reference values for alpha1-acid glycoprotein, alpha1-antitrypsin, albumin, haptoglobin, C-reactive protein, IgA, IgG and IgM during pregnancy. Acta Obstet Gynecol Scand 2008;87(10):1084-8. Doi: 10.1080/00016340802428146.
25. Milman N. Prepartum anaemia: prevention and treatment. Annals of hematology 2008;87(12):949-59. Doi: 10.1007/s00277-008-0518-4.
26. Barroso F, Allard S, Kahan BC, Connolly C, Smethurst H, Choo L, Khan K, Stanworth S. Prevalence of maternal anaemia and its predictors: a multi-centre study. European journal of obstetrics, gynecology, and reproductive biology 2011;159(1):99-105. Doi: 10.1016/j.ejogrb.2011.07.041.
27. Vandevijvere S, Amsalkhir S, Van Oyen H, Egli I, Moreno-Reyes R. Iron status and its determinants in a nationally representative sample of pregnant women. J Acad Nutr Diet 2013;113(5):659-66. doi: 10.1016/j.jand.2012.10.021.
28. Le CH. The Prevalence of Anemia and Moderate-Severe Anemia in the US Population (NHANES 2003-2012). PloS one 2016;11(11):e0166635. doi: 10.1371/journal.pone.0166635.
29. Hercberg S, Preziosi P, Galan P. Iron deficiency in Europe. Public Health Nutr 2001;4(2b):537-45.
30. Bencaiova G, Burkhardt T, Breymann C. Anemia--prevalence and risk factors in pregnancy. European journal of internal medicine 2012;23(6):529-33. Doi: 10.1016/j.ejim.2012.04.008.
31. Milman N, Taylor CL, Merkel J, Brannon PM. Iron status in pregnant women and women of reproductive age in Europe. Am J Clin Nutr 2017;106(Suppl 6):1655S-62S. Doi: 10.3945/ajcn.117.156000.
32. Khambalia AZ, Collins CE, Roberts CL, Morris JM, Powell KL, Tasevski V, Nassar N. Iron deficiency in early pregnancy using serum ferritin and soluble transferrin receptor concentrations are associated with pregnancy and birth outcomes. European journal of clinical nutrition 2016;70(3):358-63. Doi: 10.1038/ejcn.2015.157.
33. Institute of Basic Medical Sciences, University of Oslo, Norwegian Directorate of Health, Norwegian Food Safety Authority. Norway NORKOST National Food Consumption Survey 2010-2011.
34. BMJ Best Practice. Iron Deficiency Anaemia. Version current 31 March 2018. Internet: http://bestpractice.bmj.com/topics/en-gb/94/pdf/94.pdf .
35. Lilleholt K HMH, Hagve T A. Hemoglobinopathies and patients with foreign names. Tidsskr Nor Legeforen 2005;125: 1164-7.
36. Costantine MM. Physiologic and pharmacokinetic changes in pregnancy. Frontiers in pharmacology 2014;5:65. Doi: 10.3389/fphar.2014.00065.

Table 1: Sociodemographic characteristics of the total sample stratified into Western Europeans and non-Western, and further into ethnic minority groups[1]

	Total	Western Europe	Non-Western	South Asia	Middle East	Sub-Saharan Africa	East Asia	Eastern Europe
	n = 792	n = 326 (41)	n = 466 (59)	n = 198 (25)	n = 123 (16)	n = 58 (7)	n= 44 (6)	n = 43 (5)
Gestational week, mean ± SD	15.4 ± 3.5	14.7 ± 2.4	16.0 ± 4.1	15.9 ± 4.2	15.7 ± 3.3	17.5 ± 5.1	16.1 ± 4.3	15.1 ± 3.2
Age (y), mean ± SD	29.8 ± 4.8	30.9 ± 4.5	29.1 ± 4.9	28.6 ± 4.5	29.4 ± 5.5	28.4 ± 5.2	31.3 ± 4.8	28.9 ± 4.2
Parity								
0	368 (46)	172 (53)	196 (42)	83 (42)	43 (35)	24 (41)	18 (41)	28 (65)
≥1	424 (54)	154 (47)	270 (58)	115 (58)	80 (65)	34 (59)	26 (59)	15 (35)
Pre-pregnant BMI, mean ± SD	24.5 ± 4.8	24.7 ± 4.8	24.5 ± 4.8	23.8 ± 4.2	25.9 ± 5.1	25.9 ± 5.9	22.6 ± 3.8	23.7 ± 4.3
Education [2]								
≥ 10 years	658 (84)	314 (97)	344 (74)	162 (82)	77 (64)	32 (55)	36 (82)	37 (88)
< 10 years	128 (16)	10 (3)	118 (26)	35 (18)	44 (36)	26 (45)	8 (18)	5 (12)
Smoking [3]								

No / not regular	695 (89)	262 (81)	433 (94)	196 (99)	114 (93)	56 (97)	40 (91)	27 (66)
Regular	90 (11)	61 (19)	29 (6)	1 (1)	8 (7)	2 (3)	4 (9)	14 (34)
Dietary pattern [4]								
Healthy	241 (33)	183 (60)	58 (14)	16 (9)	10 (9)	9 (19)	10 (25)	13 (32)
Unhealthy	491 (67)	120 (40)	371 (86)	173 (91)	102 (91)	38 (81)	30 (75)	28 (68)
Iron supplements								
No	650 (82)	281 (86)	369 (79)	152 (77)	102 (83)	45 (78)	32 (73)	38 (88)
Yes	142 (18)	45 (14)	97 (21)	46 (23)	21 (17)	13 (22)	12 (27)	5 (12)
Chronic illness/medication								
No	716 (90)	290 (89)	426 (91)	184 (93)	110 (89)	54 (94)	38 (87)	40 (93)
Yes, with normochromic anemia [5]	23(3)	11(3)	12 (3)	3 (2)	6 (5)	2 (3)	1 (2)	0 (0)
Yes, with hypochromic anemia [6]	53 (7)	25 (8)	28 (6)	11 (5)	7 (6)	2 (3)	5 (11)	3 (7)

[1] Values are n (%) if not stated otherwise.

[2]missing information in 6 women.

[3]self-reported smoking 3 months before conception. Missing information in 7 women.

[4] self-reported dietary pattern in gestational week 28. Missing information in 60 women.

[5] women with chronic illness or medication affecting iron status and normochromic anemia (rheumatism or kidney disease, regular medication where anemia is listed as a possible side effect in their profile).

[6] women with chronic illness or medication affecting iron status and hypochromic anemia (gastrointestinal disease or use of copper intrauterine device (IUD) before the current pregnancy)

Abbreviations used: IUD; intrauterine device, n; number of observations, SD, standard deviation.

Group designation; Western Europe; participants with origin from Norway, Sweden, Denmark, other Western European countries and North America. Non-Western; participants from South Asia, Middle East, Sub-Saharan Africa, East Asia and East Europe. South Asia; participants with origin primarily from Pakistan and Sri Lanka. Middle East; participants with origin primarily from Iraq, Morocco and Turkey. East Asia; participants with origin primarily from Vietnam and The Philippines. Sub-Saharan Africa; participants with origin primarily from Somalia. East Europe participants with origin primarily from Poland, Kosovo and Russia.

Table 2: Values for serum ferritin, serum soluble transferrin receptor (sTfR), total body and sTfR concentrations) and serum hemoglobin, and prevalence of abnormal values (iron deficiency and anemia) in pregnant women in STORK-Groruddalen study

	n	Ferritin µg/L	sTfR mg/L	Total body iron mg/kg	Hemoglobin g/dL
Mean ± SD	[1]	32.5 ± 33.0	2.5 ± 1.2	4.8 ± 4.0	12.5 ± 1.0
Median (25th and 75th percentile)	[1]	21 (12, 40)	2.2 (1.8, 2.9)	4.9 (2.2, 7.7)	12.1 (11.4, 12.7)
Prevalence of abnormal values [2]					
Total sample	[1]	33 (30, 36)	6.5 (5.0, 8.5)	11 (8.9, 13)	5.9 (4.5, 7.8) [3]
By ethnic groups[4]					
Western Europe	322	15 (12, 20)	0.3 (0.04, 2.2)	0.6 (0.2, 2.5)	1.8 (0.8, 4.1)
South Asia	195	50 (43, 57)**	12 (7.9, 17)**	21 (15, 27)**	11 (7.0, 16)**
Middle East	123	43 (35, 52)**	11 (6.8, 18)**	17 (11, 25)**	7.3 (3.8, 14)**
Sub-Saharan Africa	54	55 (42, 68)**	20 (11, 34)**	28 (17, 42)**	14 (6.9, 26)**
South Asia	44	27 (16, 43)*	4.5 (1.1, 17)**	9.1 (3.3, 23)**	6.8 (2.1, 20)*
Eastern Europe	43	37 (24, 53)**	0	7.0 (2.2, 20)**	0

[1] n=792 for serum ferritin and hemoglobin, n=781 for sTfR and total body iron

[2] Abnormal values presented as percentage; 95% CI in parentheses; defined as serum ferritin <15 mcg/L, serum sTfR >4.4 mg/L, total body iron <0 mg/kg, and hemoglobin <0 mg/kg, and hemoglobin <11.0 g/dL for pregnant women in the first trimester and <10.5 g/dL in the second trimester.

[3] Hemoglobinopathy (n=3) was either self-reported, identified from the HPLC Tosoh G8, Tosoh Corporation) analysis of HbA1c or from a combination of microcytic anemia and high ferritin

[4] The differences in prevalence of abnormal values between Western Europeans and each non-Western group were tested by chi-square test; *P < 0.05, **P < 0.01

Abbreviations used: CI, confidence interval; HbA1c; glycosylated hemoglobin, n; number of observations, SD, standard deviation; sTfR soluble transferrin receptor.

Group designation; Western Europe; participants with origin from Norway, Sweden, Denmark, other Western European countries and North America. Non-Western; participants from South Asia, Middle East, Sub-Saharan Africa, East Asia and East Europe. South Asia; participants with origin primarily from Pakistan and Sri Lanka. Middle East; participants with origin primarily from Iraq, Morocco and Turkey. East Asia; participants with origin primarily from Vietnam and The Philippines. Sub-Saharan Africa; participants with origin primarily from Somalia. East Europe participants with origin primarily from Poland, Kosovo and Russia.

Table 3: Linear regression analyses of the iron indicators serum soluble transferrin receptor (sTfR) concentration and total body iron concentration (calculated from serum ferritin and sTfR concentrations), and of hemoglobin concentration in pregnant women with different ethnic origin in the STORK-Groruddalen study [1, 2]

	sTfR (mg/L)		Total body iron (mg/kg)		Hemoglobin (g/dL)	
	β, 95% CI [1]	adj β [1,2] (95% CI) $R^2 = 0.19$	β (95% CI) [1]	adj β [1,3] (95% CI) $R^2 = 0.30$	β (95% CI) [1]	adj β [1,4] (95% CI) $R^2 = 0.20$
Ethnicity (Western European ref)						
South Asia	1.0 (0.8, 1.2)**	0.9 (0.7, 1.8)**	-3.8 (-4.5, -3.2)**	-2.9 (-3.6, -2.3)**	-0.6 (-0.8, -0.4)**	-0.4 (-0.6, -0.3)**
Middle East	0.7 (0.5, 0.9)**	0.5 (0.3, 1.1)**	-3.0 (-3.8, -2.3)**	-2.0 (-2.8, -1.3)**	-0.5 (-0.7, -0.3)**	-0.4 (-0.6, -0.2)**
Sub-Saharan Africa	1.3 (0.9, 1.6)**	1.0 (0.7, 1.4)**	-4.1 (-5.1, - 3.1)**	-2.9 (-4.0, -1.9)**	-0.6 (-0.8, -0.3)**	-0.4 (-0.6, 0.1)**
East Asia	0.3 (-0.03, 0.7)	0.2 (-0.2, 0.5)	-0.2 (-1.3, 0.9)	0.8 (-0.3, 1.8)	-0.4 (-0.7, -0.1)*	-0.2 (-0.4, 0.1)
Eastern Europe	0.0 (-0.3, -0.4)	0.0 (-0.4, 0.3)	-1.6 (-2.8, -0.5)*	-1.4 (-2.5, -0.3)*	0.1 (-0.3, 0.3)	0.1 (-0.2, 0.3)

[1] n=781 for serum sTfR and total body iron, n=792 for serum hemoglobin.

[2] Univariate and multiple regression estimates for [sTfR], adjusted for gestational week, age, parity, education, smoking and dietary pattern. *; $P < 0.05$, **; $P < 0.01$.

[3] Univariate and multiple regression estimates for [total body iron], adjusted for gestational week, age, parity, education, pre-pregnant BMI, iron supplement, smoking and dietary pattern. *; $P < 0.05$, **; $P < 0.01$.

[4] Univariate and multiple regression estimates for [hemoglobin], adjusted for gestational week, parity, education, pre-pregnant BMI, iron supplement, chronic illness/medicine, smoking and dietary pattern. *; $P < 0.05$, **; $P < 0.01$.

Abbreviations: adj; adjusted, CI, confidence interval; n; number of observations, ref; reference; R2; R square, sTfR, soluble transferrin receptor.

Group designation; Western Europe; participants with origin from Norway, Sweden, Denmark, other Western European countries and North America. Non-Western; participants from South Asia, Middle East, Sub-Saharan Africa, East Asia and East Europe. South Asia; participants with origin primarily from Pakistan and Sri Lanka. Middle East; participants with origin primarily from Iraq, Morocco and Turkey. East Asia; participants with origin primarily from Vietnam and The Philippines. Sub-Saharan Africa; participants with origin primarily from Somalia. East Europe participants with origin primarily from Poland, Kosovo and Russia.

Table 4: Logistic regression analyses of serum ferritin concentration < 15 µg/dL in pregnant women with different ethnic origin in the STORK-Groruddalen study (n = 792) [1]

	OR (95% CI)	Adj OR (95% CI)[1] $R^2 = 0.26$
Ethnicity (Western European ref)		
South Asia	5.6 (3.7, 8.5)*	4.4 (2.9, 6.8)*
Middle East	4.3 (2.7, 6.8)*	3.0 (1.8, 5.0)*
Sub-Saharan Africa	7.0 (3.8, 12.7)*	4.4 (2.2, 8.6)*
East Asia	2.1 (1.0, 4.4)	1.3 (0.6, 2.8)
East Europe	3.4 (1.7, 6.7)*	3.2 (1.5, 6.7)*

[1] Univariate and multiple regression estimates for serum ferritin concentration < 15 µg/dL, adjusted for gestational week, parity, education, pre-pregnant body mass index, iron supplement, smoking and dietary pattern. *; $P < 0.01$

Abbreviations: Adj, adjusted; CI, confidence interval; OR, odds ratio, ref; reference, n; number of observations, R^2; Nagelkerke R-square Group designation; Western Europe; participants with origin from Norway, Sweden, Denmark, other Western European countries and North America. Non-Western; participants from South Asia, Middle East, Sub-Saharan Africa, East Asia and East Europe. South Asia; participants with origin primarily from Pakistan and Sri Lanka. Middle East; participants with origin primarily from Iraq, Morocco and Turkey. East Asia;

participants with origin primarily from Vietnam and The Philippines. Sub-Saharan Africa; participants with origin primarily from Somalia. East Europe participants with origin primarily from Poland, Kosovo and Russia.

Figure legends

Figure 1.

Title: Distribution of Total body iron (calculated from serum ferritin and soluble transferrin receptor concentrations) in pregnant women by ethnic group from STORK-Groruddalen study. Nine total body iron concentration intervals presented as grouped midpoint.

Nonstandard abbreviations: Group designation; Western Europe; participants with origin from Norway, Sweden, Denmark, other Western European countries and North America. South Asia; participants with origin primarily from Pakistan and Sri Lanka. Middle East; participants with origin primarily from Iraq, Morocco and Turkey. East Asia; participants with origin primarily from Vietnam and The Philippines. Sub-Saharan Africa; participants with origin primarily from Somalia. East Europe participants with origin primarily from Poland, Kosovo and Russia.

Figure 2.

Title: Median ferritin concentration (µg/L), mean serum sTfR (mg/L), and mean total body iron (mg/kg) in four hemoglobin concentration (g/dL) intervals.

Nonstandard abbreviations: Hb; hemoglobin. Group designation; Western Europe; participants with origin from Norway, Sweden, Denmark, other Western European countries and North America. South Asia; participants with origin primarily from Pakistan and Sri Lanka. Middle East; participants with origin primarily from Iraq, Morocco and Turkey. East Asia; participants with origin primarily from Vietnam and The

Philippines. Sub-Saharan Africa; participants with origin primarily from Somalia. East Europe participants with origin primarily from Poland, Kosovo and Russia.