IRON STATUS AND PREGNANCY

Gestational and postpartum iron deficiency and anaemia, and associations with ethnicity and clinical factors: a multi-ethnic population-based cohort study

Marthe-Lise Næss-Andresen

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Main supervisor Line Sletner

Co-supervisors Anne Karen Jenum and Jens Petter Berg

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Contents

Preface

As a general practitioner (GP) with a high proportion of reproductive-age women in my practice, following them through pregnancies and the postpartum period, I became increasingly aware of iron deficiency re-emerging as a medical issue. I found the prevalence of iron deficiency and anaemia among children, adolescents and adults, including pregnant and postpartum women in my practice, to be high. The Norwegian national guideline for antenatal care by the Norwegian Directorate of Health from 2005 did not recommend to screen pregnant women for iron deficiency, nor to use routine iron supplementation if they were not anaemic [1].

The previous guidelines recommended selective iron supplementation to women with inadequate iron status to meet the needs of pregnancy based on serum ferritin concentration in early pregnancy [2]. The argument to change the guidelines in 2005 was lack of evidence for benefits of iron supplementation based on low serum ferritin (SF) values without anaemia [1].

In 2012, I contacted Prof. Jørund Straand, at the time being head of the Department of General Practice at the University of Oslo, and he introduced me to Prof. Anne Karen Jenum. In 2008, she initiated a research project in Groruddalen in Oslo on pregnant women with different ethnic background and their neonates, covering a wide range of health issues. She offered me to join the STORK Groruddalen (STORK G) study group, and I was given the opportunity to use collected data from this cohort to explore iron status and anaemia in pregnant women in a multi-ethnic population.

In 2014, I joined the PhD programme at the Faculty of Medicine at the University of Oslo, worked part-time here, besides my work as GP. I soon became aware of the limitations of using serum ferritin concentrations as an indicator on iron status in pregnancy. As published work encouraged researchers to analyse other iron indicators, I applied to use the STORK G study's biobank to analyse serum transferrin receptor concentration, and thereafter calculate total body iron in addition to the already analysed serum ferritin and haemoglobin concentrations. I got access to these new data in 2016.

The research process that led to this thesis was a challenging but rewarding experience that taught me a great deal about the complexities of studying iron status.

Acknowledgements

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I would also like to thank my co-supervisor, Jens Petter Berg for your expertise and contributions in shaping this project. Your guidance into the clinical biochemistry and our discussions on assay methods, establishment of reference values and thresholds to define iron deficiency and anaemia, have been invaluable. I am grateful for the time and effort you devoted to my development as a researcher. This knowledge has also been very useful in my clinical practice.

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I would like to extend my sincere gratitude to the Norwegian Research School in General Practice, for providing an exceptional learning and networking environment. Thank you for generously funding my research stay in San Diego. The experience of working in a new environment with researchers in my field at UCSD was invaluable.

My gratitude also goes to my research colleagues in the STORK G group for all discussions and contributions in workshops, to all of the study staff for laying the groundwork for this project, and not least, to all the women participating in the STORK G study.

I would like to express my deepest gratitude to my parents, Evy and Kåre, for years of love, support, and encouragement. I would also like to thank my in-laws, Brit and Fred, for having constant belief in me.

I am also deeply thankful to my loving husband Thomas and our three wonderful children Signe, William and Frida. Thank you for your unwavering love and support, your patience and understanding throughout my PhD journey. You are my rock, my motivation, and my joy. I am so lucky to have you in my life.

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The Norwegian Research School in General Practice funded a research stay in the United States (US). I spent three months at the University of California San Diego (UCSD), meeting haematologists and other researchers working in the field of iron deficiency and anaemia.

Summary of thesis

The overall goal of this project was to increase the knowledge about iron status, iron deficiency and anaemia among pregnant women in a multi-ethnic population. We used data from the population-based cohort study STORK Groruddalen, of 823 healthy, pregnant women attending primary antenatal Child Health Clinics in Groruddalen, Oslo, Norway. Maternal data from questionnaires, clinical measurements and blood samples were collected from 2008 to 2011 at three study visits in gestational week 15 and 28, as well as 14 weeks after delivery.

We found that approximately 50% of the women with ethnic origin from South Asia, the Middle East and Africa had iron deficiency defined by low serum ferritin concentration and approximately 10% had anaemia in early pregnancy, compared with approximately 15% and 2% of Western European women. Fourteen weeks after delivery, the overall prevalence of iron deficiency was 39%, and 25% had anaemia. Also at the postpartum visit, the prevalence of iron deficiency and anaemia was higher among women from South Asia, the Middle East and Africa compared to Western European women, but the differences between ethnic groups were less pronounced than in early pregnancy. We also assessed the prevalence of iron deficiency measured by soluble transferrin receptor and calculated total body iron. We observed a larger proportion with iron deficiency defined by increased soluble transferrin receptor concentration and low total body iron in non-Western women compared to Western Europeans, although lower proportions compared with serum ferritin. Furthermore, we observed that iron deficiency by soluble transferrin receptor was associated with lower haemoglobin concentration compared to iron deficiency by serum ferritin and total body iron.

According to the study protocol, women with a haemoglobin concentration below 10 g/dL or a serum ferritin concentration below 20 µg/L at the first study visit were recommended iron supplements. The majority of these women had non-European ethnic origin. Among the women recommended daily iron supplements, the proportion who reported use of iron supplements at the follow-up visit in gestational week 28 increased. The probability of having steady high or improvement in iron status until the 14 weeks postpartum visit was also higher in those receiving the recommendation compared with those not. Use of iron supplements reduced the probability of postpartum iron deficiency. Other factors associated with postpartum iron deficiency and anaemia were low intake of iron-rich diet, no use of iron supplements during pregnancy, primiparity and postpartum haemorrhage.

The thesis has added new knowledge about iron status and anaemia in pregnant women, by simultaneously studying different ethnic groups. Iron deficiency and anaemia were

particularly prevalent among pregnant ethnic minority women, and the prevalence of postpartum iron deficiency and anaemia was high in the total study population. Certain ethnic groups may be more susceptible to iron deficiency and anaemia, and may require targeted interventions that encourage compliance with iron supplementation regimen.

Vitenskapelig sammendrag på norsk

Det overordnete målet med mitt ph.d. -prosjekt var å øke kunnskapen om jernstatus, jernmangel og anemi blant gravide i en multietnisk befolkning. Vi brukte data fra den populasjonsbaserte kohortstudien STORK Groruddalen med 823 friske, gravide kvinner som gikk til svangerskapsomsorg ved helsestasjoner i Groruddalen, Oslo, Norge. Data fra spørreskjemaer, målinger og blodprøver av mor ble i perioden 2008 til 2011 innsamlet ved tre besøk, i svangerskapsukene 15 og 28, samt 14 uker etter fødsel.

Vi fant at ca. 50% av kvinnene med opprinnelse fra Sør-Asia, Midtøsten og Afrika hadde jernmangel vurdert på grunnlag av lave på serum ferritin-konsentrasjoner i blodet og ca. 10% hadde anemi tidlig i svangerskapet, sammenliknet med henholdsvis ca. 15% og 2% av vest-europeiske kvinner. Fjorten uker etter fødselen var forekomsten av jernmangel totalt 39%, og 25% hadde anemi. Også på dette tidspunktet var forekomsten av jernmangel og anemi høyere blant kvinner fra Sør-Asia, Midtøsten og Afrika enn hos vest-europeiske kvinner, men forskjellene var mindre enn tidlig i svangerskapet. Vi undersøkte også forekomsten av jernmangel målt ved løselig transferrin reseptor og beregnet såkalt «totalt jernnivå i kroppen». Også for disse markørene observerte vi en større andel med jernmangel blant ikke-vestlige kvinner sammenliknet med vestlige, dog lavere andeler sammenliknet med serum ferritin. Videre observerte vi at jernmangel ved løselig transferrin reseptor var assosiert med lavere hemoglobin konsentrasjon sammenliknet med jernmangel ved serum ferritin og «totalt jernnivå i kroppen».

I henhold til studieprotokollen fikk kvinner med hemoglobin-konsentrasjon under 10 g/dL eller serum ferritin-konsentrasjon under 20 µg/L ved første studiebesøk råd om å ta jerntilskudd. Majoriteten av disse kvinnene hadde etnisk opprinnelse utenfor Europa. Blant kvinnene som fikk råd om jerntilskudd daglig, økte andelen som rapporterte at de tok jerntilskudd ved oppfølgingsbesøket i svangerskapsuke 28.

Sannsynligheten for å opprettholde et høyt, eller forbedre jernnivået fram til besøket 14 uker postpartum var også høyere hos de som fikk anbefalt jerntilskudd, sammenlignet med de som ikke fikk anbefalingen. Bruk av jerntilskudd ga lavere sannsynlighet for jernmangel postpartum. Andre faktorer assosiert med postpartum jernmangel og anemi var lavt inntak av jernrik kost, å ikke ta jerntilskudd i svangerskapet, være førstegangsfødende og ha stor blødning under fødsel.

Avhandlingen har tilført ny kunnskap om jernstatus og anemi hos gravide, ved å se på ulike etniske grupper samtidig. Jernmangel var spesielt utbredt blant gravide minoritetskvinner, og forekomsten av postpartum jernmangel og anemi var høy i hele studiepopulasjonen til tross

for råd om jerntilskudd til kvinner med lav jernstatus tidlig i svangerskapet. Noen etniske grupper viste seg å være ekstra sårbare for jernmangel og anemi, og synes å trenge ekstra oppfølging for å motivere dem til å ta jerntilskudd.

List of papers

Publications included in the thesis:

I. Næss-Andresen ML, Eggemoen AR, Berg JP, Falk RS, Jenum AK. Serum ferritin, soluble transferrin receptor, and total body iron for the detection of iron deficiency in early pregnancy: a multiethnic population-based study with low use of iron supplements. *The American Journal of Clinical Nutrition*, Volume 109, Issue 3, Pages 566–575. March 2019. DOI 10.1093/ajcn/nqy366

II. Næss-Andresen ML, Jenum AK, Berg JP, Falk RS, Sletner L. The prevalence of postpartum anaemia and iron deficiency by serum ferritin, soluble transferrin receptor and total body iron, and associations with ethnicity and clinical factors: a Norwegian population-based cohort study. *Journal of Nutritional Science*, Volume 11, Pages e46, June 2022. DOI 10.1017/jns.2022.45

III. Næss-Andresen ML, Jenum AK, Berg JP, Falk RS, Sletner L. The impact of recommending iron supplements to women with depleted iron stores in early pregnancy on use of supplements, and factors associated with changes in iron status from early pregnancy to postpartum in a multi-ethnic population-based cohort. *BMC Pregnancy Childbirth*, Volume 23, Issue 1, Pages 350, May 2023. DOI 10.1186/s12884-023-05668-5

The papers are referred to by their Roman numeral throughout the thesis.

Abbreviations

BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CRP	C-reactive protein
CV	Coefficient of variation
CI	Confidence interval
ECLIA	Electro-chemiluminescence immunoassay
FFQ	Food frequency questionnaire
GHO	Global Health Observatory
HbA1c	Glycated haemoglobin
Hb	Haemoglobin
ID	Iron deficiency
IDA	Iron deficiency anaemia
MCV	Mean corpuscular volume
NHANES	National Health and Nutrition Examination Survey
PCA	Principal component analysis
OR	Odds ratio
RBC	Red blood cell
SF	Serum ferritin
SEP	Socioeconomic position
SD	Standard deviation
sTfR	Soluble transferrin receptor
TBI	Total body iron
TFR	transferrin receptor
UK	United Kingdom
US	United States
WIC	Special Supplemental Nutrition Program for Women, Infants and Children
WHO	World Health Organization

1. Introduction

1.1 Iron, iron metabolism and haemoglobin

The iron-binding protein haemoglobin (Hb) is found in red blood cells (RBCs) and transports oxygen from the lungs to the rest of the body [3]. Hb consists of four polypeptide chains; two chains are α-polypeptides and two are β-polypeptides. Each polypeptide chain is associated with a haem group with an iron ion (Fe^{2+}) that can bind and transport oxygen molecules. The haem group is coloured red when it reacts with oxygen, whereas it in the deoxygenated state has a more blue or purplish colour. The average volume of RBC, the mean corpuscular volume (MCV), is about 90 fL and each RBC contains approximately 270 million Hb molecules [4].

Iron is essential for the production of Hb and for the transport of oxygen, but is also crucial in all human cells for multiple enzymatic processes, including DNA synthesis, mitochondrial respiration, hormone formation, and cellular metabolism [5]. The body ensures that iron is recycled and reused, resulting in a remarkably constant amount of body iron between 2-6 g [3,6]. Most of the iron in the body is found in Hb (70 %) and myoglobin (10%), the rest is distributed between enzymes, plasma iron, ferritin and haemosiderin [3,6]. Losses through faeces, sweat and urine constitute approximately 1 mg/day. If the loss of iron exceeds the uptake of iron, the iron stores become increasingly smaller, and iron deficiency (ID) can be the result. The most severe stage is iron deficiency anaemia (IDA), where the lack of iron prohibits adequate production of RBCs [3]. Iron is primarily stored in the liver, bone marrow, and spleen. The liver is the largest storage site for iron, where it is primarily stored as ferritin and to a lesser extent as haemosiderin. Ferritin is a protein that binds and stores iron within its structure. The production of ferritin is regulated by the body's iron stores. ID refers to low intracellular storage of iron, i.e. reduced iron stores. When the level of intracellular iron is low, the production of ferritin is also reduced. This reduction in ferritin levels can be used as an indicator of ID, measured as SF, the amount of ferritin that is circulating in the blood, which normally correlates with the amount of ferritin stored in tissues. Conversely, when iron stores are increased, the body produces more ferritin to store the excess iron. This increase in ferritin levels can also be used as an indicator of iron overload, a condition with too much iron in the body [3].

The Hb synthesis alone requires 20-25 mg of iron per day, making the bone marrow to the main consumer of iron [3]. The bone marrow contains only a small amount of iron stored as ferritin, and most of the iron used in the Hb syntheses is recycled iron [3]. In addition, iron can be released from ferritin, and transported to the bone marrow with the bloodstream bound to transferrin. The spleen also stores a small amount of iron, mainly as haemosiderin, and small amounts of iron can also be stored in other tissues, such as the heart, pancreas, and in

muscles as myoglobin. Iron stores can be depleted, resulting in ID and gradually progressing to IDA, a microcytic hypochromic anaemia.

Iron deficiency anaemia = microcytic, hypochromic anaemia [a]

Iron deficiency results in reduced synthesis of haem, which in turn reduces the synthesis of Hb and the production of RBCs in the bone marrow resulting in anaemia. The circulating RBCs with less Hb molecules have changed their appearance [5].

- Microcytic: In the peripheral blood smear, the RBCs are smaller than normal (MCV <80 fL [b])
- Hypochromic: Examined under a microscope, the RBCs contains less colour than normal (MCHC <32 g/dL [b])

[a] Other causes of microcytic anaemia are thalassemia, sideroblastic anaemia and more rare x-linked anaemias, and anaemia of inflammation

[b] MCV, MCH and MCHC are variables derived from haematology instruments

Abbreviations: *MCH* mean corpuscular haemoglobin, *MCHC* mean corpuscular haemoglobin concentration, *MCV* mean corpuscular volume

1.2 Iron requirements and regulation of iron metabolism

The recommended dietary iron intake in the adult population ranges from 8 mg per day in men, to 18 mg per day in reproductive-age women, and to 27 mg per day in pregnant women [7]. Several European and Nordic surveys have estimated the intake of dietary iron in females to be approximately 10-11 mg per day [8], including The Nordic Nutrition Recommendations from 2012 [9] (new addition planned to be published June 2023). In the Nordic diet, the dietary iron intake is mainly from cereal products, with a lower proportion from a variety of meat sources [9]. A systematic review found that the average iron intake in pregnant populations is below the nutrient recommendations in all developed countries [10].

Dietary iron consists of haem iron and non-haem iron, which represent different forms of bioavailable iron. The bioavailability of iron depends on several factors, and multiple mechanisms are involved to balance the iron loss [6]. Approximately 10-15% of the dietary iron intake is absorbed from the intestinal tract [6]. The bioavailability of iron is higher in food items containing haem (present in meat, seafood, and poultry) compared to non-haem iron (in plant sources like nuts, legumes, seeds, and some fortified grains), and ascorbic acid enhances the absorption of both haem iron and non-haem iron. Haem-iron is absorbed more efficiently in individuals with low iron status or ID [3,11,12], but is inhibited by calcium [11-13]. The absorption of non-haem iron is enhanced by haem-iron in addition to ascorbic acid, however inhibited by a range of dietary factors; phenolic compounds (e.g., tea, coffee, cocoa, and red wine), phytates (present in cereals, seeds, nuts, vegetables, and fruit) and calcium [11-13].

Lastly, the regulation of iron absorption is complex with different mechanisms and pathways involved. The absorption is higher in individuals with ID [7]. Several genes involved in the

regulation of iron metabolism have been identified [14,15]. Nevertheless, whether ethnic differences in iron metabolism are caused by differences in genetic variants and variations in the genetic expression is not known[14]. However, there are genetic diseases that cause anaemia, such as thalassemia and sickle cell anaemia that are more prevalent in Asia and Africa than in Europe [15].

1.3 Risk factors for iron deficiency and iron deficiency anaemia

ID is most common in preschool children and reproductive-age women. Risk factors for ID are inadequate iron intake, blood loss, and increased iron requirements [5]. Physiological, socioeconomic, pathological, drug-related, or genetic factors can also result in ID [3,5]. The higher prevalence of ID in low-income countries is attributed to low dietary iron content, a higher prevalence of parasitic infections, more frequent childbirths and poorer access to help for family planning, poorer access to antenatal and perinatal care with greater risk of excessive blood loss during birth and in some cases undiagnosed diseases reducing iron absorption [16].

Inadequate iron intake

A diet low in iron content, especially low haem iron content (vegetarian/vegan diet), or low dietary diversity (often caused by poverty) is associated with ID and IDA. A diet with reduced dietary iron absorption may be related to inhibitors (e.g. tea or calcium) or various conditions in the gastrointestinal tract (e.g. atrophic gastritis, use of antacids or proton pump inhibitors, helicobacter pylori infection, coeliac disease or inflammatory bowel disease) [7].

Iron loss

Reproductive-age women are at higher risk of ID compared with the rest of the adult population due to menstrual bleeding, childbirths and lactation. Chronic blood loss from hookworm and schistosomiasis is a risk factor in low-income countries, while blood losses from blood donation and intestinal lesions (e.g. ulceration, cancer, use of aspirin and other non-steroidal anti-inflammatory drugs) are risk factors in high-income countries [17,18].

Increased iron requirements

Infants, preschool children and adolescents have higher iron requirements than adults due to their rapid growth, and women have increased requirements from the onset of menarche [19]. During pregnancy, women's iron requirements increase with each trimester, from 1.2 mg per day in first trimester, 4.7 mg per day in second trimester, to 5.6 mg per day in third trimester to meet the maternal and foetal production of RBCs, the development and function of the placenta, and foetal growth [20,21]. An increased requirement of iron in postpartum women, is caused by the blood loss during childbirth and for the production of breast milk [20].

1.4 Definitions of anaemia and iron deficiency

ID is the most common micronutrient deficiency across the globe and the leading cause of anaemia [22]. Anaemia is a condition in which the blood Hb concentration is lower than normal. The threshold used to define anaemia during pregnancy are different from non-pregnant women. During pregnancy, the production of RBCs increases to support the transport of oxygen and nutrients to the developing foetus, and to support the increased metabolic demands of maternal organs. The increase in plasma volume during pregnancy is also important, as it increases cardiac output and the blood flow to the uterus and placenta, maintains maternal blood pressure, and prevents dehydration. During pregnancy, the body produces more plasma than RBCs, causing a physiological hemodilution. To account for these changes, different thresholds to diagnose anaemia during pregnancy [23-25] and postpartum [25-28] as suggested shown in Table 1.

Table 1: Definition of anaemia in pregnancy

First trimester:	Haemoglobin <11.0 g/dL [a]
Second trimester:	Haemoglobin <10.5 g/dL [b]
Third trimester:	Haemoglobin <11.0 g/dL [a]
Post-partum:	Haemoglobin <10.0 g/dL [c, d]
More than 8 weeks after delivery	Haemoglobin <12.0 g/dL [d]

[a] Suggested thresholds for anaemia in pregnant women by WHO [23]
[b] Suggested trimester-specific thresholds for anaemia in pregnant women by WHO [24,25]
[c] Suggested threshold for postpartum anaemia when measured immediately after and in the first five weeks after delivery [24,26].
[d] Suggested threshold more than 8 weeks postpartum [27,29]

Most reference ranges and thresholds are derived from non-pregnant adults. 'Prussian Blue' staining of bone marrow smears is the "gold standard" for the diagnosis of IDA, however, this method is both invasive and inappropriate for clinical use in unselected patients, for screening and in epidemiologic studies [30]. According to present standards, the most frequently used indicator of an individual's iron status in clinical practice is SF concentration [31]. SF levels are influenced by inflammation and infection [32], and by the plasma volume expansion during pregnancy [33]. Therefore, it is important to interpret SF in the context of other clinical and laboratory findings to make a diagnosis of ID.

Transferrin receptor (TfR) is a protein found on the surface of cells, which binds and internalizes transferrin-bound iron into cells. If the cells are exposed to ID, they respond with an increased number of TfR on the cell surface [3]. Soluble transferrin receptor (sTfR) is derived from the cleavage of membrane-bound TfR on the surface of cells and these levels correlate [3]. sTfR can therefore be used as an indirect measure of the number of TfR on cell surfaces, and consequently the cell's need for iron. When iron stores are low, the body produces more TfR to increase iron uptake resulting in elevated sTfR concentrations. sTfR is

commonly used to evaluate IDA and distinguish it from anaemia from other causes such as haemoglobinopathies, inflammation, and infection [3]. sTfR testing is not a routine part of antenatal care, but can be used among pregnant women in certain situations [21].

Table 2: Comparison of biochemical indicators, their function, limitations and threshold used for the diagnosis of iron deficiency

Biomarker	Function of iron indicator	Limitations	Thresholds used to define ID
Prussian Blue staining of bone marrow smears	The most accurate measure of ID, diminished / absent stainable iron in bone marrow aspirate	Invasive, expensive, may require sedation, traumatic	No visible iron under high power magnification [30]
Hb	Measure of anaemia and proxy of functional iron	Adjustment of thresholds needed for age, sex, pregnancy, altitude, smoking and some ethnic groups	Differ by population: <12 g/dL in women <13 g/dL in men [34]
MCV	MCV decreases in ID (microcytic anaemia)	Decreased in thalassaemia and inflammation	No set cut-offs
MCH	MCH decreases in ID (hypochromic anaemia)	Slow to respond to ID	No set cut-offs
Serum or plasma iron	Circulating transferrin-bound iron; indicator of iron supply to the bone marrow/other tissues	Varies from day-to-day and after meals. Low in chronic disease, infection and inflammation	<50-60 µg/dL [35,36]
SF	Size of iron stores	Increased in inflammation and infection, liver disease and malignancy. Decreased in expanded plasma volume, e.g. in pregnancy	<12-15 µg/L [35,36]
sTfR	Iron-deficient erythropoiesis	Limited availability, expensive, assay differences, minor increase in chronic disease	Assay-dependent [37]
TBI	Reflection of range of iron status	Requires two measurements	<0 mg/kg body weight [38]
Zinc protoporphyrin	Iron supply to bone marrow; indicator of lack of iron to developing RBCs	High day-to-day variation Increased in iron deficiency	>40-80 µg EP/dL of RBSs [36]
Total iron binding capacity	Total capacity of circulating transferrin bound to iron, increase in ID	Large overlap between normal values and values in ID. Low in inflammatory disorders	No set cut-offs
Transferrin saturation	Iron-deficient erythropoiesis	Requires two measurements	<15% [35]
Ratio sTfR to SF	Reflection of range of status	Requires 2 measurements	No set cut-offs
Hepcidin	Regulator of iron absorption from gut	Experimental and under development	No set cut-offs

[a] *MCH;* Mean cell haemoglobin

Calculated total body iron (TBI) is another indirect method for estimating the body's total iron stores. This method uses the ratio of sTfR to SF to estimate the amount of iron that is

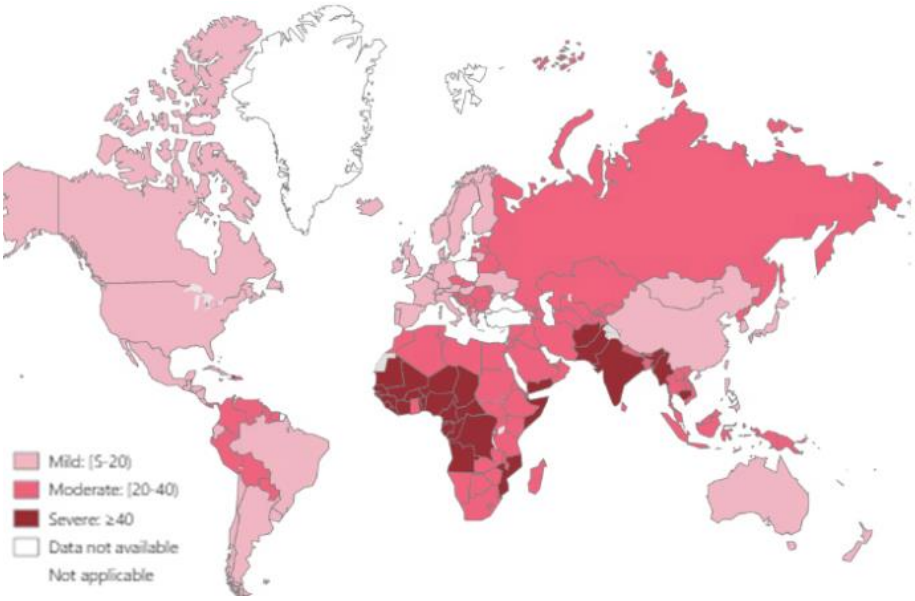
available for erythropoiesis and storage. Calculated TBI based on sTfR and SF is not widely used in clinical practice, and its performance has not been extensively evaluated in various populations.

In addition to Hb, SF, sTfR and TBI, several other biomarkers reflecting iron status are available, some listed in Table 2. The sensitivity and specificity of all the biomarkers to detect ID depend on several factors, including the population being tested for ID and the threshold used to define ID. Several studies have conducted receiver operating characteristic (ROC) analysis to determine the diagnostic utility to detect ID [39-41]. Some of the biomarkers in Table 2 are rarely used in clinical setting, however with increasing interest and research, use of other biomarkers may change in the future.

1.5 Prevalence of anaemia and iron deficiency

WHO defines anaemia as a global public health issue, with ID as the leading cause (40-50% of the cases) of anaemia [42]. Groups particularly at risk are preschool children, and reproductive-age and pregnant women.

Figure 1. Prevalence of anaemia in women of reproductive age (aged 15-49) (%) [22]



WHO, being the UN's global health agency with 194 member countries, has for decades focused on the high prevalence of anaemia and ID, studied the development and initiated several measures to reduce the prevalence [21,35]. All member states appoint representatives to the World Health Assembly, which meets annually and sets WHO guidelines. In 2012, the World Health Assembly endorsed six global nutrition targets, one being a 50% reduction of anaemia in reproductive-age women by 2025 [43]. The Global Health Observatory (GHO) is a public health observatory established by WHO to share data on global health from the 194

countries that covers specific diseases and health measures. GHO publishes yearly prevalence data on anaemia in different populations and by regions or country and can be followed at https://data.worldbank.org/.

A moderate decrease in global prevalence rates of anaemia has been observed in preschool children and pregnant women after 2000, however, not in reproductive-age women [22,44]. The latest reports estimate that 30% of reproductive-age women, 37% of pregnant women, and 60% of preschool children worldwide have anaemia [22]. According to the latest estimates by the GHO, the prevalence of anaemia in reproductive-age women in Europeans countries was 18.8% (range 14.5-25 %) [22] and ID remains a common nutrition deficiency in high-oncome countries, especially among high-risk groups [34,45]. The highest prevalence rates are in South Asia and Central- and West Africa, as illustrated in Figure 1.

1.6 Associations between anaemia and iron deficiency and health outcomes

ID and anaemia have been linked to adverse outcomes in the mother, fetus and infant, however with various quality of evidence and small numbers of studies.

Anaemia

Symptoms of anaemia can be dyspnoea, fatigue, palpitations, and cognitive impairment, affecting the wellbeing in individuals [46]. Maternal anaemia is reported to be associated with poor maternal outcomes, including increased morbidity related to haemorrhage [47,48], and antenatal and postnatal sepsis [48,49]. A decreased Hb concentration can result in impaired uterus contraction and a prolonged antenatal bleeding [48]. The mechanism by which anaemia increases the risk of puerperal sepsis is not fully understood. However, it has been suggested that anaemia can weaken the immune system, making women more susceptible to infections, and can also lead to poor wound healing and impair the body's ability to fight infections [47]. A WHO multi-country survey with more than 300 000 pregnancies, found the odds of maternal death to be doubled in those with severe anaemia (Hb <7 g/dL) compared with those without severe anaemia [50].

Maternal anaemia has also been linked to an increased risk of several adverse perinatal and neonatal outcomes, including perinatal and neonatal mortality, low birth weight, and preterm birth [48], although the evidence grade have been described as low to moderate [51]. These outcomes are of great concern as they can have long-term effects on the health and development of the offspring. Several studies have reported a U-shaped association between maternal Hb concentrations and birth weight, where also increased Hb concentration has been associated with low birth weight, a condition that usually indicate poor plasma volume expansion [52,53].

Iron deficiency

There is less evidence for associations between maternal non-anaemic ID and adverse birth outcomes, however, trials on women with ID showed that iron supplementation was associated with increased birth weight compared to those not exposed to supplements [54]. Non-anaemic ID is also linked to clinical impairments, however with low grade of evidence [51], such as fatigue [55], impaired physical performance [56], decreased work productivity [57], and sub-optimal brain development in the offspring [58], delayed neurocognitive development and even psychiatric illness in the offspring [59]. Postpartum ID has also been linked to increased risk of postpartum depression and poorer mother-child interaction [27]. A systematic review found iron supplementation in individuals with ID to significantly improve fatigue, however inconsistent findings were reported on the effects of iron supplementation on exercise tolerance, educational attainment, infant development [60].

1.7 Clinical guidelines

WHO recommends universal 30-60 mg daily oral iron supplementation to pregnant women to prevent maternal anaemia, puerperal sepsis, low birth weight, and preterm birth [54]. In populations with mild prevalence of anaemia (see Figure 1), or if daily iron supplementation causes strong side effects, WHO suggests intermittent oral iron supplementation with 120 mg per week [61,62].

Table 3. Recommendations for supplements to prevent and treat iron deficiency and anaemia

Organization	The recommended daily iron intake		Average iron intake	Recommendations		
	Reproductive-age women	Pregnant women		To all pregnant women	To pregnant women with anaemia	To pregnant women with ID
BSH [48]	~ 15-18 mg	~ 27 mg	10 mg/d [48]		UK: 40-80 mg [48]	UK: 40-80 mg [a]
D-A-CH [63]			11.8 mg/d [64]		Germany: 40 – 100 mg/day [65]	
NIH [66]			14.7 mg/day [67]	US: 27 mg/day [68,69]	US: 60-120 mg/day [68,70]	
NNR [9]			10.2 mg/d [9]	Denmark: 40-50 mg/day	Denmark / Norway [b]	Norway: 40-60 mg [71]
WHO [54]			-	30-60 mg/day*	120 mg/day [54]	

BSH; British Society for Haematology. D-A-CH abbreviation for the German-speaking countries of Germany (D), Austria (A), and Switzerland (CH). NIH; National Institutes of Health. NNR; Nordic Nutrition Recommendations.

[a] only at-risk women screened [48]

[b] consider high dose oral treatment, referral to obstetrician for intravenous administration of iron and fetal growth control [72,73]

The nutritional programs in the Nordic countries, the German-speaking countries, the United Kingdom (UK) and the US recommend iron supplementation, with a somewhat varied approach (Table 3).

IDA is generally considered as a common and important health concern, particularly during pregnancy, and appropriate screening, prevention, and treatment are fundamental for maternal health and her offspring. However, the antenatal care guidelines for preventing and treating IDA differ between countries based on factors such as local prevalence of IDA and dietary habits. Some countries recommend routine iron supplementation during pregnancy for all women [73], while others recommend supplementation only for women at high risk for ID, or when anaemia is diagnosed [48], or recommend dose-specific supplements dependent on iron status early in pregnancy [71]. Similarly, recommendations for the treatment of IDA vary between countries (Table 3).

Antenatal care and clinical guidelines in Norway

In Norway, a list-based system entitles all inhabitants with a regular GP. The GPs act as the patients' main point of contact with the health service and are responsible for the inhabitants' primary medical needs. Most pregnant women use either their GP, a midwife at the Child Health Clinics, or both, for antenatal care. The antenatal care at the GP or the Child Health Clinics are free of charge and easily available. Some women cover expenses for follow-up at a private obstetrician. Some women with specific diseases and high-risk pregnancies are recommended follow-up at hospitals [74]. During our data collection, the national guideline on antenatal care (Svangerskapsomsorgen from 2005) recommended to screen women by Hb concentration at the first antenatal visit and in gestational week 28, and further to recommend iron supplementation if anaemia was detected [1].

The current national guideline on antenatal care published in 2018 [71], recommends that all pregnant women are screened with Hb and SF concentration at the first antenatal visit, preferably in the first trimester. Women with SF concentration below 12 µg/L are subsequently recommended 60 mg of daily iron supplement, and women with SF below 30 µg/L are recommended daily supplements of 60 mg of iron from 18-20 weeks of gestation. Women with SF concentration 30-70 µg/L are recommended daily supplement of 40 mg iron from 18-20 weeks of gestation until delivery, while women with SF concentration above 70 µg/L are not recommended supplements. If the blood samples are drawn after 15 weeks of gestation; or if iron status is not measured, women are recommended daily supplements of 40 mg iron from 18-20 weeks of gestation. IDA is by Norwegian Gynaecological Associations recommended daily supplement of 60-200 mg iron [72].

In Norway, Hb is not routinely measured after delivery, but is recommended in women with anaemia in pregnancy, symptoms of anaemia, and in women with more than 500 mL blood loss after the birth [75]. According to the Norwegian Gynaecological Associations guidelines, women with diagnosed postpartum anaemia by the obstetrician, should be recommended

iron supplementation, and be advised to book an appointment for follow-up visit 6 weeks postpartum with her GP to control Hb concentration [75].

1.8 Ethnicity and migration from low- and middle income countries

The word “ethnicity” derives from the Greek word *“ethnos”,* meaning *“nation, people or tribe”.* The term ethnicity covers cultural traditions important for identity and inherited biological characteristics, and are related to country of birth, or country of birth of parents or ancestors [76]. However, also within a country there may be different native ethnic groups. The term ethnicity is thus complex. In health research, country of birth, or country of birth of parents, is often used as a proxy for ethnicity [76]. The term ethnic minority population is often used for inhabitants with different ethnicity than the majority population, and they may have poorer health for a range of health conditions [76].

Cultural traditions related to dietary habits, religion, language and lifestyle may differ between ethnicities [76]. ID and anaemia are more common in immigrants from low-income and middle-income countries compared to the majority population [77,78], and in people on low incomes [79].

According to the latest update from the Statistics Norway, immigrants accounted for 16% of the total population in Norway and those who are Norwegian-born to immigrant parents accounted for 3.9% [80]. Polish, Lithuanian, Swedish and Syrian immigrants comprise the largest groups, and most who are Norwegian-born of immigrant parents have background from Pakistan, Somalia and Poland.

Figure 2: The city districts of Oslo [82]

The proportion of immigrants in Norway varies widely across the country, with Oslo having the largest proportion of immigrants (33%).

The highest proportion of immigrant inhabitants (>50%) live in the city districts Søndre Nordstrand, Stovner, Alna and Grorud [81].

2. Aims of the thesis

The overall goal of this thesis was to increase the knowledge on iron status and gestational and postpartum anaemia and ID in different ethnic groups, and factors associated with these conditions.

We used a multi-ethnic population-based cohort from Oslo, Norway to address the specific objectives:

I. Assess the prevalence of anaemia and ID in early pregnancy by the three iron indicators: SF, sTfR, and TBI, and associations with ethnicity in a population with low use of iron supplements.

II. Assess the prevalence of anaemia and ID by three iron indicators (SF, sTfR, TBI) 14 weeks after delivery, including the relations between the ID indicators and Hb, and their associations with ethnicity, and other maternal and clinical factors.

III. Evaluate the impact of recommending iron supplements to women with depleted iron stores in early pregnancy on supplement use later in pregnancy, and maternal and clinical factors associated with changes in iron status from early pregnancy to postpartum.

3. Methods

3.1 Study design

In the three papers included in this thesis, data from the STORK G cohort study was used. Table 4 present an overview of study designs, variables and study samples in each paper.

In Paper I, a cross-sectional design was used, while in Papers II and III a prospective design was applied. Additionally, a pre-post design (with no control group) was used in Paper III to assess the impact of a simple intervention recommending iron supplementation to women with low concentrations of Hb and/or SF.

Table 4. Overview of characteristics for each paper (STORK G)

Characteristics	Paper I	Paper II	Paper III
Design	Population-based Cross-sectional	Population-based Prospective longitudinal cohort	
Primary outcomes	SF, sTfR, TBI, and Hb concentration. Prevalence of ID by three iron indicators and of anaemia in early pregnancy.	SF, sTfR, TBI, and Hb concentration postpartum. Prevalence of ID by three iron indicators and of anaemia postpartum.	Changes in iron stores measured by change in the concentrations of SF, sTfR, TBI, and Hb from GW 15 to 14 weeks postpartum
Aims	Ethnic differences in outcomes	Ethnic differences in outcomes. Relations between the ID indicators and Hb. Factors associated with postpartum ID and anaemia.	Impact of recommending iron supplementation on later use of supplements. Factors associated with changes in iron stores.
Primary explanatory variable	Ethnicity	Ethnicity, parity, gestational ID/anaemia, dietary pattern, use of supplements and haemorrhage	The simple intervention (recommendation) and use of iron supplements
Other covariates	See Table 5		
Simple intervention (recommendation)		Recommended iron supplements 30-50 mg per day if SF <20 µg/L or Hb <10 g/dL	
Setting	Primary antenatal Child Health Clinics, Groruddalen, three city districts in Oslo		
Participants	Pregnant women followed in primary antenatal care		
Years of data collection	2008-2010		
Ethnic groups	Western Europe, South Asia, Middle East, Sub-Sahara Africa, East Asia and Eastern Europe		
Age range	19-45 years		
Sample size	792	573	
Questionnaires	Yes		
Blood samples and analyses	SF and Hb analysed consecutively + sTfR from biobank in 2016. Calculated TBI from SF and sTfR concentration		
Biobank	Yes		
Ethics Committee Registration number	REK 2007/894 REK 2015/1035C		

Hb, haemoglobin; SF; serum ferritin, ID; iron deficiency, GW; gestational week

3.2 Recruitment of participants to STORK G

STORK G is a population-based multi-ethnic cohort of pregnant women attending antenatal care at three Child Health Clinics located in the three city districts Stovner, Grorud, and Bjerke in Groruddalen, Oslo, Norway and their offspring [83]. Information about the study was widely distributed in the study districts, and GPs were asked to refer women to the Child Health Clinics early in pregnancy. All information material was translated into Arabic, English, Sorani, Somali, Tamil, Turkish, Urdu and Vietnamese and quality checked by bilingual health professionals. The women attending the Child Health Clinics were met by the STORK G study staff, given oral and written information about the study and invited to participate if found eligible. From May 6th 2008 to May 15th 2010, 1918 pregnant women attended the Child Health Clinics for antenatal care (constituting the source population for this study). Of these, 1114 (58%) were invited to the study and 823 women were included. The overall participation rate was 74% [83]. In the total sample, 59% were of ethnic minority background (see Table 6). A flow chart of invited and participating women has previously been published [83].

Inclusion criteria

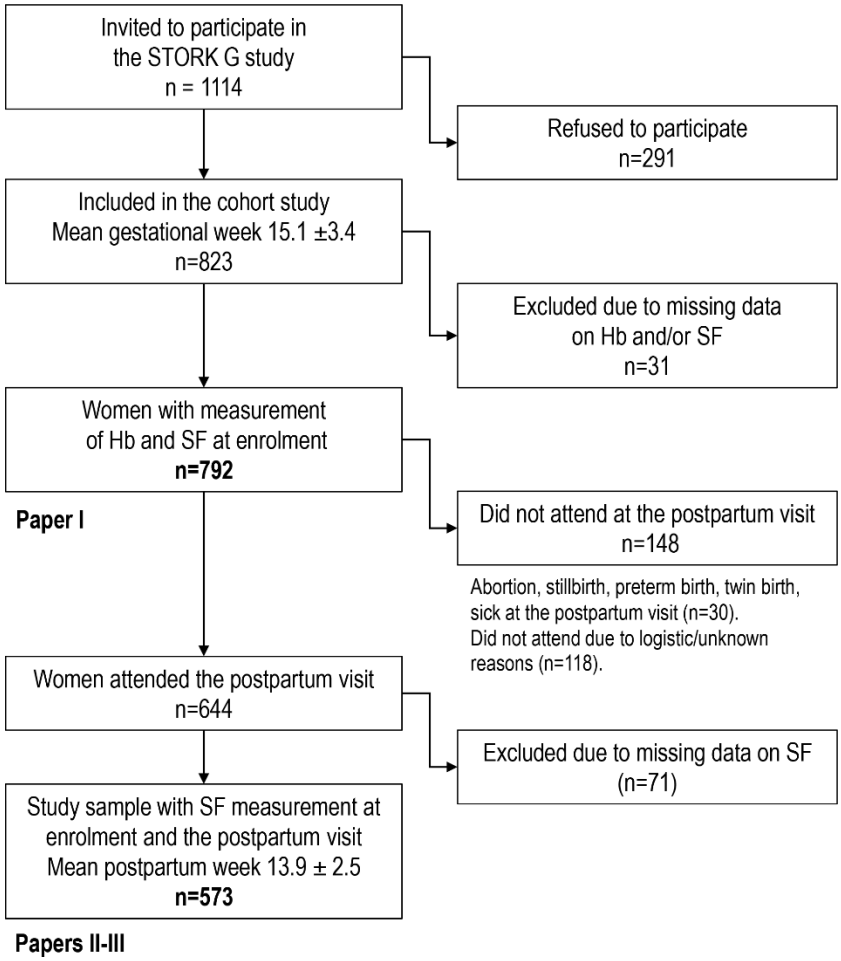
Pregnant women attending the Child Health Clinics for antenatal care were eligible if they lived in the study district and planned to give birth at either Akershus University Hospital or Oslo University Hospital Ullevål. Further, women who were less than 20 weeks of gestation and not in need of intensive hospital follow-up during pregnancy were eligible. Participating women had to be able to communicate orally in Norwegian or any of the specified languages, and to provide written consent to participate.

The study staff registered age, parity, and ethnic origin routinely in all women attending the Child Health Clinics for antenatal care, and monitored the participation to identify challenges in the recruitment process.

Participants

In Paper I, the final sample of study participants comprised 792 women with measurements of Hb and SF concentration at enrolment. In Papers II and III, the sample comprised 573 women with Hb and SF measurements both at enrolment and at the postpartum visit. An overview of the study samples in the papers is presented in Figure 3.

Figure 3. Flow chart. Overview of study samples in Paper I-III



3.3 Data collection

Data used in the three papers were collected from questionnaires, clinical measurements, and blood samples during three study visits, and from the hospital records. The variables used are listed in Table 5.

The first study visit was at enrolment in mean gestational week 15. In Paper I, this is referred to as ‘early pregnancy’. The second study visit was scheduled to the beginning of the third trimester, gestational week 28. Information from hospital records were used to obtain data on delivery mode, estimated blood loss, and birth complications. The third visit was scheduled to be approximately three months after delivery, aiming to achieve a high attendance rate, as at this point, most mothers have established routines on feeding practices and the baby’s sleeping periods and have recovered from operative delivery or other birth injuries. This study visit is referred to as the "postpartum visit" in this thesis and took place at mean 14 weeks after delivery.

Table 5. Main maternal data collection relevant to this thesis (STORK G)

	Visit 1	Visit 2	Visit 3
Questionnaires			
Date of birth (maternal age)	X	X	X
Date of first date in last menstruation (gestational week)	X		
Ethnicity (country of birth; parents' country of birth)	X		
Childhood socioeconomic factors	X		
Adult socioeconomic factors	X		
Obstetric history	X		
Medical history and use of medication	X		
Dietary pattern		X	
Use of supplements	X	X	X
Other demographic factors, family, smoking	X	X	X
Data from study-hospitals birth records			
Delivery date (postpartum week), delivery mode, haemorrhage and birth complications [b]			X
Measurements			
Maternal pre-pregnancy weight, height, pre-pregnancy BMI	X		
Blood samples			
Blood samples (Hb and SF)	X	X	X
Biobanked samples (CRP, sTfR)	X		X

[a] Calculated from first day of last menstruation or routine ultrasound in gestational week 17-19
[b] From hospital medical records

Fasting blood samples were drawn and a number of questionnaire data were collected at all study visits [83]. Validated questions from other Norwegian or international surveys were used if available. The questionnaires were pilot tested for clarity, and the study logistics for feasibility. Bilingual health professionals quality-checked the translations of the questionnaires. Study personnel were certified after extensive training, and study personnel, assisted by professional interpreters when needed, collected information through interviews.

3.4 Outcome variables, explanatory variables and covariates

The explanatory variables of primary interest are referred to as explanatory variables. Variables that can affect the relationship between the explanatory variables of primary interest and the outcome variable are called covariates.

Paper I

Values for the three iron indicators, SF, sTfR, TBI, and Hb, in addition to ID defined by the iron indicators and anaemia in early pregnancy, were outcome measures.

Maternal ethnic origin, defined as Western European (reference), South Asia, Middle East, Sub-Saharan Africa, East Asia, and Eastern Europe was the primary explanatory variable.

Gestational week, age, parity, pre-pregnant BMI, education, smoking, dietary pattern, use of supplements, and chronic illness and medication associated with anaemia were covariates in the regression analyses.

Paper II
SF, sTfR, TBI, and Hb at the postpartum visit, in addition to postpartum ID defined by three iron indicators and postpartum anaemia, were outcome measures.

The variables of primary interest were maternal ethnic origin and the predefined important clinical factors parity, gestational ID/anaemia, dietary pattern, use of supplements and haemorrhage.

The following variables were covariates: postpartum week, age, pre-pregnant BMI, adult socioeconomic position, early life socioeconomic position and level of integration, chronic illness and medication associated with anaemia, delivery mode, and birth complications were covariates.

Paper III
Changes in SF, sTfR, TBI, and Hb concentrations and in iron status from enrolment to the postpartum visit were outcome measures. The changes were calculated by subtracting the concentration at the postpartum visit from that at enrolment for each indicator.

Participants with depleted iron stores (SF <20 µg/L) at enrolment were recommended iron supplementation. This recommendation and the use of supplements were the primary explanatory variables. The impact of the recommendation was measured on the use of supplements later in pregnancy, and the effect of use on the changes in iron status.

Covariates were postpartum week, age, parity, pre-pregnant BMI, adult socioeconomic position, early life socioeconomic position, dietary pattern, use of supplements in 28 weeks of gestation, chronic illness and medication associated with anaemia, gestational ID or anaemia, delivery mode, birth complications and haemorrhage.

Outcome variables

Haemoglobin and anaemia
Hb was analysed consecutively at the Department of Multidisciplinary Laboratory Medicine and Medical Biochemistry in Akershus University Hospital (Oslo, Norway), using a sodium lauryl sulphate method (XE 5000, Sysmex) with an inter-assay CV of 0.7%. The laboratory is accredited by Norwegian Accreditation according to ISO standard 15189. Hb was normally distributed.

The following trimester-specific and postpartum thresholds to define anaemia (see section 1.4) was used in the papers in this thesis:

- Hb concentration <11 g/dL in the first trimester
- Hb concentration <10.5 g/dL in the second trimester
- Hb concentration <12.0 g/dL more than 8 weeks postpartum

Serum ferritin and iron deficiency

SF concentration was analysed consecutively at the Department of Multidisciplinary Laboratory Medicine and Medical Biochemistry in Akershus University Hospital (Oslo, Norway), using an electro-chemiluminescence immunoassay method (Unicel DxI 800, Beckman Coulter) with an interassay CV of <7%. The distribution of SF was skewed to the left. ID by SF was defined as SF concentration <15 µg/L.

Soluble transferrin receptor and iron deficiency

Biobanked serum samples was used to analyse sTfR. The samples were collected through the study period, directly frozen and stored at −80°C. In 2016, the samples were thawed and analysed at the Department of Medical Biochemistry at Oslo University Hospital (Oslo, Norway) using an ELISA method by Roche assay (Modular P800, Roche) with an interassay CV of <5%. sTfR was normally distributed. ID was defined according to the manufacturers guideline [84] being sTfR >4.4 mg/L, and in line with another epidemiological publication on ID by sTfR using this assay [85].

Total body iron and iron deficiency

TBI is a measure of the estimated amount of iron in the body based on the levels of SF and sTfR concentration. The formula for calculating TBI:

$$-[\log 10\ (\mathrm{sTfR} \times 1000 \div \mathrm{SF}) - 2.8229] \div 0.1207$$ [38,85].

This equation's sTfR is determined by Flowers assay. Therefore, sTfR was converted from Roche assay levels to Flowers assay levels by the following conversion equation:

$$\text{Flowers sTfR} = 1.5 \times \text{Roche sTfR} + 0.35\ \text{mg/L}$$

The equation is derived from a previous comparison of the two assays [85,86]. TBI was normally distributed, and ID was defined as TBI <0 mg/kg [38].

Primary explanatory variables

Ethnicity

The questionnaires covered several aspects of ethnicity (see appendix, Case Record Form 1), which was the explanatory variable of interest in Papers I and II. Ethnic origin was defined by the participant's country of birth, or mother's country of birth, if she was born outside Europe or North America. Ethnic origin was categorised into the following six regions: Western Europe, South Asia, the Middle East, Sub-Sahara Sahara Africa, East Asia (from South-East Asia and East Asia) and Eastern Europe. Table 6 presents the study population and the countries of origin in the total sample and in Papers I-III.

Table 6. Ethnic origin of the women included The STORK G cohort, and in the study samples in Paper I-III

Ethnic origin of participants included in STORK G (n=823)							
Ethnic groups	Western Europe	South Asia	Middle East	Sub-Saharan Africa	East Asia [a]	Eastern Europe	South America
Number of participants (%)	336 (41%)	200 (24%)	126 (15%)	62 (8%)	44 (5%)	43 (5%)	12 (2%)
Country of origin	Norway (93%) Sweden/ Denmark (4%) Other (3%)	Pakistan (63%) Sri Lanka (31%) India/ Bangladesh (6%)	Iraq (30%) Morocco 22%) Turkey (22%) Afghanistan (10%) Iran (5%) Other (11%)	Somalia (65%) Nigeria (8%) Ethiopia (7%) Gambia (5%) Other (15%)	Vietnam (41%) Philippines (30%) Thailand (11%) China (7%) Other (11%)	Poland (16%) Kosovo (14%) Russia (14%) Other (56%)	Other (100%)
Ethnic origin of participants in Paper I-III [b]							
Paper I (n=792)	n=326 (41%)	n=198 (25%)	n=123 (16%)	n=58 (7%)	n=44 (6%)	n=43 (5%)	Excluded [c]
Papers II & III (n=573)	n=217 (38%)	n=157 (27%)	n=94 (16%)	n=38 (7%)	n=33 (6%)	n=34 (6%)	

[a] East-Asia and South-East Asia are merged into one group
[b] Participants with measurement of SF and Hb concentration at enrolment in early pregnancy (Paper I) and at both study visits (Paper II – III)
[c] Excluded due to low number of participants

Parity

Parity was defined as the number of previous pregnancies lasting more than 22 weeks and thereafter dichotomised. Women with no previous births were called nulliparous in Paper I and primiparous in Papers II-III, whereas women with one or more previous births were called multiparous.

Dietary patterns

A food frequency questionnaire was developed for the STORK G by experienced nutritionists to reflect the variation in the diet of ethnic minority groups [87]. Frequencies of intake for 67 food and beverage items were given at gestational week 28 (see appendix, STORK G diet). Four clusters were extracted using the Ward's method, and the dietary pattern was dichotomized into "healthy and unhealthy dietary pattern". The "healthy dietary pattern" contained more frequent intake of fruit, vegetables, wholegrain bread with pate and meat spread, and meat, i.e. food items with relatively high iron content, compared with the other patterns.

The simple intervention with iron supplements based on iron status at enrolment

In accordance with our protocol for ethical reasons [88], letters to women with low Hb (<10.0 g/dL) during pregnancy and/or SF concentration (<20 µg/L) at enrolment were sent, with recommendations to use iron supplementation 30-50 mg/day, and consult the GP for follow-up (see appendix, Letters with recommendations). In cases with severe anaemia, the women's GP were contacted by telephone to ensure that she would be taken care of.

Iron supplements

At all study visits, participants were asked about their intake of iron supplements during the past two weeks. In addition, participants were asked about the number of tablets per day and per week, and type of the compounds used (see appendix, Case Record Form 2). The exact iron intake could be calculated in about 40% of cases in gestational week 28, therefore use of iron supplementation was dichotomized into 'no intake' and 'intake of iron supplementation', the latter covering daily or intermittent iron supplement use.

Postpartum haemorrhage

Postpartum haemorrhage was defined as blood loss greater than 500 mL blood within 24 hours of the birth [89], derived from the hospital birth records.

Covariates

Gestational week and postpartum week

Gestational age at enrolment was derived from the first day of the woman's last menstrual period. However, if this information was uncertain or if the estimated term differed by more than 14 days from the term estimated at the ultrasound, gestational age derived from ultrasound was chosen. The postpartum week was calculated from the date of the postpartum visit and date of birth of the child.

Maternal age
Maternal age was calculated at enrolment and cross-checked with information from the Child Health Clinic records.

Pre-pregnancy BMI
Pre-pregnancy BMI (kg/m^2) was calculated from self-reported weight before pregnancy and height measured at enrolment.

Education
Self-reported maternal education was dichotomized as <10 years or ≥10 years. Education < 10 years reflects low education, and was used as a proxy for low socioeconomic position in Paper I.

Adult maternal socioeconomic position and level of integration
The following factors were in 2012 entered into a principal component analysis (PCA): education, employment status, occupational class (ISCO-08), housing tenure, house type, number of rooms in household, number of persons in household, level of household crowding, marital status, time of residence, self-reported Norwegian skills, need of interpreter at doctor's appointment, frequency of reading Norwegian newspapers or watching Norwegian television, frequency visited by ethnic Norwegian (see appendix, Case Record Form 2) [90]. Ethnic Norwegians were given the highest value on the last five questions.

Two components were extracted from the PCA:

1. The variable "adult maternal socioeconomic position" consists of components with high correlations with the predefined individual and household markers of socioeconomic status. This score was continuous and normal distributed (mean=0, SD=1).
2. The variable "level of integration" consists of components with high correlations with the predefined markers of integration. This variable had a distribution skewed to the left [90].

   To assess the potential impact of low social integration, the variable was dichotomized into "low social integration" (40% with the lowest scores) and "high social integration" (60% with highest scores).

Early life socioeconomic position
A separate PCA with variables reflecting the participants' early life socioeconomic position was conducted. The following three childhood socio-demographic variables, from the women at age of 10 years, were used: the highest parental occupational class in household, the level of household crowding and family ownership of car or not (see appendix, Case Record Form 2). The analysis resulted in one component that was normally distributed.

Smoking
The participants were asked if they did smoke during the last three months before the actual pregnancy. Smoking was of interest as it is associated with increased Hb concentration [24,35]. Smoking was dichotomized into “regular smoking” and “non-smoking/not regular smoking” three months prior to the pregnancy. This variable was used in regression analyses in Paper I.

Medical history possibly associated with iron deficiency and anaemia
The questionnaires covered information on the participant’s medical history including factors known to be associated with anaemia. We summarized this information into a variable categorized into three groups:

- no chronic illness and/or medication
- chronic illness and/or medication associated with normochromic anaemia
- chronic illness and/or medication associated with hypochromic anaemia

Copper intrauterine device is associated with increased blood loss through longer menstrual periods. Twenty-six participants reported use of intrauterine device prior to the current pregnancy, of whom six reported hormone intrauterine advice, 10 copper intrauterine advice, and 10 types were unknown. We found few participants with medical conditions that may be associated with anaemia such as rheumatism, chronic kidney disease or the use of medication (n<15), of whom <15% had anaemia. The number with of self-reported disease (unspecified type) from GI tract disease was larger (n=48), and we found that 20% of participants in the latter group had IDA.

Delivery mode and birth complications
We have detailed data on delivery mode and birth complications extracted from hospital birth records. We categorized delivery mode into normal vaginal delivery, instrumental vaginal delivery (forceps or vacuum assisted vaginal delivery), elective caesarean section and emergency caesarean section. Both caesarean sections and instrumental vaginal deliveries are associated with more excessive bleeding [75].

Lastly, we used extracted information on the following complications from the birth records; episiotomy, third- or fourth-degree perineal tear, obstructed labour, and manual removal of placenta. We constructed a composite variable reflecting the presence of at least one of these complications due to small numbers of each type of birth complications.

3.5 Statistical analyses

All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 24 and 25, and Stata (Statistics and data) version 15.0.

Descriptive statistics

Descriptive statistics for categorical variables were expressed as frequencies with proportions, and for continuous variables as means with standard deviations if normally distributed and medians with interquartile ranges if not normally distributed. To compare prevalence rates of ID and anaemia between ethnic groups, we used the chi-squared test. To compare changes in SF from early pregnancy to 14 weeks postpartum, we used Wilcoxon signed rank test.

We have used graphs or diagrams to explore and illustrate:

- the distribution of TBI concentrations in early pregnancy by ethnicity (Paper I)
- the relation between SF-, sTfR-, and TBI-concentration and four Hb concentration intervals by ethnicity (Papers I and II)
- the degree of overlap between measures of ID, and the relation between mean Hb concentration and ID defined by the different iron indicators (Paper II)
- changes in SF concentration from enrolment to postpartum in five groups with different SF concentration at enrolment in early pregnancy, including one group being exposed to a simple intervention (recommendation) (Paper III)

Regression models

In all three papers we used regression analyses to study associations. In Paper I, ethnicity was the main explanatory variable, whereas in paper II, ethnicity, and maternal factors before and during pregnancy and birth complications were explanatory variables. Separate linear regression model analyses were performed to assess the relationship between the main explanatory variables and the concentration of sTfR, TBI and Hb at enrolment and postpartum, and logistic regression models were performed to assess the relationship between the explanatory variables and SF <15 µg/L (Papers I and II). Factors of particular clinical relevance were forced into the models, being ethnicity in Paper I, and ethnicity, gestational anaemia and ID, postpartum haemorrhage, parity, dietary pattern and iron supplementation in Paper II. Other potentially relevant factors for analyses were included into the multiple regression analyses if having *P*-value <0.2 in the univariate analyses, however, after a stepwise backward elimination process, only included in the final model if still statistically significant (*P*-value <0.05) associated with the outcome.

In Paper III, we conducted a multinomial logistic regression model to investigate factors associated with belonging to “steady low”, “improvement”, “deterioration” and “steady high”,

using the latter group as the reference. The four groups reflect SF concentration in early pregnancy (≥20 µg/L) and postpartum (<15 µg/L). A priori, we adjusted for ethnicity, gestational age, maternal age, parity, socioeconomic position, diet, use of iron supplementation in gestational week 28 and postpartum, postpartum haemorrhage and birth complications.

Supplementary and sensitivity analyses

Supplementary analyses were conducted in addition to the main analysis with the intent to provide additional insights into the understanding of the association between iron status and ethnicity, and between iron status and inflammation. Sensitivity analyses were used to assess the robustness of the findings by using different thresholds for ID by SF.

Supplementary analyses in a subgroup (Paper I)

We excluded women with trimester-specific elevated CRP concentration, and analysed prevalence rates in the total sample and by ethnic origin in the subsample with no CRP elevation. We chose trimester-specific thresholds for elevated CRP, as reported in a study that assessed reference values for CRP in pregnancy: [91]

- 12 mg/L in gestational weeks 8-16
- 14 mg/L in gestational weeks 17-23
- 20 mg/L in gestational weeks 24-27
- 37 mg/L in gestational weeks 28-31

Supplementary analyses replacing ethnicity with level of social integration and sensitivity analyses using different threshold for ID by SF (Paper II)

We analysed prevalence rates on postpartum anaemia and ID in the total sample and by ethnic origin in the subsample with CRP <5 mg/L (no CRP elevation). We performed multivariable regression analyses replacing the ethnicity variable with the dichotomous variable “low” and “high” social integration. In addition, we conducted supplementary analyses in a dichotomised sub-sample ‘South Asian’ or ‘other’ ethnic origin, after excluding Western European. We conducted sensitivity analyses to estimate prevalence rates of postpartum ID by SF >12 µg/L as an alternative to SF >15 µg/L.

In Paper II, we assessed the association between low social integration and the outcome variables as an alternative explanatory variable to ethnicity (see appendix, Supplemental Table 2 Paper II). We also conducted sensitivity analyses in ethnic minority women only (*n*=332), dichotomised into ‘South Asian’ or ‘other’ ethnic origin, and explored if the continuous variable social integration could explain the differences observed between ethnic minority groups (see appendix, Supplemental Table 3 Paper II).

Supplementary analyses on the outcomes in women exposed to our intervention or not (Paper III)

We performed stratified analysis based on whether women were exposed to our simple intervention (recommendation) or not. In each stratum, we performed two sets of regression analyses. Linear regression analyses were performed to assess the associations between use of supplementation in gestational week 28 and changes in SF, sTfR, TBI, and Hb concentrations from enrolment to the postpartum visit. Logistic regression analyses were performed, when postpartum ID (defined by SF, sTfR and TBI) and anaemia were the outcomes. Adjustments were made for gestational week, age, parity, SEP, diet, ethnicity, and use of iron supplements in pregnancy. In addition, SF concentration at enrolment was included to reduce dilution of regression to the mean. In a final model we also adjusted for use of supplements postpartum, postpartum haemorrhage, and birth complications although these were not true confounders, but potentially strongly associated with the outcomes.

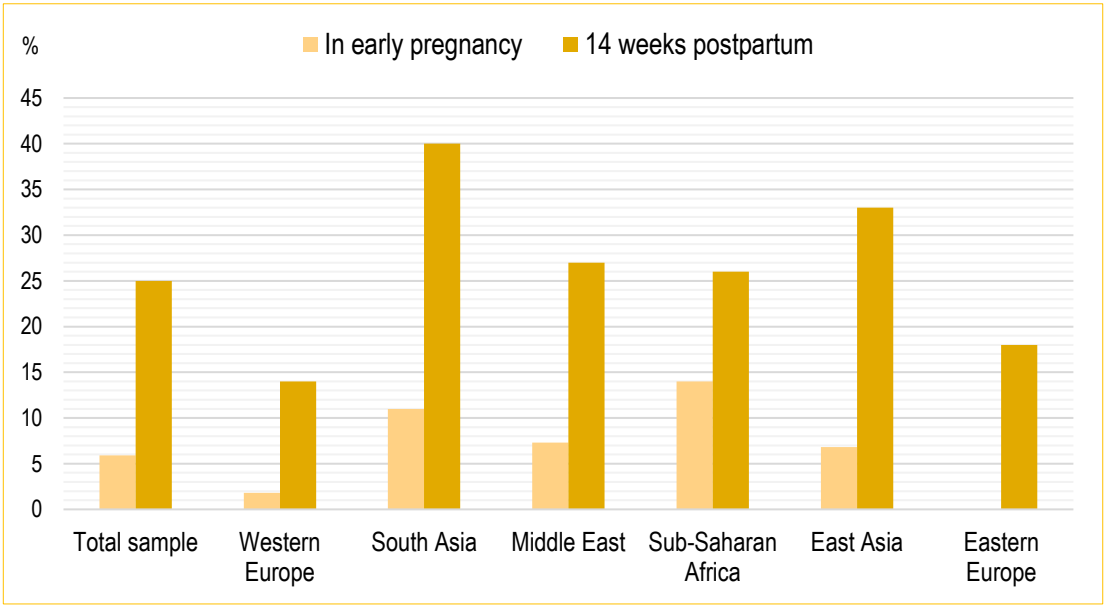
4. Summary of results

4.1 Crude prevalence of anaemia overall and by ethnic origin (Papers I and II)

In the total sample, 5.9% had anaemia in early pregnancy, and 25% had anaemia 14 weeks after delivery. The crude prevalence rates of anaemia in Western European women were 1.8% in early pregnancy and 14% at the postpartum visit, while women with non-European origin had significantly higher prevalence at both time points (in early pregnancy 6.8-14% and 14 weeks after delivery 26-40%).

The prevalence of anaemia was significantly higher in women with origin from South-Asia, the Middle East, Sub-Saharan Africa, and East Asia in early pregnancy (Paper I) and at the postpartum visit (Paper II).

Figure 4. Prevalence of anaemia (%) in STORK G in early pregnancy and 14 weeks postpartum



4.2 Adjusted associations between exposure variables and Hb concentration (Papers I and II)

After adjustment for possible covariates in our regression analyses, we found that the association between ethnic origin and Hb concentration in early pregnancy persisted for South-Asians, Middle Easterners, and Sub-Saharan Africans (Paper I).

After adjustment for possible covariates, we found a significant association between postpartum Hb concentration and the following exposure variables of interest; South Asian ethnic origin, gestational anaemia, use of supplements in gestational week 28 and postpartum haemorrhage. We found no significant associations between postpartum Hb concentrations and the other ethnic groups, nor parity or dietary pattern (Paper II). Other

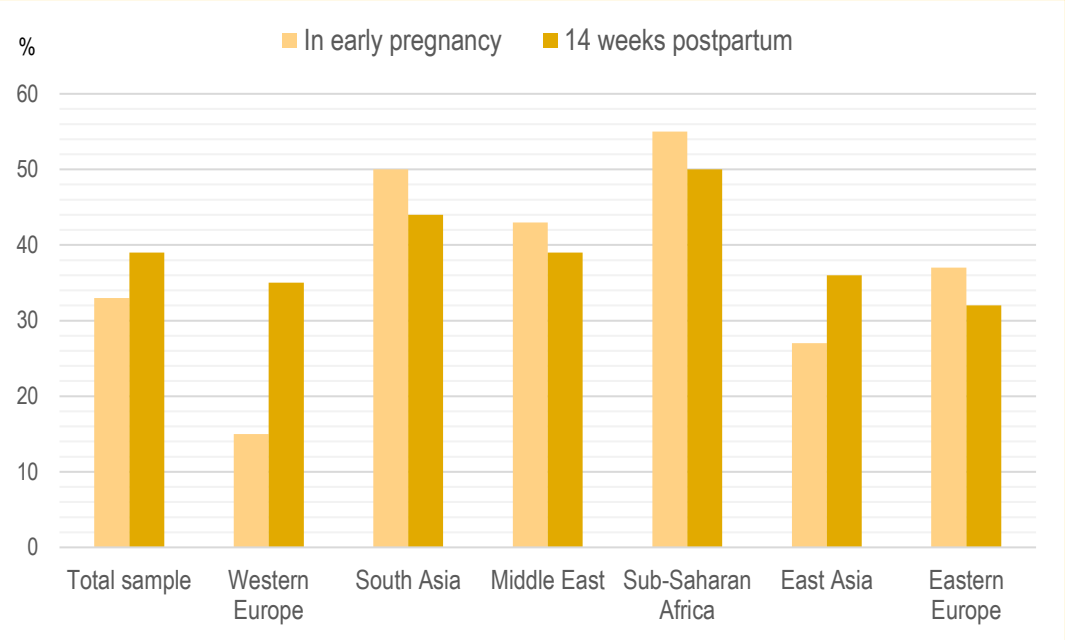
independent risk factors were low maternal age, low early life socioeconomic position, and chronic illness associated with normochromic anaemia (Paper II).

4.3 Crude prevalence of ID by SF in early pregnancy and 14 weeks postpartum, overall and by ethnic origin (Papers I and II)

The prevalence of ID by SF was 33% early in pregnancy and 39% at the 14 weeks postpartum visit.

We found significantly higher prevalence of ID by SF in early pregnancy in all ethnic minority groups compared with Western Europeans (Paper I). At the postpartum visit, although nominally higher, the prevalence of ID by SF did not differ significantly between the ethnic groups (Paper II). However, we observed that among Western European and East Asian women, the prevalence of ID by SF increased considerably from early pregnancy until postpartum.

Figure 5. Prevalence of iron deficiency (%) by SF in STORK G in early pregnancy and 14 weeks postpartum



4.4 Adjusted associations between exposure variables and ID by SF (Papers I and II)

After adjustment for possible covariates, we found that except for women with East Asian background, women with non-Western ethnic origin had higher odds of having ID by SF in early pregnancy compared to Western European women (Paper I).

No ethnic origin was significantly associated with higher odds of having postpartum ID by SF compared to Western Europeans after adjustment for possible covariates (Paper II). Primiparity, gestational ID by SF, no use of iron supplements in gestational week 28,

unhealthy dietary pattern and haemorrhage were significantly associated with higher odds of having postpartum ID by SF.

4.5 Crude prevalence of ID by sTfR and TBI in early pregnancy and 14 postpartum in the total sample and by different ethnic origin (Paper I and II)

The crude prevalence of ID in the total sample was 6.5% by sTfR and 11% by TBI in early pregnancy, which increased to 19% and 22% respectively 14 weeks postpartum. The prevalence of ID by sTfR and TBI increased in all ethnic groups from early pregnancy until the postpartum visit.

Figure 6. Prevalence of iron deficiency (%) by sTfR in STORK G in early pregnancy and 14 weeks postpartum

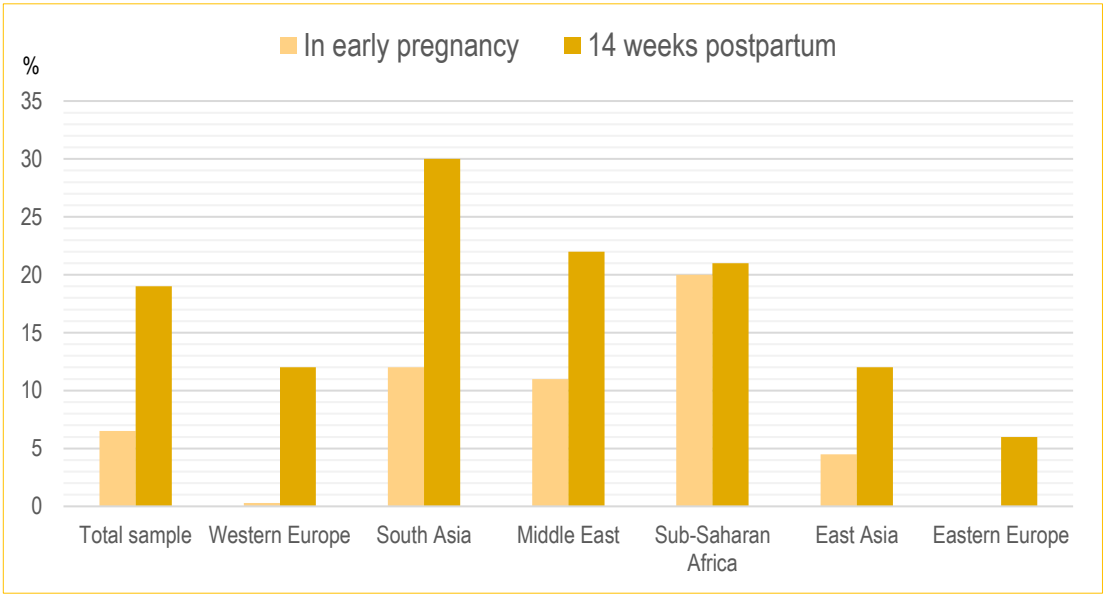
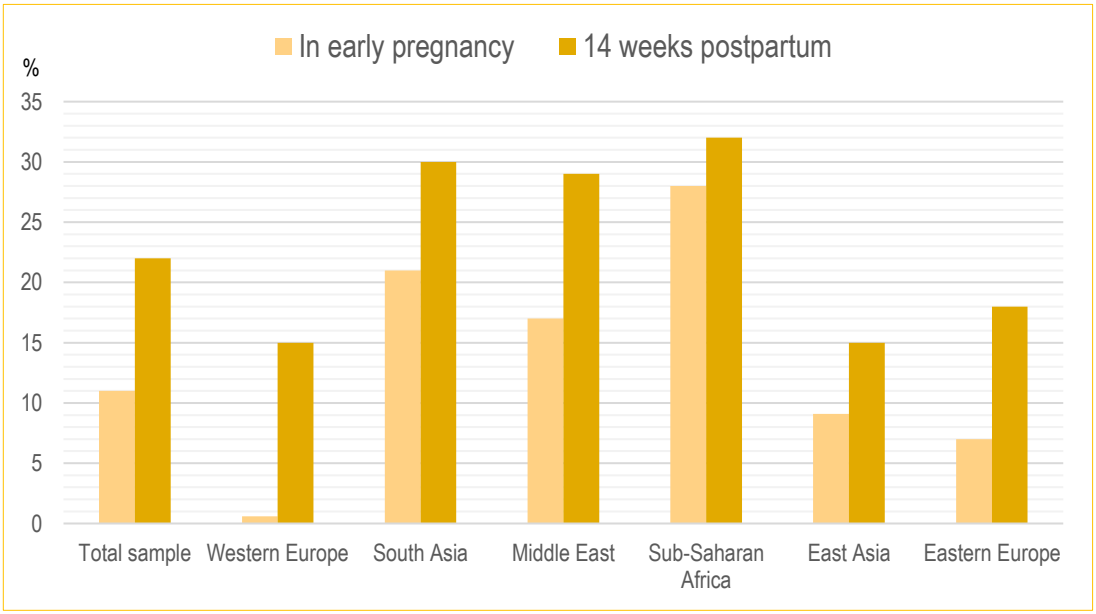


Figure 7. Prevalence of iron deficiency (%) by TBI in STORK G in early pregnancy and 14 weeks postpartum



Compared to Western Europeans, we found significantly higher prevalence of ID by sTfR early in pregnancy in non-Europeans, and of ID by TBI in all non-Western groups (Paper I).

Compared with Western Europeans, the prevalence of postpartum ID was significantly higher in women of South Asian and Middle Eastern origin when defined by sTfR and TBI, and in Sub-Saharan African women when defined by TBI (Paper II).

4.6 Adjusted associations between exposure variables and soluble transferrin receptor and total body iron (Papers I and II)

After adjustment for possible covariates, ethnic origin from South Asia, the Middle East and Sub-Saharan Africa were significantly associated with higher sTfR and lower TBI early in pregnancy compared to Western Europeans. In addition, Eastern European origin was associated with lower TBI in early pregnancy (Paper I).

After adjustment for possible covariates, only South Asian origin was associated with increased sTfR concentration postpartum compared to Western Europeans. In addition, ID in early pregnancy, primiparity, no use of supplements, unhealthy dietary pattern, and haemorrhage were significantly associated with adverse sTfR and TBI (Paper II).

We explored whether level of integration could explain South Asians' higher sTfR and lower Hb concentration postpartum. Our supplementary analyses showed that the higher prevalence in South Asians compared with the other minority groups persisted when including social integration into our regression model (see appendix, Supplemental Table 2 Paper II). Further, in the ethnic minority subsample, no significant association between low social integration and poor postpartum iron status or haemoglobin concentration was observed (see appendix, Supplemental Table 3 Paper II).

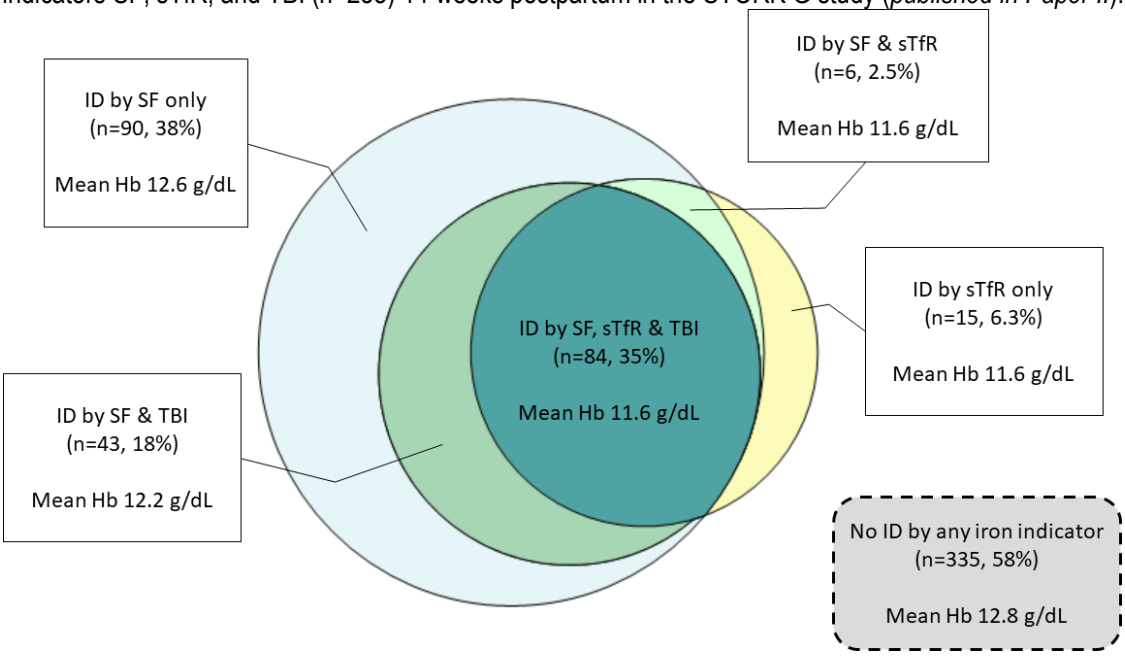
4.7 The relation between SF, sTfR and TBI, and the relation between the iron indicators and Hb (Papers I and II)

Within the four Hb concentration intervals (presented as grouped at the midpoint), we observed a poorer iron status in the lowest Hb intervals (Figure 2 in Papers I and II). We also observed different distributions by ethnicity. In all four Hb concentration intervals, women with origin from South Asia, the Middle East and Sub-Saharan Africa had consistently poorer iron status early in pregnancy and postpartum, and women of Western European and East Asian origin had consistently higher iron stores (Papers I and II).

The relations between ID by SF, sTfR and TBI, and mean Hb concentration are illustrated in the scaled VENN diagram (Figure 8). Overall, 42 % of the study sample had ID by any indicator. The lowest mean Hb concentration (11.6 g/dL) was observed among women with

ID by sTfR (sTfR alone or in combination with ID by SF and/or TBI). The highest mean Hb concentration (12.6 g/dL) was observed in those having ID by SF only. Only 6.3% had ID by sTfR only (mean Hb 11.6 g/dL), while none had ID identified by TBI only.

Figure 8. Venn diagram for postpartum women with iron deficiency by ≥1 of the three iron indicators SF, sTfR, and TBI (n=238) 14 weeks postpartum in the STORK G study (*published in Paper II*).



4.8 The impact of recommending iron supplements, and the change in iron status from early pregnancy to 14 weeks postpartum (Paper III)

We found only minor ethnic differences in the prevalence of ID 14 weeks postpartum. In addition, compared to women from South Asia, the Middle East and Sub-Saharan Africa, we observed a more adverse development in iron status among Western Europeans and East Asians with their relatively more robust iron stores early in pregnancy. This prompted us to study the impact of our simple intervention and to assess factors associated with change in iron stores from early pregnancy to postpartum.

Of the 252 women exposed to our simple intervention, 81% were non-Western Europeans. The median SF concentration increased slightly from early pregnancy until postpartum in women recommended supplements, while a reduction from early pregnancy until postpartum was observed in women not exposed for our intervention (Figure1 Paper III).

From mean gestational week 15 to 28, the use of supplements increased from 25% to 65% in the group receiving a letter recommending supplements and from 13% to 26% in the group not exposed for this intervention. The results of the changes in iron status are summarized in Table 7.

We observed that self-reported use of iron supplements in gestational week 28 was associated with higher iron stores by all indicators and higher Hb concentration postpartum. In addition, supplement use in gestational week 28 was associated with lower odds of postpartum ID, but not of postpartum anaemia.

In our multinomial logistic regression model, we investigated factors associated with belonging to either “steady low”, “improvement”, or “deterioration” compared to “steady high”, reflecting SF concentration in early pregnancy and postpartum (Paper III).

Postpartum haemorrhage
Postpartum haemorrhage was positively associated with “steady low” (OR 5.4, 95% CI 1.5-19.2) and “deterioration” (OR 4.7, 95% CI 1.6-14.0)

Dietary iron intake (diet and supplements)
Unhealthy dietary pattern was positively associated with “steady low” (OR 4.0, 95% CI 1.7-9.5) and “deterioration” (OR 2.8, 95% CI 1.5-5.1). Use of iron supplementation in gestational week 28 was positively associated with “improvement” (OR 5.2, 95% CI 3.0-9.0) and negatively associated with “deterioration” (OR 0.3, 95% CI 0.2-0.6).

Parity
Primiparity was positively associated with “steady low” (OR 2.7, 95%CI 1.5-4.8), however stronger associated with “deterioration” (OR 2.7, 95% CI 1.5-5.1). Primiparity was also negatively associated with “improvement” (OR 0.5, 95% CI 0.3-0.9)

Ethnic origin
South Asian origin was associated with “improvement” (OR 2.7, 95% CI 1.2-5.9), however stronger associated with “steady low” (OR 3.7, 95% CI 1.6-8.7).

Table 7. Change in iron status from early pregnancy to 14 weeks postpartum,
and factors associated with "steady low", "improvement" and "deterioration" iron status (STORK G)

Total sample n = 573	At enrolment in early pregnancy	Change from early pregnancy until postpartum	14 weeks postpartum	
	SF concentration **< 20 µg/L** n = 252 78% non-Western **Simple intervention; Recommended daily iron supplements 30-50 mg**	Δ SF 11 µg/L Δ sTfR 0.8 mg/L Δ TBI 0.9 mg/kg Δ Hb 0.7 g/dL	Postpartum SF concentration <15 µg/L n = 110 **"Steady low"**	Adj. OR Use of supplements in GW 28: OR 2.7 (95% CI 1.5-4.8) Unhealthy dietary pattern: OR 4.0 (95% CI 1.7-9.5) Postpartum haemorrhage: OR 5.4 (95% CI 1.5-19.2) South Asian origin: OR 3.7 (95% CI 1.6-8.7)
			Postpartum SF concentration SF ≥15 µg/L n = 111 **"Improvement"**	Adj. OR Use of supplements in GW 28: OR 5.2 (95% CI 3.0-9.0) Primiparity: OR 0.5 (95% CI 0.6-0.9) South Asian origin: OR 2.7 (95% CI 1.2-5.9)
	SF concentration ≥ 20 µg/L n = 321 22% non-Western	Δ SF - 26 µg/L ΔsTfR 1.4 mg/L Δ TBI - 4.6 mg/kg Δ Hb 0.4 g/dL	Postpartum SF concentration SF <15 µg/ n = 114 **"Deterioration"**	Adj. OR Use of supplements in GW 28: OR 0.3 (95% CI 0.2-0.6) Unhealthy dietary pattern: OR 2.8 (95% CI 1.5-5.2) Primiparity: OR 2.7 (95% CI 1.5-5.1) Postpartum haemorrhage: OR 4.7 (95% CI 1.6-14.0)
			Postpartum SF concentration ≥15 µg/L n = 207 **"Steady high"**	Reference

5. Methodological considerations

Methodological considerations are an important aspect to discuss, to ensure that the research is well-designed and the findings are valid and reliable.

5.1 Study design

In this thesis, one paper uses a cross-sectional design (Paper I) and two papers use a prospective longitudinal study design (Papers II-III). The papers are population based, aiming to study and improve maternal health in a multi-ethnic population. Observational studies may be biased by the study sample selection on how well it represents the study population [92]. Further, cohort studies are likely exposed to loss to follow-up. Different biases affect the external validity of the results and whether the findings can be generalized outside the study setting [93].

Population-based studies have their limitations. For example, they may not include certain groups of people who are not easily reachable, such as ethnic minority women or illiterate women. We strived to include a representative sample of the population in order to ensure the generalizability of the study findings by making several adaptions. We used bilingual staff and interpreters to facilitate communication with participants to reduce the language barrier. One other limitation of observational studies is due to the uncertainty of whether an observed association is causal or rather a reflection of the association with other variables (confounding bias) [92]. Even though causal effects are hard to be concluded from observational studies alone, such studies may contribute with important findings that may lead to further studies.

On the other hand, population-based studies can provide more accurate estimates of the prevalence of certain health issues in a particular population, however the method to collect data is crucial to present data on a representative sample of the population. In population-based studies, individuals are sampled from the population of interest, and the prevalence of the health issues of interest is estimated. This can provide a more accurate estimate of the prevalence than studies that only recruit participants with a specific condition or those who seek medical attention.

Population-based observational studies are important for studying the prevalence of health issues, risk factors and to assess factors associated with these health issues. Overall, studying health issues in diverse populations is critical to promoting health equity, improving generalizability, identifying risk factors, addressing cultural factors, and improving the health care. It is important to understand how health problems affect different populations and to identify inequalities in health outcomes. Studies carried out in heterogeneous populations

may be more generalizable and be of higher relevance to real-world settings. Heterogeneous populations often offer a wider range of risk factors and different cultural factors compared to homogeneous populations. Studying a population with a diverse ethnic and socioeconomic background can help to identify risk factors and cultural factors of importance for health, and can be used for targeted prevention and intervention efforts.

5.2 The sampling and recruitment strategy

We aimed to recruit pregnant women with a diverse ethnic and socioeconomic background into STORK G. Groruddalen was chosen because this urban area in the north-eastern part of Oslo covers a multi-ethnic population with diverse socioeconomic status (see Figure 2).

The recruitment strategy for pregnant women to STORK G was to invite women who used the public free-of-charge Child Health Clinics for antenatal care. Previous studies have shown that most women (75-85%) living in these city districts in Oslo prefer the Child Health Clinics for this purpose [83], which suggests that there is little selection regarding pregnant women who visit the Child Health Clinics for their antenatal care. Lastly, the GPs was engaged to recruit pregnant women for our study, which most likely contributed to both higher attendance at the Child Health Clinics in general, and towards a higher participation rate.

Both logistics regarding attendance in cohort studies and lack of confidence in health system may contribute to lower participation rate in cohort studies, especially among ethnic minorities. The most frequent reasons to decline participation in STORK G, was lack of time because of work obligations or care of small children[83] . Age, parity, and ethnic origin between the 291 women who declined to participate were not different from the study sample [90]. We did not have permission to collect other data on the invited participants who chose not to attend. Self-selection bias, i.e. that voluntary participants in a research study often differ from those who do not agree to such participation, cannot be ruled out.

However, despite that participation in our cohort study was time-consuming, we believe that the use of trained midwives and translators trained and familiar with the questionnaires implied reduced barriers for inclusion of ethnic minority women and illiterate women, and contributed with our successful participation rate. STORK G achieved a high participation rate of 74%, and varied between 83% and 64% among the largest ethnic groups. Although some ethnic minority groups had a slightly lower attendance rate compared with ethnic Norwegians, the attendance rate in STORK G was still higher compared to other Norwegian population-based studies [94,95]. The Norwegian Mother and Child Cohort Study (MoBa) is a large cohort inviting pregnant women attending their routine ultrasound examination, aiming to detect causes of disease among mothers and children, had a total participation rate of

41% [95]. The Oslo Health Study (HUBRO) is another large cohort that studied the health status of Oslo residents, adapted to include ethnic minority participants, with a total participation rate of 46% (39% participation rate of invited non-Western) [94]. In the “Romsås in Motion" (MoRo) cohort, a project to promote physical activity in a multi-ethnic local community in east Oslo, the total participation rate was 48% [96]. STORK G strength is its high participation rate, therefore the study results may be generalizable to the larger population.

The population studied, lived in the city districts with the highest proportions of non-Western population in Norway/Oslo (Figure 2). We therefore believe that the women attending the Child Health Clinics were representative for reproductive-age women from these ethnic groups. In addition, the ethnic composition, age, and parity were similar with data from the Norwegian Medical Birth Registry [83,97]. One in five of all non-Western European women rated their Norwegian language performance as poor/somewhat poor and were in need of an interpreter during interviews, indicating that we succeeded to reduce barriers to ensure inclusion of ethnic minority women.

The main reason for being excluded from the STORK G project was being in the second half of the pregnancy (37%) [83]. Other reasons were not speaking any of the eight languages covered by the questionnaires, planning to give birth at another study hospital than our two, or requiring excessive hospital care. Women who require excessive hospital care may have different health outcomes compared to the general population. Excluding them from the study sample may increase the representativeness of the study findings to the wider population. In some cases, it may be more appropriate to stratify the sample based on healthcare utilization or to conduct a separate analysis for this subpopulation. Nevertheless, the decision to exclude women in need of excessive hospital care resulted in improved feasibility.

5.3 The sample size

This multi-ethnic population-based cohort was primarily set up to study obesity, gestational diabetes mellitus, and cardiovascular disease. However, STORK G was also designed to cover a range of health issues through the collected, large variety of different variables.

In statistics, "power" refers to the probability of correctly rejecting the null hypothesis when it is false, the default assumption being that there is no significant difference between groups or no significant relationship between variables. A larger sample size generally results in greater statistical power, reduce the impact of random errors and increase the precision of the effect estimates, which means that there is a higher probability of detecting a true effect if it exists. The statistical justification of sample size was based on power calculations related to identify ethnic differences in the prevalence of gestational diabetes, being at least 800 women which would give 100 women with gestational diabetes [97]. In this study, we took

advantages of the collected date and performed regression models based on the available sample size. The largest sample of ethnic minority groups were those from South Asia and the Middle East, while the samples of Sub-Saharan African, East Asian and Eastern Europe were small. Drawing conclusions in the smaller groups must therefore be done with caution.

Study sample Paper I (n=792)

At enrolment, measurements of Hb and SF concentration were missing in 31 participants. There were no significant differences between the study sample and the excluded women regarding ethnicity, age, gestational week, parity, pre-pregnant BMI or education. Further, with missing data in only 31 participants (3.8%) in the study sample, we do not suspect substantial selection bias.

Study sample in Paper II-III (n=573)

Among the 644 women who attended the postpartum visit (81% of included), a slight selection towards ethnic Norwegians was observed [98], as seen in similar multi-ethnic cohorts [99]. In general, participation rate above 80% in large prospective cohort studies is considered well [100].There were largely logistical or medical reasons for non-attendance, pregnancy complications, such as abortion, stillbirth, or preterm birth, twin birth, or reported to be sick at the postpartum visit, and sick leave by study staff. The rest did not attend because they had moved out of the study district, changed birth hospital, or unknown reason (see Figure 3).

At the postpartum visit, SF concentration was missing in 71 women, resulting in 573 participants (72% of total follow-up sample). In cases of sick leave by study staff, ethnic minority women were prioritized over Western European women for blood samples. This was considered necessary to maintain adequate sample sizes in the ethnic minority groups.

Table 8. Comparison of subject characteristics in postpartum week 14 between study sample (n=573) and the women who did not attend due to logistic/unknown reasons (n=250) in STORK G. Numbers are mean (d) or n (%).

	Study sample Paper II n=573	Excluded sample (n=250)
Gestational week at inclusion, *mean* (SD)	15.1 (3.4)	15.0 (3.3)
Age at inclusion (*years), mean* (SD)	29.7 4.8	30.2 (5.0)
Parity		
Primiparous, *n* (%)	260 (46)	118 (47
Multiparous, *n* (%)	304 (54)	132 (53
Pre-pregnant Body Mass Index, (*kg/m²), mean* (SD)	24.6 (4.8)	24.5 (4.9
Maternal adult socioeconomic position, *mean* (SD)	0.01 (1.0)	-0.03 (1.0
Early life socioeconomic position*, mean* (SD)	- 0.01 (1.0)	0.03 (1.1
Ethnic group		
Western Europe, *n* (%)	217 (38)	123 (49)
South Asia, *n* (%)	157 (27)	44 (18)
Middle East, *n* (%)	94 (16)	32 (13)
Sub-Saharan Africa, *n* (%)	38 (7)	24 (10)
East Asia, *n* (%)	33 (6)	14 (6)
Eastern Europe, *n* (%)	34 (6)	13 (5)

The Western European women not invited to undergo blood test at the postpartum visit were randomly selected, thus no selection bias is expected within this group. This resulted in a slightly larger proportion of ethnic minority women compared to the study sample in Paper I. However, there were no significant differences between the 573 women in the study sample and the 250 excluded women regarding age, parity, pre-pregnant BMI and socioeconomic status (Table 8).

5.4 Biochemical measurements

The methods used to analyse blood samples will be discussed in this section. Accurate measurements are dependent on proper sample collection and handling, and equipment used in the laboratories must be regularly controlled. Some of our analyses lack standardized assay methods. Further, physiological pregnancy changes and states of inflammation or infection can affect the levels of several of our measurements, and need to be taken into account when interpreting results.

Haemoglobin and serum ferritin

Reliable and well-established methods were used to analyse Hb (CV <0.7%) and SF (CV <7%), as presented in section 3.4. Internal and external quality controls were included for all biochemical analyses, and were conducted for each reagent lot used throughout the study period, for SF eight different lots.

Our analysis methods are not suspected to have any systematic errors, as women were recruited consecutively, and blood sampling and analyses were analysed consecutively.

Soluble transferrin receptor and calculated total body iron

The Roche assay used to analyse sTfR was the same assay used by the Centers for Disease Control and Prevention (CDC), the national public health agency of the US. Even though different assays exist and there is no standardized method, the sTfR assay from Roche is well-established [85,101,102] . This method had an acceptable interassay CV (CV <5%), and all analyses were performed as one "batch" within the same lot. The batchwise analyses and elimination of lot-to-lot variation reduces the analytical CV. In order to compare sTfR and TBI from different studies, conversion formulas must be used. The equation to convert sTfR by Roche to Flowers values, and to calculate TBI is described in section 3.4.

As the analyses and calculation method was done equally for all the participants, we suspect no systematic error in the conversions and calculations.

The impact of increased plasma volume on the biochemical iron indicators

An accurate measurement of iron status during pregnancy is challenging to obtain because of the plasma volume expansion [31], and there are no simple biomarkers that provide good estimates of the extent of plasma volume expansion. Different methods to determine the

plasma volume are available, however none are considered reliable [103]. Lobigs et al. conclude that by evaluating the results of eight different biochemical tests, it is possible to explain 68% of the changes in plasma volume in healthy men [104]. The ideal time to measure gestational iron status is preferably in first trimester before the plasma volume expansion. The relationship between gestational age and plasma volume expansion in physiological pregnancy is by de Haas et al. illustrated as proportional [105], with an average increase from 2.4 to 2.8 L from the time before pregnancy to gestational week 19 [105]. Four weeks postpartum, the plasma volume is at the same level as before pregnancy [105].

To facilitate inclusion of Pakistani and Somali women, a change in the study protocol was performed after six months, allowing inclusion if gestational week <25. Of the 21 (17%) women with Pakistani origin and 15 (38%) women with Somali origin were included in gestational week ≥ 20. In our cohort, the gestational age at enrolment ranged from 9 to 30 weeks (interquartile range 13-17 weeks), hampering the interpretation of the analysed blood samples. In the total study sample, 88 (11%) included women were in gestational week ≥20. According to de Haas et al., the plasma volume expansion increases with gestational week [105]. Women with the higher gestational age can be assumed to have a more diluted plasma volume compared to the other ethnic groups. Considering the adjustment of inclusion criteria regarding gestational week at enrolment for women with Pakistani and Somali background, we compared mean gestational week in these groups with the rest of the included participants, using ANOVA Bonferroni test (Table 9).

Table 9. Comparing mean gestational week in women with ethnic origin from Pakistan and Somalia with the rest of the study sample, using ANOVA Bonferroni test in the total sample of STORK G (n=823)

		Gestational week at inclusion			
		N	mean	± SD	p*
	The rest of the study sample	656	14.8	± 3.0	
	Pakistan	127	15.4	± 3.9	0.20
	Somalia	40	17.8	± 5.4	<0.001

We found significantly higher gestational age in women with Somali origin. Therefore, caution must be taken in drawing conclusions when comparing crude prevalence of gestational iron status (Paper I) among the ethnic groups. Somali women constituted the majority (64%) in the Sub-Saharan Africa group. The crude prevalence rate of gestational ID by SF in Sub-Saharan Africans is most likely lower than our estimates due to the expanded plasma volume caused by significantly higher gestational week when enrolled in the study.

In our regression analyses, we adjusted for gestational week, and consequently plasma volume expansion, and found that the ethnic differences persisted (Paper I). Further, the

main reason to analyse sTfR from the biobank and calculate TBI, was to estimate iron status by iron indicators that are not to the same extent affected by the expanded plasma volume. These analyses also showed significantly different prevalence rates by ethnicity. We therefore conclude that despite these limitations, the prevalence rates of ID and anaemia are higher in ethnic minority groups.

In Paper III, we assessed factors associated with change in iron status by SF from enrolment until postpartum. In addition, we examined the effect of supplementation in gestational week 28 on the change in SF, sTfR, TBI, and Hb concentrations from enrolment until postpartum, and on the odds of having anaemia or ID (by ST, sTfR or TBI). The plasma volume is on average about 20% greater in gestational week 15 than 14 weeks postpartum [105]. The change, calculated by subtracting the concentrations at the postpartum visit from that at enrolment for each indicator, will be more or less over-estimated because of the enrolment value being affected by expanded plasma volume. We adjusted for gestational week at enrolment in our regression analyses and observed minor changes on our results.

Nevertheless, with the plasma volume expansion, it is difficult to give a good quantitative measure of how big the improvements were. However, we found an association between iron supplement use and improvement of iron status (Table 2 and 3 in Paper III), after adjusting for gestational week and consequently plasma volume expansion. Therefore, it is unlikely that the plasma expansion is an important explanation for our findings.

The impact of inflammation on the biochemical iron indicators

Acute-phase proteins increase secondary to infections and inflammations, and also physiologically during pregnancies [106]. SF is considered an acute phase protein [107]. There is an ongoing debate on which SF concentration to use as threshold to diagnose ID in pregnancies [31], some suggest to raise the threshold for SF that defines ID from 15 µg/L to 30 µg/L [106]. Another method is to exclude individuals with elevated CRP concentrations from prevalence calculations based on SF. Alternatively, regression correction approaches may be used to adjust SF concentrations for inflammation. Lastly, additional measurements of iron status are recommended, such as sTfR [31].

Supplementary analyses were conducted (see section 3.5). In Paper I, more than two in five had elevated CRP (≥5 µg/L) at enrolment, however by using gestational age specific thresholds, one in ten had elevated CRP. The prevalence of gestational ID was only slightly lower in the study sample after excluding women with possible inflammation from the regression analyses (see appendix, Supplemental Table 1 Paper I). This implies that our high prevalence rates of ID by SF cannot be explained by the physiological rise in acute phase proteins secondary to pregnancies or infections/inflammations. Also in Paper II, we

conducted supplementary analyses on a subsample with no inflammatory response (CRP <5 µg/L), and observed only minor changes in the mean and median values and in the prevalence of postpartum ID and anaemia across ethnic groups in these analyses. We concluded that inflammatory response cannot explain the differences observed in our population.

Our second approach was to estimate ID by sTfR and calculated TBI. Although sTfR have been presented as a better indicator not being influenced by plasma volume expansion, infections or inflammations [32], recent research found inflammatory states to affect the sTfR concentration [108]. However, the impact was minimal and not consistent [108]. In addition, the impact of inflammation on calculated TBI has been assessed by others, and found that in areas with a high prevalence of inflammation or infections, the prevalence of ID by TBI is underestimated if not adjusted by inflammation [109].

5.5. Thresholds used to define anaemia and iron deficiency

Anaemia

An accurate assessment of anaemia and ID depends on an appropriate threshold value in the population being studied. During pregnancy, an increase in plasma volume contributes to reduced Hb and SF levels, which makes interpretation difficult. In addition, different threshold values based on ethnicity have been proposed, because several studies have shown different reference ranges of Hb between different ethnic groups. This will be discussed in the next section.

The thresholds used to define anaemia proposed by a WHO study group on nutritional anaemias in 1968 [23], remained unchanged for decades, being Hb <13.0 g/dL in men, Hb <12.0 g/dL in women and Hb <11.0 g/dL in pregnant women. New thresholds defining mild, moderate and severe anaemia were presented in 1989 [110], and modified threshold among smokers and individuals living high above sea level was suggested in 2001 [35].

The normal Hb distribution vary with different stages of pregnancy, decreasing from the first trimester, proportionally with higher gestational age until the end of the second trimester, then gradually increase during the third trimester [24]. In 1989, the CDC developed month-specific and trimester-specific thresholds based on data from four European surveys between 1975-1982 of healthy pregnant women taking iron supplements [24]. These modified thresholds for anaemia in pregnant women, being Hb <11.0 g/dL in first and third trimester, and Hb <10.5 g/dL in second trimester, were suggested used by the WHO in 1993 [25]. This means that the current Hb thresholds defining anaemia in pregnancy are based on historical normal values, and not based on bone marrow staining or clinical outcomes, promoting an ongoing debate as to the applicability of these values [46].

In addition, questions have been raised as to whether the threshold can be applied to all population groups. Several studies have reported lower Hb concentrations in blacks than in whites (regardless of SF concentration) in the US and proposed adjustments for ethnicity to the WHO threshold values defining anaemia [21,111-113]. WHO has initiated a comprehensive project to review the evidence for defining anaemia, and to collect updated data on normal ranges of Hb in different populations [114,115]. The results will eventually be summarized and presented to a development group for WHO's guidelines.

Further research is necessary to assess which Hb concentration reflects postpartum anaemia. Most guidelines in Europe and Australia define postpartum anaemia as Hb <10 g/dL (24-48 hours after delivery) [48,75,116,117], however France define postpartum anaemia using a higher threshold, Hb <11 g/dL [118]. The guidelines in the US and Switzerland define postpartum anaemia as <12.0 g/dL (4-6 weeks after delivery) [69,119]. A systematic review from 2020 comparing different clinical guidelines supports to use Hb <10.0 g/dL the first 5 weeks after delivery [26], and two systematic reviews suggest to use Hb <12.0 g/dL as threshold for postpartum anaemia more than 8 weeks after delivery [27,29]. The definition of postpartum anaemia measured shortly after delivery is hampered by the pregnancy-related plasma expansion. After 6-8 weeks the plasma volume has returned to normal and a threshold for anaemia is the same as for non-pregnant women [29,105]. As our analyses were conducted in mean 13.9 ±2.5 weeks after delivery, we used the threshold Hb <12.0 g/dL to define postpartum anaemia.

Iron deficiency

Also the interpretation of gestational iron status by SF in our pregnant population was hampered by high gestational age in some women. The use of SF to define ID has potentially led to a higher prevalence of ID by SF in Paper I. Further studies to assess SF concentrations during pregnancy and establishment of threshold values by trimester is recommended [31]. To ease comparison to other studies, and between the iron indicators, we conducted sensitivity analysis using SF <12 µg/L as the threshold to define iron deficiency (Paper II). By using this threshold, the proportion of ID between the indicators were more comparable by using the higher threshold 15 µg/L.

Because no widely accepted threshold for sTfR exists, the interpretation and comparison of ID by sTfR is hampered. sTfR can be analysed through various assay methods, e.g. enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and chemiluminescent immunoassay (CLIA). sTfR may measure functional ID or tissue ID, and decreased Hb concentration measure a severe ID [120]. WHO is currently working to establish a reference method to reduce the variability between commercially available methods, but not yet succeeded [121].

Skikne, Flowers and Cook observed that TBI (mg/kg) could be estimated by measurements of SF and sTfR concentrations in a study of serial of phlebotomies [120]. The logarithm of the concentrations of sTfR and SF illustrated a fairly linear relation with estimated iron stores. The authors presented an equation to calculate TBI from the concentrations of SF and sTfR [38]. TBI as an iron indicator is mainly used in research, and is not established as a clinically used analysis.

WHO has suggested to measure SF and sTfR concentration in combination in population surveys for interpreting iron status in populations, and proposed interpretation of low SF (<30 µg/L) and high sTfR concentrations (>10% abnormal sTfR concentration) in population surveys [21]. According to this proposal, our population is iron depleted in early pregnancy, and iron deficient 14 weeks postpartum.

5.6 Data collection

There are also several types of measurement bias that can occur in data collection methods using questionnaires. Misclassification of participants' group affiliation can result in bias and incorrect estimates of the association between the exposure and outcome. When the probability of individuals being misclassified is equal across all groups in the study, non-differential misclassification occurs. Differential misclassification occurs when the probability of being misclassified differs between groups in a study [122]. Participants may have difficulties accurately recalling past events (recall bias), or provide answers based on interpretation of the question or the desire to please the researcher (response bias), or provide answers that they believe are socially acceptable or desirable, rather than their true beliefs or behaviours [122]. In addition the language or cultural context of the questionnaire may be unfamiliar or inappropriate for some, leading to inaccurate responses. With use of interviewers, and in some cases professional translators, possible expectations of the interviewer might interfere with the judgement of the interviewer (interviewer bias), and the design of the questionnaire may influence the responses of the respondents, for example, by providing leading questions or using unclear language [122].

The quality control of the data in our cohort study involved series of steps to ensure that the data collected was accurate, reliable, and consistent [122]. The study staff was trained to systematically review the data set, identify and correct errors, and validate the data. The data was assigned codes to categorical variables and numerical values to continuous variables to ensure consistency across the data set. Data entry was performed by experienced personnel familiar with the study protocol in a standardized and consistent manner. The quality and completeness of the data was continuously monitored to detect any problems or errors early.

Classification of ethnicity

It is important to identify vulnerable groups in order to contribute to better health. To capture the cultural, language and dietary factors that may persist after migration and over generations, we used the country of birth extending it by also using the mother's country of birth if she was born outside Western Europe. However, the concept ethnicity is complex and classified in several ways. Country of birth is considered an objective, crude method for classification of ethnicity [123] and often comprise heterogeneous populations. Other authors use self-defined ethnicity, and in countries with a long history of immigration and a diverse population such as Australia, the US and UK, this method might better reflect the population's ethnicity as ethnic inequalities may be reduced over time.

To assess the difference of health outcomes by ethnicity, the sample must be large enough. Due to small numbers of participants for most countries, we merged country of origin with geographical regions, which resulted in even more heterogeneous populations. When merging, the groups must have more or less similar findings, and there must be reason to assume that they are similar. Our South Asian group consisted of 124 (63%) Pakistani and 61 (31%) Sri Lankan women. The majority of Pakistani population are Muslims, and the Sri Lanka society is multi-ethnic and multi-religious, but the majority of Sri Lankans living in Norway are from the Tamil, primarily Hindu, minority. The prevalence of anaemia is according to GHO high in both countries [22], being 35% (20-48%) in Sri Lanka, and 41% (32-51%) in Pakistan [22].

There are likely genetic differences between populations in these two countries, and within the countries. From the literature, the East-Asian region seem to differ from the other regions in relation to iron status and metabolism. However, as most genetic studies linking to iron metabolism have been performed in ethnic European populations, there is a lack of evidence regarding whether genetic differences explain ethnic or regional differences in iron status [14]. A Norwegian study on dietary pattern by ethnic origin, found no significant differences in the dietary intake of haem iron or non-haem iron, however, described a higher content of iron absorption inhibitors in bread and chapatti in the Pakistani group compared to Norwegians [124].

By defining group affiliation based on participants' country of birth, we cannot rule out that the composition of our group differs from other studies. However, we believe STORK G is representative for the study population, and suggest that our findings are representative for similar ethnic groups in multiethnic populations outside Oslo, achieving external validity.

Iron intake through supplements and diet

The following self-reported data may be hampered by recall bias, interviewer bias or response bias. The simple intervention is described in section 3.4. The participants received

a letter with recommendations on iron supplementation. The letter was not translated, but written very simple in Norwegian and the women were recommended to contact their GP. Also, when we used the integration variable (where language is an important component), we did not see differences in postpartum anaemia. We have not investigated whether the integration variable has an effect on the use of supplements in gestational week 28. We lack information on whether our recommendations were followed or not, how the information was interpreted, and if the women made appointments with their GP. In addition, we lack information on duration and type of supplements used. Despite the limitations mentioned, our results imply that our simple intervention had a positive impact on supplemental use.

The questionnaires used to assess use of iron supplements at all study visits are described in section 3.4 (see appendix, STORK G diet). To reduce the risk of recall bias regarding supplementation use, we asked about intake during the previous two weeks instead of a longer period. We lack information on exact iron intake in approximately 60% of the cases. In the cases where we were able to calculate frequency dose intake of iron, the results were a range from 30 mg to 1400 mg of iron per week. In Norway, supplements with a low iron content (≤30 mg) are commercially available in grocery stores and in pharmacies, whereas preparations with higher iron content (65 mg or more) are only available in pharmacies (without prescription). Of those reporting type of iron supplements, 57% used ferrous gluconate, 39% ferrous sulphate, and 4% ferrous fumarate. The calculation of dose x frequency intake of iron was not possible, and we therefore dichotomized the variable into yes/no, covering daily or intermittent iron supplement use. Obviously, it would have been an advantage with more accurate measurements of iron intake. Nevertheless, use of supplements was positively associated with change in all iron indicators from enrolment to postpartum, lower probability with postpartum ID, however not with postpartum anaemia. With more accurate information on iron content, one would be able to categorize the use of iron supplements better, and with a higher probability find an association between iron supplementation and reduced odds of anaemia as shown in other studies [54].

The participants' dietary pattern was characterised using a food frequency questionnaire (FFQ) in gestational week 28 (see appendix, Case Record Form 2), which might be hampered by recall bias, response bias and interviewer bias. To reduce the potential recall bias, we limited the questions to assess the dietary intake to the previous two weeks. The "healthy dietary pattern" represents a diet with higher intake of foods with relatively high iron content and foods rich in vitamin C, which again improves the bioavailability of iron. We therefore believe that we to some extent have managed to dichotomize a lower/higher dietary iron intake variable. The dietary pattern was found associated with postpartum iron status (Paper II), and change in iron status (Paper III). The validity of dietary pattern concerning our

aim to assess factors associated with ID and anaemia might be hampered by other limitations. The FFQ was developed by nutritionists to capture pattern associated with obesity and type 2 diabetes, and not with dietary iron. Ideally, other questions that better would cover dietary iron intake should have been chosen. Retrospectively, it is possible that by going through the questionnaires in detail, a better assumption of iron intake could be estimated by using frequency consumption of different foods. In any case, measuring food intake is both very challenging and demanding by data collection and interpretation of data.

A more accurate information of dietary iron content (supplements/diet) between ethnic groups would be of interest to assess its association of ID and anaemia. Despite some acknowledged methodological weaknesses, we find that this data adds important knowledge related to dietary pattern and prevalence of ID in multi-ethnic populations of pregnant women.

If the cohort study had been planned with the primary aim to assess iron status in a pregnant multi-ethnic population, the following changes should have been undertaken: to develop a food frequency questionnaire to capture the iron content in the diet, and more detailed information about the dose of specific iron supplements used, and focus on ethnic groups of specific interest, preferably with larger samples.

Gestational age, maternal age, parity and pre-pregnancy BMI

Iron status is affected by expanded plasma volume that increase with gestational age. Therefore, if the primary aim of this well-planned cohort study had been to assess iron status in a pregnant population, blood sampling in first trimester would be an inclusion criteria. By using the ultrasound-derived gestational age in cases where calculated term differed by more than 14 days until ultrasound term, we attained more accurate data on gestational age. Further, we have no reason to assume that the information about maternal age is misreported. Our collected data on parity were based on several detailed questions about previous pregnancies and births. We therefore have no reason to believe that parity is over- or underreported.

Compared to normal-weight women, the dietary iron absorption is reduced in overweight and obese women [21,125,126]. One possible explanation is that obesity leads to chronic low-grade inflammation, which leads to elevated levels of hepcidin and can result in reduced absorption of dietary iron [126]. On the other hand, underweight, undernutrition and malabsorption are also associated with ID and anaemia [21]. Pre-pregnancy BMI was based on self-reported pre-pregnancy weight. To reduce recall and response data, we asked the participants about their pre-gestational body weight shortly after being weighed. There was a strong correlation between pre-pregnant body weight and measured body weight, which indicated low

misclassification of self-reported information and good internal validity [122]. We therefore find it unlikely that the uncertainty around self-reported weight affected our results.

Health literacy, education and socioeconomic position

Health literacy, defined as "the degree to which individuals have the capacity to obtain, process and understand basic health information and services needed to make appropriate health decisions" [127], may influence immigrants' iron status. Low socioeconomic status, low education, and financial deprivation, younger and older age, are associated with low health literacy [127]. Ethnic minorities often have poor communication skills in the majority language, and lower education level implying less knowledge of the body as well as the nature and causes of disease. Lower health literacy among ethnic minority groups compared to the general population, may contribute in part to ethnic health disparities [127,128]. Our data on education, socioeconomic position and integration that can be linked to health literacy.

In Paper I, we used education in our regression models. There were few missing data regarding education (n=6), and the distribution of education for ethnic Western Europeans in our sample was quite similar to the national data on the education level in ethnic Norwegian women in the same age group [90]. We observed slightly skewed distribution towards having a higher education, which often is observed in populations in rural areas [90]. Other authors use income level to reflect socioeconomic position, however correct data on income level is difficult to collect. Income can reflect paid employment and self-employment, however also capital gains, and transfers such as housing benefit, child benefit, and unemployment benefit. In addition, it is not given that the woman has knowledge of her husband's income. The questionnaire contained questions on how many completed years in school, with multiple options for answers. The content of education and the quality of the educational system varies widely in different cultures and geographic regions, therefore the education variable might not be equally valid across ethnic groups. However, we dichotomized the variable into more than or less than ten years of education, and believe that in this way we formed a rough picture of the level of education.

We also extracted components reflecting adult socioeconomic position and integration and early life socioeconomic position from our collected data, with the intention to capture several factors affecting socioeconomic position (see section 3.4). These variables were used in Papers II and III. We cannot rule out that we might not have captured the variation of socio-economic variables sufficiently.

Delivery mode, birth complications, and haemorrhage and the postpartum visit

We extracted data from hospital birth records and have complete data on variables which are also reported to the Norwegian Medical Birth Registry [83,97]. We have no reason to assume that delivery mode or birth complications are biased.

Postpartum haemorrhage is defined as a blood loss of more than 500 mL immediately after the birth of the baby or within the first 24 hours after delivery [75]. Our reported blood loss reflects primarily blood loss during and immediately after delivery. Despite these uncertainties, any inaccuracy affects everyone equally, and is not considered a systematic bias.

The postpartum period is often defined as the first six weeks after delivery [26]. Our follow-up was 14 weeks after delivery, referred to as "the postpartum visit". There are some advantages by assessing the iron status after 14 weeks of "recovery-time" after delivery. First, the highest rates of anaemia in an US survey, assessing iron status among women with different postpartum length, were 12-18 weeks postpartum [129]. Second, treatment of anaemia in women exposed to great blood loss giving birth, or having symptoms of anaemia, was most likely initiated at the hospital [75]. Lastly, the pregnancy-related physiological changes would have returned to normal by this time [28], implying that the analyses of biomarkers were no longer affected by these changes. We therefore believe that our results reflect the prevalence of postpartum anaemia and ID in a presumably "normal and healthy" population, with few methodological obstacles or limitations.

To conclude, we consider that despite the mentioned limitations, our study method and data collection were appropriate and effective in answering the research questions at hand. The core strengths of this study were its population-based multi-ethnic design and the high attendance rates. We have succeeded in including hard-to-reach women in our cohort study by language support and use of interpreters when needed. We had a high participation rate with minor loss to follow-up visits throughout the study. For those who participated, very little data was missing. We believe STORK G is representative for the study population, and suggest that our findings are representative for similar ethnic groups in multiethnic populations outside Oslo, achieving external validity.

5.7 Causality

In observational studies, causal relationships are hard to demonstrate, but causality can be suggested. When analysing causal relationships between variables in epidemiology, it is important to consider several concepts, including confounding, colliders, mediators and moderators/interactions [122]. Several methods can be used to consider these concepts statistically [122].

Potential confounders are variables that affect both the exposure and outcome, and should be adjusted for [122]. One way to control for confounding, is to use multivariable regression analysis, where the confounding variables are included as covariates in the model. There are no real confounders to ethnicity. We therefore conducted explanatory models and examined

the influence of covariates, resulting in adjusted associations between ethnicity and the outcome variables (ID and anaemia) i.e. direct effects. Uncertainties in the adjusted estimates may be caused by potential residual confounding, i.e. unidentified factors, or imprecise variables (e.g. dietary pattern, socioeconomic position and birth complications) [122].

In our regression analysis in paper I and II, we used stepwise backward elimination, a statistical technique used for variable selection. First, the multivariable model includes all of the independent variables with p <0.2 in the univariate analyses, and then repeatedly removes the variable with the highest p-value >0.05. When all variables that not meet the significance criterion (0.05) are removed from the model, the result is a model with the remaining variables found statistically significant with the outcome variable [130]. The main advantage of stepwise backward elimination is that it can help to identify the most important independent variables associated with the outcome variables, and can produce a model that is easy to interpret [130]. However, this statistical technique also has potential drawbacks, such as overfitting the model to the data, and the risk of excluding important variables that may not meet the significance criterion. Because the method selects variables based on their presentation in the current dataset, rather than their true relationship with the outcome, the model may perform well on the data used, but may not generalize well to new data. Further, if the selected variables are not truly associated with the outcome, but are correlated with other variables in the model, stepwise backward elimination can result in biased estimates of the regression coefficients [130]. Therefore, it is important to use stepwise backward elimination judiciously and with caution. A good a *priori* knowledge and theory used to select variables for inclusion in the model, reduces the probability of bias. We forced the most important variables into our model based on a *priori* knowledge, even though the associations were not statistically significant in our dataset, to control for confounders weakly associated with outcome.

In Paper III we used a multinomial logistic regression model to study a categorical dependent variable with four categories. The dependent variable reflected iron status at enrolment and postpartum, with "steady high" (SF >20 µg/L at enrolment, and >15 µg/L postpartum) as reference category. The model was used to identify which independent variables were associated with each category of the dependent variable. Multinomial models are useful for categorical outcomes variables with more than two categories, allow inclusion of both continuous and categorical independent variables, and estimate probabilities (odds) for each category of the dependent variable.

When performing regression analysis it is important that all prerequisites of the analysis have been checked and found satisfactory. The women included should be independent of each

other. Variables included in the model should be potential confounding factors, but not colliders (i.e. variables caused by both the exposure and the outcome). Mediators (i.e. variables on the causal pathway between the exposure and the outcome) may be included based on the question of interest. For example, in our study investigating the relationship between ethnic origin and anaemia, dietary pattern may act as a mediator. Moderators refer to variables that modify the relationship between the exposure and the outcome. To test for moderators, we included interaction terms in the regression analysis to determine if the association of the independent variable varies across levels of the moderator variable. For example, in our study we were interested in examining whether the associations with our simple intervention and clinical factors differed by ethnic origin. Interactions with ethnicity were examined graphically and by entering cross-product terms one by one. No significant interactions were observed. Stratification is one way of illustrating different associations in various subgroups. Stratification involves dividing the sample into subgroups based on levels of the confounding variable and then analysing the data separately within each stratum. For example, in our analyses, we performed two sets of regression analysis in stratified data, based on whether women were exposed to our simple intervention (recommendation) or not.

Further, the sample size (n) should be large compared to the number of variables included in the analyses, otherwise we may suffer from overfitting if the number of independent variables are large relative to the number of observations [122]. This was considered before performing the multivariable regression analysis in the papers. For example, in the logistic regression model studying ID by SF in Paper I, we applied the “one-in-ten rule”, i.e. there should be at least ten observations in the least frequent group per variable estimated. There were 261 participants categorized as ID by SF, which means that maximum 26 variables could be included in the model at the same time. We included 12 parameters into the model (5 for ethnicity and 7 adjustments variables), thus we are well within the number of variables that reliably can be estimated from the data. Furthermore, the choice of the reference categories should be chosen with care. For example, in Paper III, we chose "steady high" as the reference as this was a "natural reference", and the largest group.

Linearity refers to the linear relationship between the continuous independent and the dependent variables. This linearity assumption was explored by plots and by dividing the variables into quartiles. E.g. for gestational age we observed a linear increase in SF concentration by increasing age group. Multi-collinearity is a phenomenon in which two or more independent variables in a regression model are highly correlated with each other. The multi-collinearity was examined by calculating variance inflation factors. Residuals helps to assess model fit and to evaluate the unexplained variability in the data. A normal probability

plot was used to assess the normality of the variables. We found that SF was skewed to the left, and therefore categorized this variable.

In conclusion, the choice of method and its assumptions seems appropriate and contributes to good internal validity of the performed studies and its findings.

6. General discussion

This thesis adds important knowledge on the prevalence of anaemia and ID among pregnant and postpartum women in a multi-ethnic population in a Western European country, associations with ethnic origin, the use of iron supplements, the impact of our simple intervention, and factors associated with postpartum iron status and change in iron status.

When comparing the findings from our multi-ethnic, population-based study with high participation rates with other studies, we are primarily interested in studies representing other population-based samples. Further, when interpreting our findings, it is also important to be aware of the ethnic composition of the samples in other studies, as our study included 59% ethnic minority women. In this section, first we discuss our results regarding the overall prevalence of anaemia compared with other studies, and second, the prevalence of anaemia by ethnicity as reported by others. Thereafter, we follow the same order when discussing the prevalence of ID.

6.1 Anaemia

Anaemia in pregnancy

The overall prevalence of anaemia in early pregnancy (5.9%) in our study is low in a global context, however comparable to reports from other high-income countries. According to the WHO, prevalence rates between 5% and 20% indicate that anaemia is a mild public health problem in the population being studied [21].

According to GHO, the last updated (2019) prevalence of anaemia in pregnancy worldwide is 37% [22,131], in line with a systematic review and meta-analysed by Karami et al describing the overall prevalence based on 52 studies between 1991 and 2021 [44]. The prevalence of anaemia in pregnant women is by GHO estimated to 18% in Europe [132] and 12% in the US [133]. Karami et al. report substantially different prevalence rates between high-income countries (16%) and low- and middle-income countries (53%), and by trimester [44]. In the first trimester, prevalence rates range from 3% (USA) to 19% (Pakistan), and in the second trimester from 9% (Belgium) to 65% (Nigeria). The prevalence is affected by economic and sociological factors, as well as access to nourishing food and health care [44].

Masukume et al reported a prevalence of 2.2% of anaemia in pregnant women in gestational week 14-16 in a large prospective, multicentre cohort study from New Zealand, Australia, England and Ireland. However, included women were 'low risk' women primarily of European ancestry, and 37% reported use of iron supplementation from first trimester [134]. Barroso et al published a multicentre study from the UK, where the prevalence of anaemia in pregnant women (n=2103) in mean gestational week 12 was 7%, increasing to 19% and 15% in second and third trimester [135]. Vandevijvere et al performed a multistage proportionate-to-

size stratified sampling of pregnant women in gestational week 10 in Belgium, and reported a 4.2% prevalence of anaemia [136]. Massot et al. estimated the prevalence of anaemia to be 2.8% in first trimester in Belgium, by using already analysed blood samples in a laboratory matched with dates of birth, and calculating gestational age. In a Swizz prospective longitudinal study by Bencaiova et al, 6.5% had IDA in gestational week 16-20 [137]. A systematic review by Hercberg et al. from 2001, reported that the prevalence of IDA in Europe ranged from 6% to 30%, the highest levels in countries where routine iron supplementation was not recommended [138]. A Canadian retrospective cohort, using laboratory test records of female patients to detect positive β-HCG and measurement of Hb concentration, reported a 8% prevalence of anaemia (n= 33446, no information of gestational age) [139]. In the US, the Centre for Disease Control (CDC) conducts yearly population-based cross-sectional surveys in randomly selected residents, called The National Health and Nutrition Examination Survey (NHANES), providing epidemiologic data on different conditions. The crude prevalence rates on gestational anaemia were 5-9% in the periods 1999-2006 and 2003-2012 (both missing data on gestational age) [78,85].

To conclude, the prevalence rates differ between regions and populations [42,44], and increases with trimester [44,85,140]. They are also dependent on the study design and population. Our population-based study which included high-risk and hard-to-reach women often excluded in research, contribute with prevalence rates that are relevant in increasingly multi-ethnic societies.

Anaemia in the postpartum period

Our finding of a 25% overall prevalence rate of anaemia 14 weeks postpartum is in line with reports from GHO and WHO [22]. Prevalence rates between 20% and 40% indicate that anaemia is a moderate public health problem in the population being studied [21].

The WHO Guideline “Iron supplementation in postpartum women” highlights that limited data on the prevalence of postpartum anaemia exists, referring to five studies in high-income countries that estimate the prevalence of postpartum anaemia to be 10-30% [141]. Based on data on reproductive aged women, prevalence rates above 50% in low- and middle-income countries are presumed [141]. GHO has estimated the prevalence of anaemia in reproductive aged women to be 30% worldwide [22].

The majority of prevalence estimates for postpartum anaemia are based on measurements 24-48 hours after delivery, using Hb <10 g/dL to define postpartum anaemia, hampering direct comparisons with our result difficult. At a university hospital in Madrid, 29% of the women in a pre-selected group were detected with postpartum anaemia [142]. In a big

retrospective cohort from a university hospital in Berlin, with Hb measurement in 90% of all births between 1993 and 2008 (n=43807), 22% were detected with postpartum anaemia [143].

In two RCTs from Denmark, the prevalence of postpartum anaemia eight weeks postpartum was examined. One RCT reported that 8% had postpartum anaemia among iron treated women, and 16% among the placebo treated women [144]. In the second RCT, receiving different doses of iron supplements, the total prevalence of postpartum anaemia was 5.7% [145]. Milman suggested that anaemia should be defined by Hb <12.0 g/dL at 8 weeks postpartum based on the results of these two RCTs [28].

Bodnar et al used data from NHANES in the period 1988-1994 and estimated the crude prevalence of anaemia (Hb <12.0 g/dL) 0-6 months after delivery to be 10% [146]. The prevalence was 22% among low-income women (defined as below 130% of poverty) an 6% among women with higher income (defined as above 130% of poverty) (6%) [146]. The Special Supplemental Nutrition Program for Women, Infants and Children (WIC) is a federally funded program in the US that provides nutrition education, healthy food and support to low-income women and their offspring up to five years of age. WIC participants receive financial support assigned for the purchase of nutritious food. One criterion for being accepted into the program was to undergo blood testing. Bodnar et al. have used WIC data to conduct a retrospective cohort study, assessing the prevalence of anaemia (Hb <12.0 g/dL) among low-income women 4-26 weeks after delivery, and reported that the prevalence in the total sample ranged from 25 to 33%, with increasing prevalence rates with increasing weeks postpartum, reaching the highest levels at 12-18 weeks [129].

To conclude, direct comparison of prevalence rates is difficult as it depends on several factors, including the population being tested, thresholds used to define anaemia and number of weeks postpartum for the measurement. We have assessed the prevalence of anaemia 14 weeks after delivery in a multi-ethnic population-based cohort, with low use of iron supplements. Our overall prevalence rate is comparable to prevalence rates in low-income women living in the US [129,146]. We suspect that low iron supplement use in our population at least partly explain the significantly higher prevalence rates compared with those reported in the RCT's in Denmark and the US' NHANES survey [144-146].

Anaemia by ethnic origin

We found that the prevalence of anaemia differed substantially between ethnicities. The prevalence of anaemia in early pregnancy was five times higher in non-Western ethnic women (9%; range 7-14% between ethnic groups) compared with Western European women (1.8%). The prevalence of postpartum anaemia was more than doubled in women with ethnic minority origin compared to Western Europeans (14% vs 32%), and women with South Asian

ethnic origin had the highest prevalence (40%). The level of integration could not explain the differences we found between South Asians and the other ethnic minority groups.

Prevalence rates for gestational and postpartum anaemia in high-income countries stratified by ethnicity are scarcely reported. Our findings seem to reflect prevalence rates of anaemia by geographic region published by the WHO and GHO [131,147].The countries with the highest reported rates of anaemia are in the South Asian and Sub-Saharan African region, however, with differences between countries within these regions [147].

Table 10. GHO estimated the prevalence of anaemia in reproductive aged and pregnant women in the different regions (updated last 2019) to be the following (https://data.worldbank.org/) [131]

	Worldwide	Europe	United States	South Asia	Middle East	Sub-Saharan Africa	East Asia
Reproductive age women	30%	14%	12%	49%	31%	40%	19%
Pregnant women	37%	18%	12%	48%	31%	46%	27%

Our prevalence rates for postpartum anaemia by ethnic origin, seem to some extent reflect those reported in their country of origin [148], although the prevalence rates reported within South Asian countries differ considerably (23-62%) [42]. Both cultural practices and genetic factors must be considered when interpreting the prevalence rates of anaemia among different ethnic groups.

Nybo et al. found the prevalence of anaemia to be 20% among women from the Eastern Mediterranean, African and Asian regions (women were without hemoglobinopathy and in gestational week 16) living in Denmark [149]. In two publications using NHANES data, reporting crude prevalence rates for gestational anaemia (across trimesters) in the periods 1999-2006 and 2003-2012 by ethnicity, pregnant Non-Hispanic Black women had the highest proportions of anaemia (18-24%) and non-Hispanic white women the lowest (2.2-3.1%) [78,85]. Kanu et al. used 2008-2018 WIC data to assess the prevalence of anaemia among pregnant low-income women (across trimesters) [150]. They reported considerable variation by ethnicity, the highest rates of 21% were found in Non-Hispanic Black women, and approximately 6-11% in the other ethnicities [150]. Barroso et al reported that all non-White ethnicities in the UK had higher odds of early anaemia (measured before 32 weeks gestation) than Whites (odds ratios 1.37-2.89 by different non-White ethnic origin) [135].

We did not find any studies from Europe reporting prevalence rates for postpartum anaemia stratified by ethnic groups. In the US, Bodnar et al used NHANES data from 1988-1994 and WIC data from 1996 to assess prevalence of postpartum anaemia [129,146], and found that the prevalence of postpartum anaemia in non-White ethnicities (37-48%) were higher than in non-Western Europeans in our study (8-33%).

To conclude, even though direct comparisons between studies are difficult, anaemia is generally reported to be more prevalent in ethnic minorities than in the majority population. Our findings from a population-based cohort study contribute with data on scarcely reported prevalence rates of gestational and postpartum anaemia in women with different ethnic origin, which includes women considered to be at high-risk and hard-to-reach.

6.2 Iron deficiency

Iron deficiency in pregnancy and in the postpartum period

In our cohort with low use of iron supplementation, we found that every third women had ID by SF in early pregnancy (33%) and even more 14 weeks postpartum (39%).

A systematic review by Milman on iron status in pregnant women and reproductive age women in Europe, concluded that 10-32% had ID by SF depending on the thresholds used [151]. We used SF <15 µg/L as the threshold to define ID in early pregnancy. We found three European studies using this threshold. A Belgium population-based study by Vandevijvere et al., reported that 6% had ID, and in a Swiss prospective longitudinal study by Bencaiova et al., 22% were detected with ID in gestational week 16 [152]. Lastly, in a UK multicentre study, 23% had ID in first trimester [135].

Other studies have used SF <12 µg/L as threshold to define ID. In one Swizz population-based study by and one German population-based study, the prevalence of ID was 19% [153,154]. In one US survey using NHANES data (1999-2006), the crude prevalence rate on gestational ID was 7% in the first and 24% in the second trimester. In an Australian Cohort study, the prevalence of ID was 20% in first trimester [85,155]. In a Canadian retrospective cohort, 24% of pregnant women had ID by SF [139]. Limitations in some of these studies are uncertainty on trimester and lack of demographic variables including ethnicity and socioeconomic position.

To the best of our knowledge, our study is the first population-based multi-ethnic study on postpartum ID. When we used SF <12 µg/L as threshold to define postpartum ID (instead of <15 µg/L), the prevalence of ID was reduced from 39% to 29% (see appendix, Supplemental Table 1 Paper II). Bodnar et al. used the NHANES to estimate the crude prevalence of ID in women who reported to have had a childbirth the last 24 months. ID was in this study defined as abnormal values for ≥2 of 3 iron indicators (SF, free erythrocyte protoporphyrin, and transferrin saturation). They found the prevalence of postpartum ID to be 6.9% and 30% in high-income and low-income women respectively (above or below 130% of poverty women) **[156]**. Using the same material and definition, Bodnar reported the prevalence rates of ID for women 0-6, 7-12, and 13-24 months postpartum to be 12.7, 12.4 and 7.8%, respectively [156].

In our supplementary subgroup analyses after having excluded 75 women with elevated trimester-specific CRP in early pregnancy (n=717), the prevalence rates remained relatively unchanged (see appendix, Supplemental Table 1 Paper I). We observed the same in the supplementary analyses of ID postpartum. The BRINDA (Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia) database and the VMNIS (Vitamin and Mineral Nutrition Information System) database contains data on vitamin and mineral deficiencies of populations around the world. The data from these databases provide prevalence rates of ID and on inflammation-adjusted ID. One publication using these databases, reported various prevalence rates on ID (SF <12 µg/L) in reproductive age women in different regions of the world (1.3%-41%; inflammation-adjusted 1.4%-56%) [157]. The following three publication used the BRINDA database to assess the prevalence rates on ID and inflammation-adjusted ID by SF, sTfR and TBI in reproductive age women and in pre-school children. Namaste et al. found 13-20% of women in this age to have ID by SF in the African region [33], and that the inflammation-adjusted prevalence rates ID were higher (particularly in children and not so much in reproductive age women). Rohner et al. found that 22-32% of such women had ID by sTfR in Africa, and 5-15% in East Asia, and the inflammation-adjusted prevalence rates were relatively unchanged, but not consistently across surveys [108]. Mei et al. and found that 13-20% of reproductive age women had ID by TBI in the African region, with a higher prevalence rate after inflammation-adjustment in areas with high prevalence of inflammation or infections (particularly in children and not so much in reproductive age women) [109]. In summary, in areas with high prevalence of inflammation, prevalence rates were underestimated if it is not adjusted by inflammation.

To conclude, the prevalence rates of ID by SF in early pregnancy and postpartum were higher in our study compared to other comparable studies, also in the subsample excluding women with elevated CRP in early pregnancy and postpartum, and in analyses using SF <12 µg/L to define ID postpartum. In addition to the ethnic composition of our study sample with 59% ethnic minority women, our findings might also reflect the low proportion of women using iron supplements.

Iron deficiency in pregnancy and postpartum by sTfR and TBI

Overall, in our cohort we found 6.5% to have ID by sTfR in early pregnancy and 19% postpartum. The distribution of TBI in early pregnancy (presented as grouped at the midpoint), was shifted towards lower TBI-concentration in the non-Western groups compared with Western Europeans (Figure 1 Paper I). When using TBI to define ID, we found 11% to have ID in early pregnancy and 22% at the postpartum visit.

sTfR is not routinely recommended as part of the standard antenatal screening tests for iron deficiency anaemia in the US or in European countries. sTfR measurements may be useful in

some situations, such as when the diagnosis of ID is uncertain and in populations where inflammation is common [108]. TBI is not a commonly used test in clinical practice, however, is suggested to be used for research purposes or in certain clinical situations [109]. Few population-based studies have used sTfR and TBI values to assess ID in pregnant women. An assessment of iron status in pregnant women in the US using 1999-2006 NHANES data, found that the crude prevalence of gestational ID by sTfR was 6% in the first and 11% in the second trimester. In the same survey, ID by TBI <0 mg/kg was 7% and 14% in the first and the second trimester respectively [85]. Khambalia et al. reported prevalence rates of ID by sTfR and TBI in an Australian cohort study to be 15.3% by sTfR and 15.7% by TBI in the first trimester [155]. A Belgian study found 4% of pregnant women to have ID across trimesters, 0.5% in the first and 6.8% in the third trimester [136].

The iron indicators seem to identify different stages or forms of ID. In our total sample, we observed two-five times lower prevalence rates of ID by sTfR compared to ID by SF. Further the Hb concentration was lower in participants with ID by sTfR (Hb 11.6) compared to ID by SF (Hb 12.6). This indicates that increasing sTfR concentration reflects a more severe stage of ID, being a biomarker that is associated with iron-deficient erythropoiesis [120].

To the best of our knowledge, this is the first population-based multi-ethnic study on postpartum ID. We have not found other studies assessing postpartum iron status defined by sTfR or TBI. Comparison of prevalence rates are hampered by different use of assays to determine sTfR concentrations. Our findings are similar to the NHANES data (used Roche assay for sTfR analyses, identic to our assay). Our prevalence rates are, however, lower compared to the Australian data (used Quantikine assay) and higher compared to the Belgian data (used Ramco assay). Our results show that the prevalence of ID differs depending on the iron indicators being used and their thresholds, as well as different assays used.

Iron deficiency by ethnic origin

ID is frequent in populations with low socioeconomic position (affordable diet being iron-poor, and with poorer health), and among vegetarians/vegans (iron-poor diet) [21], and if the diet contains compounds that inhibit the absorption of iron [13]. The prevalence estimates of ID in low- and middle-income countries are uncertain because of the high proportion of inflammatory conditions [158].

Only a few population-based studies have assessed the iron status in pregnant women with different ethnic backgrounds, and various iron indicators have been used. Therefore, to ease the comparison with other studies, we provide additional data on the mean/median values of

Hb and SF value in early pregnancy, and prevalence of gestational IDA (anaemia and SF <15 µg/L) by ethnicity (Table 11).

Table 11. Mean Hb and median SF concentration in early pregnancy, and prevalence of gestational IDA (anaemia and SF <15 µg/L) in STORK G

	Hb, mean (SD)	SF median (IQR)	IDA [1] (%)
Western Europe	12.3 (0.9)	31 (18-54)	0.6%
South Asia	11.7 (1.0)	14.5 (9-25)	8.5%
Middle East	11.9 (0.9)	17 (9.5-30)	5.6%
Sub-Saharan Africa	11.7 (1.0)	12.5 (7.5-26)	12%
East Asia	11.9 (0.9)	28.5 (13-90)	4.5%
Eastern Europe	12.4 (0.8)	16 (13-40)	0%

In our cohort, the median SF concentration in early pregnancy was 21 µg/L and the crude prevalence of ID by SF <15 µg/L was 33% (15% in Western Europeans and 27-55% in non-Westerners). The ethnic disparities in iron status in Table 11, are consistent with our findings on the ethnic differences in prevalence of ID and anaemia (Paper I).

In a Dutch cross-sectional, population-based study assessing iron status in gestational week 13 in different ethnic groups, the median SF concentration was 61 µg/L in Dutch women, and 32-60 µg/L in women with migrant background **[159]**. Further, the overall prevalence of ID by SF <15 µg/L was 7%, and the highest prevalence rates were observed in the Turkish, Moroccan and Surinamese-Hindustani (ethnic South-Asian) groups (around 18%), and the lowest in Dutch women (3.5%) **[159]**. In a Belgian study on gestational iron status, the median SF concentration was 48 µg/L, and women from the African region had lower SF and higher sTfR concentration compared to Dutch women [136]. In general, the iron status reported in these two studies are better compared to our findings, which probably reflects the difference in use of supplements.

In a Swiss prospective, longitudinal study with a high proportion of immigrant women, IDA was detected in 1.3% Western Europeans and in 7.7% of women with origin from the African, Asian, Middle Eastern and South American region [152], comparable to our findings. A Danish study assessing iron status in pregnant women with origin from Eastern Mediterranean, African and Asian regions, the median SF concentration was 16 µg/L, also comparable to our results.

Our results are in line with the results in other European studies, women migrated to Western Europe from non-Europeans countries are generally more iron deficient compared to the majority population. Our findings identified ethnic minority women as at-risk women, having higher crude prevalence of ID compared to Western European. Women of South-Asian origin had consistently higher risk of ID and anaemia in early pregnancy and postpartum, and

compared to other ethnic groups, they had higher odds for “steady low” iron status despite recommendations on supplements. Our supplementary analyses showed that the level of integration could not explain the differences we found between South Asians and the other ethnic groups.

To conclude, ID is more prevalent in ethnic minorities. Our findings provide important new data on iron status in pregnancy and postpartum, including high-risk and hard-to-reach women, that previously has been underexplored in the literature.

6.3 Factors associated with anaemia and iron deficiency, and with change in iron status from early pregnancy to postpartum

In early pregnancy, the overall prevalence of anaemia was 5.9%. As previously described, WHO estimates that half of the cases of anaemia are caused by ID (see section 1.4). In early pregnancy, we found that 6.5-33 % had ID by the different iron indicators.

Several factors can increase the risk of developing postpartum anaemia and ID. Women who experience significant blood loss during delivery, have ID or anaemia in pregnancy, poor nutrition (e.g. poverty, vegetarian/vegan diet), or pre-existing medical conditions, and women who undergo Caesarean section are more likely to develop postpartum anaemia [21,29,141]. Our findings on factors associated with adverse postpartum anaemia are consistent with these described risk factors, except we did not find operative delivery associated with postpartum anaemia.

High maternal age, obesity and primiparity are associated with postpartum haemorrhage and may contribute to postpartum anaemia and ID [75,160]. Advanced maternal age is associated with postpartum anaemia, especially women above the age of 35, may be more disposed to pre-existing medical conditions, pregnancy related conditions, higher rates of operative assisted and complicated births [161]. We found higher age to be associated with higher postpartum Hb concentration. The age range in our cohort was 19-42 years, one percent being teenagers and 14.5% being above 35 years of age. We can only speculate whether this age effect may be related to physiological or to unmeasured socioeconomic or nutritional factors. Primiparity is a risk factor for postpartum anaemia because first-time mothers are more likely to experience complications during delivery, including haemorrhage [162], which can lead to anaemia. On the other hand, multiparity is a risk factor for postpartum anaemia due to the cumulative effect of multiple pregnancies on iron stores [75]. With each pregnancy, the total demand for iron is increased, and if iron intake is inadequate, iron stores can become depleted, leading to anaemia. Multiparous women may also have pre-existing conditions or damages from previous childbirths that may lead to excessive bleeding during delivery, which can contribute to anaemia. We found primiparity associated with adverse postpartum iron

status development and being multipara (≥1 previous births) to be associated with improvement in iron status from early pregnancy to postpartum, which supports findings presented from other studies. In our cohort, few women with multiple births (>3) previous participated. Lack of time because of care of small children was a frequently used reason for declining participation in STORK G [83]. Our results may therefore have changed somewhat if we had more multipara women in our cohort. However, we adjusted for ethnicity, age, BMI, birth complications and haemorrhage, and found that it explained only some of the differences.

Socioeconomic position is a well-known determinant of health, and is through several pathways associated with anaemia. First, a limited access to nutritious foods can lead to nutritional deficiencies (including iron, folate, and vitamin B12) and nutritional anaemias [21]. Second, poor sanitation and hygiene can increase the risk of infectious diseases associated with anaemia (e.g. malaria, hookworm, and schistosomiasis - all rare conditions in Western Europe) [21]. An association between low socioeconomic position background and anaemia is observed both in low-, middle- and high-income countries [21,156]. Interestingly, we observed that women with a high early life socioeconomic position had higher Hb concentrations after delivery, also when adjusting for other factors. This is a finding that needs further investigation and to be re-examined in another population to clarify whether this is a random finding or a result, for example, of epigenetic downregulated or upregulated expression of genes that regulate the iron metabolism.

Other important factors that can result in anaemia are haemoglobinopathies, other nutritional deficiencies (e.g. folate and vitamin B12), and infectious diseases (e.g. malaria, tuberculosis, HIV and parasitic infections) [147]. We detected only three participants with haemoglobinopathies via laboratory analysis or by self-reporting. In the follow-up study (STORK G 2 initiated in 2019), a fourth participant with thalassemia was discovered. Of these four, two had anaemia. The number of participants with haemoglobinopathies might be underestimated because we did not run specific tests for these conditions. However, another Norwegian study found that 35% of the patients with a ‘foreign name’ and MCV value <70 fL had a heterozygous haemoglobinopathy, mainly β-thalassemia minor. If we apply the use of ‘foreign name’ and MCV value <70 fL (n=8, all non-Wester women) to estimate the number of participants with haemoglobinopathy, this would result in three participants having haemoglobinopathies.

The factor with the highest odds of “steady low” and “deterioration” iron status was postpartum haemorrhage, while the factor with the highest odds of “improvement” iron status was use of supplements.

6.4 The impact of our simple intervention of recommending iron supplements to women with depleted iron stores

Most antenatal care guidelines recommend routine screening for anaemia in pregnancy, however, recommendations for routine ID screening and iron supplements are variable [163]. The guidelines in the UK and Germany recommend routine screening of Hb concentration, and iron supplements to women diagnosed with anaemia [48,164], however, with additional empirical iron supplementation and/or screening to high-risk women. The guidelines in the US, Canada and Denmark recommend a daily iron supplementation dose to all pregnant women throughout pregnancy [68,69,73,165]. In Norway, routine screening for SF in addition to Hb are now recommended, and women with SF concentration below 70 µg/L are recommended supplements [71]. According to the current Norwegian guidelines, in retrospect 89% of the participants in our study should have been recommended supplementation.

A Cochrane review by Peña-Rosas et al. concludes that low-dose supplementation of iron during pregnancy improves maternal hematologic parameters and reduces the likelihood of ID at term [166]. Use of supplements in gestational week 28 was associated lower odds of ID and with improved iron status by SF, sTfR, and TBI concentration. Use of supplements in gestational week 28 was also associated higher Hb concentration, however, not with anaemia.

We observed an increase in supplement use among women that were actively recommended supplements according to the study protocol (from 25% to 65%). The change in iron status by SF concentration from early pregnancy until postpartum was illustrated in Figure 1 in Paper III, with an improvement of SF concentration among women recommended supplements and a decrease in women not exposed to this recommendation. Table 12 shows the results from our study of additional analyses on change by sTfR, TBI and Hb, being consistent and in line with our findings reported on change in SF concentration.

Table 12. Change in SF, sTfR, TBI and Hb concentration from early pregnancy to postpartum in women with different SF concentration at enrolment (STORK G)

	Groups derived from the SF concentration at enrolment in early pregnancy				
	SF <20 µg/L[a]	SF 20-29 µg/L	SF 30-49 µg/L	SF 50-69 µg/L	SF >70 µg/L
Delta SF	11 µg/L	- 5 µg/L	- 12 µg/L	- 37 µg/L	- 74 µg/L
Delta sTfR	0.8 mg/L	1.4 mg/L	1.4 mg/L	1.4 mg/L	1.5 mg/L
Delta TBI	0.9 mg/kg	-3.2 mg/kg	- 4.0 mg/kg	- 6.0 mg/kg	- 6.7 mg/kg
Delta Hb	0.7 g/dL	0.4 g/dL	0.4 g/dL	0.4 g/dL	0.5 g/dL

[a] Exposed to our simple intervention (recommendation)

Among women not exposed to our recommendation, the sTfR concentration increased twice as much compared to the intervention group. Further, TBI was reduced as SF, and lastly Hb concentration improved less compared to the group exposed to our recommendation. On the other hand, women with SF <20 µg/L at enrolment and being recommended supplements, still had the highest prevalence rates of postpartum anaemia (31%) and ID (25-44% by the different indicators) (see appendix Supplemental Table 1 Paper III).

Bothwell et al. have illustrated the progressive increase of iron requirements throughout the pregnancy [20], and Fenton et al. that supplementation improve the SF concentration [167]. Our findings indicate the need to investigate the optimal management of iron supplementation in pregnancy, including methods to ensure that recommendations are being followed.

Our findings showed that routine screening of SF detects suboptimal iron stores. The VENN diagram showed that 6.3% had ID by sTfR, without at the same time having ID by SF, indicating that SF is a reliable measurement in our population. Further, the prevalence of ID by SF was unchanged in the subsample excluding women with elevated CRP. WHO suggests to define ID in pregnancy as SF <30 µg/L [21], and to combine SF and sTfR measurements when studying iron status in populations [21].

Our findings support the current Norwegian guideline to offer routine screening with SF to all pregnant women. However, given the associated costs and the limitations of SF measurement in co-occurrence with inflammation or infection, universal screening may not be recommended in all populations. Clinical guidelines do not recommend universal screening of Hb concentration shortly after delivery or at a later postpartum visit [26]. Most guidelines recommend selective Hb screening in cases with haemorrhage and symptoms of anaemia [48,116,117,168], however, the estimation of blood loss is often inaccurate, and symptoms of anaemia are unspecific.

For most women, there is a general agreement that dietary iron alone will not ensure sufficient iron intake during pregnancy [9]. Our cohort offered a unique opportunity for studying ethnic differences in iron status in women in early pregnancy with different dietary habits and low use of iron supplements. In total, 44% of participating women were exposed to a simple intervention (recommendation), in a setting comprising free antenatal health care and wide availability of iron-rich food.

To conclude, we found routine screening with SF useful to detect depleted iron stores and ID. The use of supplements increased in women being exposed to our recommendations. We found that iron supplements improved iron status in pregnant women until postpartum, and reduced the odds of having postpartum ID. The prevalence of postpartum anaemia and ID remained high in the group with depleted iron stores in early pregnancy. In our study sample

with low use of supplements, a high prevalence of postpartum anaemia was detected. We observed an adverse development in iron stores in women not recommended supplements. This implies the need to investigate the optimal management of postpartum anaemia and ID, including to assess whether universal postpartum iron status screening is beneficial.

7. Ethical considerations

The Regional Committee for Medical and Health Research Ethics for South-Eastern Norway (REK 2007/894 and REK 2015/1035C) and the Norwegian Data Inspectorate approved the study protocol. Participation was based on informed, written consent from each woman on behalf of herself and her offspring. The study was conducted in agreement with the Helsinki Declaration. Personal identifying information was removed from all data prior to analysis. To access the STORK G database, the researchers have to go through several security steps.

There are several ethical issues to consider when designing a study. The use of professional interpreters and translated material also helped participants with linguistic challenges to understand all the information they needed to decide whether to participate. Further, we suspect that the three extra ultrasound measurements during pregnancy that participants were provided contributed to accepting the invitation for some women.

For ethical reasons, we decided to inform participants of their iron status by predetermined low Hb and SF concentrations (see appendix, Letters with recommendations). In cases of severe deviations, we also contacted their GP to ensure proper diagnostics and treatment.

In 2018 we sent a letter to all study participants with information about the main results that had been published from STORK G so far, the planned follow-up study (STORK G 2) and about the STORK G website where they could find more information.

8. Concluding remarks

When investigating iron status in a pregnant multi-ethnic population from Oslo, Norway in 2008-2011 we found that the crude prevalence of ID was high in early pregnancy (33%) and postpartum (39%). The prevalence of postpartum anaemia was high (25%), while few had anaemia in early pregnancy. Women with ethnic origin from South-Asia, the Middle East and Sub-Saharan Africa had an overall poorer iron status, and higher odds of having ID and anaemia in early pregnancy and postpartum compared to Western Europeans.

Ethnic minority background, ID and anaemia early in pregnancy, unhealthy dietary pattern and no use of iron supplements, primiparity, and haemorrhage were identified as factors associated with postpartum ID and anaemia.

The prevalence of ID differed considerably depending on which of the three iron indicators used (SF, sTfR, or TBI). While ID by SF seems to reflect iron depletion, ID by sTfR seems to reflect a more severe stage of ID associated with reduced Hb concentration.

Ethnicity, iron intake (diet/supplement), parity and haemorrhage were identified as factors associated with changes in iron status from early pregnancy to postpartum ("steady low", "improvement", and "deterioration" of iron status). Two thirds of women recommended iron supplements on the basis of iron depletion, reported use of supplements later in pregnancy, compared to one in four in the group not recommended supplementation

Our findings indicate that ID and anaemia in pregnancy and postpartum still represent considerable public health problems in our population, providing support to the current Norwegian antenatal guideline on routine screening of both Hb and SF concentration early in pregnancy. Further research is needed to assess the optimal treatment for women identified with ID and anaemia early in pregnancy, including methods to ensure the treatment is being followed.

Overall, this thesis provides valuable information for health care personnel. By following a large group of pregnant women until 14 weeks postpartum, risk factors and protective factors for maternal iron status were identified. This information can be used by policymakers for the development of evidence-based guidelines for antenatal and postpartum care, and screening procedures. The results may have a significant impact on patient care. At-risk women seem to need more targeted and culturally sensitive information during pregnancy in their own language, and adequate screening, treatment and follow-up to reduce the high prevalence of ID and anaemia during pregnancy and postpartum.

Future perspectives

Thresholds to define anaemia and ID by ethnic origin and during pregnancies, the prevention, treatment and optimal management of gestational and postpartum anaemia and ID are still considered unresolved controversies in the field. WHO is currently reviewing the evidence for thresholds used to define anaemia, including the need to adapt thresholds to different populations [114].

Blood tests should be taken early in pregnancy, preferably in first trimester, to be interpreted correctly. sTfR may be helpful in contexts and clinical situations when SF is less reliable.

More research is particularly needed to:

- Develop and validate reliable screening methods for identifying ID in pregnant women.
- Develop a standardized (and less expensive) assay for determining sTfR concentrations, and validating a standardized assay for sTfR measurement in pregnancy.
- Investigate the optimal management on iron supplementation during pregnancy, including methods to ensure that recommendations are followed.
- Investigate the optimal management on postpartum iron status

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Appendix

Online Supporting Material (Paper I)

Supplemental Table 1 Paper I

Sensitivity analyses in sample excluding women with elevated CRP concentration [1]; values for serum ferritin, serum sTfR, total body iron (calculated from serum Ferritin and sTfR concentrations), and 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	717	32.8 ± 33.5	2.5 ± 1.2	4.9 ± 4.0	12.1 ± 1.0
Median (25th and 75th percentile)	717	21 (12, 40)	2.2 (1.8, 2.8)	4.9 (2.2, 7.7)	12.1 (11.4, 12.7)
Prevalence of abnormal values [2]					
Total sample	717	33 (29, 36)	5.9 (4.5 ,7.8)	11 (8.9, 14)	5.7 (4.2, 7,7)
By ethnic groups [3]					
Western Europe	304	15 (12, 20)	0.0	0.7 (0.2, 2.6)	2.0 (0.9 - 4.3)
South Asia	169	52 (44, 60)**	12 (8.2, 18)**	21 (16, 29)**	10 (6.3, 16)**
Middle East	112	43 (34, 52)**	9.8 (5.5, 17)**	16 (10, 24)**	6.2 (3.0, 13)**
Sub-Saharan Africa	47	53 (39, 67)**	19 (10, 33)**	32 (20, 47)**	17 (8.5, 31)**
East Asia	44	28 (16, 44)*	2.3 (0.3, 16)**	9.3 (3.4, 23)**	7.0 (2.2, 20)*
Eastern Europe	42	36 (22, 52)**	0	7.1 (2.2, 21)**	0

[1] Trimester-specific CRP cutoffs; 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

[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 <11.0 g/dL for pregnant women in the first trimester and <10.5 g/dL in the second trimester. *P < 0.05, **P < 0.01, chi-square with Western Europeans as reference

[3] 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: CI, confidence interval; CRP, C-reactive protein; n; number of observations, sTfR, soluble transferrin receptor.

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.

Online Supporting Material (Paper II)
Supplemental Table 1 Paper II

Sensitivity analyses of the prevalence of iron deficiency by serum ferritin (SF) <15 µg/L and serum ferritin (SF) <12 µg/L 14 weeks postpartum in the STORK-Groruddalen study, and the relative change of prevalence of iron deficiency when the lower thresholds for serum ferritin is chosen [1]

	n	SF <15 µg/L %	SF <12 µg/L %	Relative change %
Total sample	573	39	29	-26
Ethnic group				
Western Europe	217	35	24	-31
South Asia	157	44	35	-20
Middle East	94	39	30	-23
Sub-Saharan Africa	38	50	40	-20
East Asia	33	36	24	-33
Eastern Europe	34	32	29	-9

[1] The STORK-Groruddalen multi-ethnic pregnancy cohort from Oslo, Norway, 2008 – 2010.

Online Supporting Material (Paper II)
Supplemental Table 2 Paper II

Multivariable regression analyses exploring the potential effect of participants' level of integration as an alternative to ethnicity. Logistic regression analysis of SF <15 µg/L, and linear regression analyses of sTfR, TBI, and Hb concentration 14 weeks postpartum in the STORK-Groruddalen study [1]

	SF <15 µg/dL		sTfR, *mg/L*		TBI, *mg/kg*		Hb, *g/dL*	
	adj OR	95% CI	adj β	95% CI	adj β	95% CI	adj β	95% CI
	R^2 = 0.13			R^2 = 0.12	R^2 = 0.15		R^2 = 0.12	
Low social integration (high = reference) [2]	1.5	1.0, 2.2	0.02	-0.3, 0.3	-0.7	-1.4, -0.1 *	-0.1	-0.3, 0.1
Postpartum week			-0.1	-0.1, -0.02 **				
Age, *per 5 years*					0.5	0.2, 0.9 *	0.1	0.02, 0.2 *
Multiparous (primiparous = reference)	0.5	0.4, 0.8 **	-0.3	-0.6, -0.01 *	0.7	-0.1, 1.4	-0.2	-0.4, 0.0 *
Early life socioeconomic position [3]							0.2	0.1, 0.3 **
Gestational ID or anaemia (no = ref) [4]	1.5	0.9, 2.5	1.9	1.3, 2.6 **	-2.7	-3.7, -0.6 **	-1.0	-1.4, -0.6 **
Iron supplementation use in GW 28 (no = reference) [5]	0.5	0.3, 0.8 **	-0.5	-0.8, -0.2 **	1.2	0.6, 1.8 **	0.1	-0.03, 0.3
Dietary pattern (healthy = reference) [6]	1.6	1.1, 2.5 *	0.7	0.4, 1.1 **	-1.7	-3.7, -1.8 **	-0.1	-1.4, -0.7
Chronic illness/medication associated with normochromic anaemia (no = reference) [7]							-0.8	-1.4, -0.3 **
Chronic illness/medication associated with hypochromic anaemia (no = reference) [8]							-0.05	-0.3, 0.2
Postpartum haemorrhage (<500 mL = reference) [9]	3.1	1.4, 6.7 **	0.2 (-0.4, 0.8)		-1.9	-3.1, -0.6	-0.3	-0.7, 0.003 *

Adj, adjusted; GW, gestational week; Hb, haemoglobin; ID, iron deficiency; SEP, socioeconomic position; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI; total body iron.

[1] The STORK-Groruddalen multi-ethnic pregnancy cohort from Oslo, Norway, 2008 – 2010. Only adjusted values presented.
[2] Variable derived from a principal component analysis of predefined markers reflecting integration such as language skills, time of residence, social interaction with ethnic Norwegians and use of Norwegian media, with a higher score reflect higher social integration. "Low social integration" represents participants belonging to the 40% with the lowest scores.
[3] Variable derived from a principal component analysis of three childhood sociodemographic variables representing maternal SEP at age 10 years, with a higher score reflecting higher socioeconomic position.

[4] Gestational iron deficiency by 1) SF <15 µg/L; 2) sTfR >4.4 mg/L, or 3) TBI <0 mg/kg; and gestational anaemia by trimester-specific haemoglobin < 10.5 or 11.0 g/dL, analysed in mean gestational week 15.1.
[5] Self-reported intake of iron supplements during the past two weeks in GW 28.
[6] Data from food frequency questionnaires collected in GW 28; four clusters were extracted using the Ward's method. Clusters were referred to as "a healthier dietary pattern" vs. three "less healthy dietary patterns"; here dichotomized into "healthy" and "unhealthy" dietary pattern.
[7] Self-reported chronic illness or medication associated with normochromic anaemia (i.e. kidney or rheumatic disease, use of carbamazepine or infliximab).
[8] Self-reported chronic illness or medication associated with ID and hypochromic anaemia (i.e. gastrointestinal disease or Copper intrauterine device use before conception).
[9] Excessive blood loss (≥500 mL) after delivery.
* $P<0.05$, ** $P<0.01$.

Online Supporting Material (Paper II)
Supplemental Table 3 Paper II

Logistic regression analysis of serum ferritin <15 µg/L, and linear regression analyses of soluble transferrin receptor, total body iron, and haemoglobin concentration 14 weeks postpartum in a sub-sample of non-Western women in the STORK-Groruddalen study [1]

	SF <15 µg/dL				sTfR, *mg/L*				TBI, *mg/kg*				Hb, *g/dL*			
	OR	95% CI	adj OR	95% CI	β	95% CI	adj β	95% CI	β	95% CI	adj β	95% CI	β	95% CI	adj β	95% CI
			R^2=0.12				R^2=0.12					R^2=0.16				R^2 = 0.15
Non-Western ethnic origin except South Asians [1]																
South Asia	1.2	0.8, 1.8	1.2	0.7, 1.9	0.5	0.1, 1.0 **	0.5	-0.9, -0.1 *	-0.7	-1.5, -0.1	-0.5	-0.3, -1.4	0.5	-0.7, 0.3 **	0.5	-0.7, 0.3 **
Postpartum week	1.0	0.9, 1.0			-0.1	-0.1, -0.01 *	-0.1	-0.2, -0.001	0.1	-0.04, 0.2			0.02	-0.01, 0.05		
Age, *per 5 years*	0.8	0.7, 1.0 *			-0.2	-0.4, -0.1 **			0.7	0.4, 1.0 **	0.5	0.1, 1.0 *	0.1	-0.01, 0.02	0.1	-0.03, 0.2
Multiparous (primiparous = reference)	0.6	0.4, 0.8 **	0.6	0.4, 0.9 *	-0.2	-0.5, 0.1	-0.4	0.8, -0.03	-0.1	-0.4, 0.2	1.0	0.1, 1.9*	-0.03	-0.1, 0.1	-0.2	-0.4, 0.1
Pre-pregnant Body Mass Index, *per 5 kg/m²*	1.0	0.8, 1.2			0.2	0.03, 0.3 *			-0,1	-0.4, 0.4			0.03	-0.1, 0.1		
Adult socioeconomic position [2]	0.8	0.7, 0.9 *			-0.3	-0.4, 0.1 **			0.6	0.3, 0.9 **			0.2	0.1, 0.2 **		
Social integration [3]	1.0	0.9, 1.2	1.2	1.0, 1.5	-0.2	-0.3, -0.01 *	0.03	-0.2, 0.2	0.2	-0.2, 0.5	-0.2	-0.6, 0.1	0.1	0.03, 0.2 **	0.05	-0.05, 0.1
Early life socioeconomic position [4]	0.9	0.7, 1.0			-0.2	-0.4, -0.1 **			0.3	0.01, 0.6 *			0.2	0.1, 0.3 **	0.1	0.01, 0.3 *
Gestational ID or anaemia (no = reference) [5]	1.3	0.9, 1.8	1.5	0.9, 2.4	2.0	1.4, 2.6 **	1.8	1.0, 2.5 **	-2.5	-3.4, -1.5 **	-2.6	-3.8, -1.3 **	-0.9	-1.3, -0.6 **	-1.0	-1.4, -0.7 **
Iron supplementation use in GW 28 (no = reference) [6]	0.6	0.4, 0.9 *	0.6	0.4, 1.0 *	-0.3	-0.6, -0.004 *	-0.7	-1.2, -0.3 **	0.8	0.1, 1.4 *	1.5	0.7, 2.3 **	-0.002	-0.2, 0.2	0.2	-0.04, 0.4
Dietary pattern (healthy = reference) [7]	2.2	1.5, 3.3 **	5.2	2.1, 12.7 **	0.6	0.3, 1.0 **	0.9	0.3, 1.6 **	-1.5	-2.2, -0.8 **	-2.7	-2.1, -0.5 **	-0.2	-0.4, -0.1 **	-0.1	-0.4, 0.2
Chronic illness/medication associated with normochromic anaemia (no = reference) [8]	1.5	0.5, 4.4			0.8	-0.1, 1.7			-0.1	-2.0, 1.9			-0.8	-1.3, -0.2 **	-1.0	-1.7, -0.4 **
Chronic illness/medication associated with hypochromic anaemia (no = reference) [9]	0.7	0.4, 1.3			-0.2	-0.7, 0.3			0.4	-0.7, 1.4			-0.1	-0.3, 0.2	0.1	-0.3, 0.5
Operative delivery (no = reference) [10]	1.3	0.9, 1.9			-0.03	-0.3, 0.3			-0.5	-1.2, 0.1			0.01	-0.2, 0.2		
Postpartum haemorrhage (<500 mL = reference)	2.9	1.4, 6.0 **	4.0	1.3, 12.8 *	0.3	-0.3, 0.9	0.3	-0.6, 1.3	-1.6	-2.9, -0.3 *	-2.1	-3.9, -0.2 *	-0.2	-0.5, 0.2	-0.4	-0.9, -0.05
Birth complications (no = reference) [11]	1.4	0.9, 2.2			0.07	-0.3, 0.5			-0.8	-1.6, -0.04 *			-0.1	-0.3, 0.2		

Adj, adjusted, GW, gestational week; Hb, haemoglobin; ID, iron deficiency; SEP, socioeconomic position; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI; total body iron.

[1] The STORK-Groruddalen multi-ethnic pregnancy cohort from Oslo, Norway, 2008 – 2010. Multivariable regression analyses exploring the potential effect of participants' socioeconomic position and integration. Western Europeans were excluded from this model. Non-Western women were stratified into South Asian and other ethnic minority origin.
[2] Variable derived from a principal component analysis of predefined individual and household markers of socioeconomic position (SEP), with a higher score reflect higher SEP.
[3] Variable derived from a principal component analysis of predefined markers reflecting integration such as language skills, time of residence, social interaction with ethnic Norwegians and use of Norwegian media, with a higher score reflect higher social integration.
[4] Variable derived from a principal component analysis of three childhood sociodemographic variables representing maternal SEP at age 10 years, with a higher score reflecting higher SEP.
[5] Gestational iron deficiency by 1) SF <15 µg/L; 2) sTfR >4.4 mg/L, or 3) TBI <0 mg/kg; and gestational anaemia by trimester-specific haemoglobin < 10.5 or 11.0 g/dL, analysed in mean gestational week 15.1.
[6] Self-reported intake of iron supplements during the past two weeks at all three study visits dichotomized into "yes", covering daily or intermittent iron supplements, and "no".
[7] Data from food frequency questionnaires collected in GW 28; four clusters were extracted using the Ward's method. Clusters were referred to as "a healthier dietary pattern" vs. three "less healthy dietary patterns"; here dichotomized into "healthy" and "unhealthy" dietary pattern.
[8] Self-reported chronic illness or medication associated with normochromic anaemia (i.e. kidney or rheumatic disease, use of carbamazepine or infliximab).
[9] Self-reported chronic illness or medication associated with ID and hypochromic anaemia (i.e. gastrointestinal disease or Copper intrauterine device use before conception).
[10] Operative delivery: Caesarean section (elective and emergency) or assisted vaginal delivery (forceps or vacuum). Normal vaginal delivery reference.
[11] A composite variable created by combining following four birth complication; episiotomy, third- and fourth degree perineal tear, obstructed labor and manual removal of placenta.
* $P<0.05$, ** $P<0.01$.

Online Supporting Material (**Paper III**)
Supplemental Table 1 Paper III

Average SF, sTfR, TBI and Hb concentration in early pregnancy and postpartum, and prevalence of postpartum iron deficiency/anaemia in participants with different SF concentration in early pregnancy [1]

	Total	SF < 20 µg/L in early pregnancy	SF ≥ 20 µg/L in early pregnancy		
	n = 573	n = 252 (44)	n = 106	n = 106	n = 46
	n = 573	SF < 20 µg/l n = 252 (44)	SF 20-29 µg/L n = 106	SF 30-49 µg/L n = 106	SF 50-69 µg/L n = 46
Early pregnancy (mean GW 15.1)					
Median SF (IQR)	23 (12, 41)	11 (7,15)	25 (22, 27)	37 (33, 43)	59 (52, 63)
Mean sTfR (95% CI)	2.5 (2.4, 2.6)	3.1 (2.9, 3.3)	2.3 (2.1, 2.4)	2.1 (2.0, 2.2)	1.9 (1.7, 2.1)
Mean TBI (95% CI)	4.9 (4.6, 5.3)	1.4 (1.1, 1.8)	5.4 (5.2, 5.6)	7.2 (7.0, 7.4)	9.1 (8.8, 9.4)
Mean Hb (95% CI)	12.0 (11.9, 12.1)	11.8 (11.6, 11.9)	12.1 (12.0, 12,3)	12.3 (12.1, 12.5)	12.2 (11.9, 12.5)
Postpartum (mean week 13.9 after delivery)					
Median SF (IQR)	18 (10, 32)	16 (9, 31)	17 (9, 26)	20 (12, 36)	19 (11, 32)
Mean sTfR (95% CI)	3.7 (3.5, 3.8)	3.9 (3.6, 4.1)	3.7 (3.4, 4.0)	3.5 (3.2, 3.7)	3.3 (2.9, 3.8)
Mean TBI (95% CI)	2.7 (2.4, 3.0)	2.3 (1.8, 2.8)	2.1 (1.5, 2.8)	3.2 (2.6, 3.9)	3.1 (2.2, 4.0)
Mean Hb (95% CI)	12.6 (12.5, 12.6)	12.4 (12.3, 12.6)	12.6 (12.4, 12.8)	12.7 (12.5, 12.9)	12.6 (12.4, 12.9)
Prevalence of ID by SF (<15) [2] n (%)	224 (39 %)	110 (44%)	43 (41%)	36 (34%)	19 (41%)
Prevalence of ID by SF (<12) [2] n (%)	167 (29%)	82 (33%)	37 (35%)	25 (24%)	13 (28%)
Prevalence of ID by sTfR [3] n (%)	106 (19%)	61 (25%)	18 (17%)	13 (12%)	6 (13%)
Prevalence of ID by TBI [4] n (%)	127 (22%)	65 (28%)	27 (26%)	17 (16%)	8 (17%)
Prevalence of anaemia [5]	114 (25%)	77 (31%)	23 (22%)	19 (18%)	12 (26%)

[1] The STORK-Groruddalen multi-ethnic pregnancy cohort from Oslo, Norway, 2008 – 2010.
95% CI 95% confidence interval; *Hb* haemoglobin; *ID* iron deficiency; *SF* serum ferritin; *sTfR* soluble transferrin receptor; *TBI* total body iron.
[2] Iron deficiency by SF defined as SF concentration <15 µg/L and <12 µg/L.
[3] Iron deficiency by sTfR defined as sTfR concentration >4.4 mg/L.
[4] Iron deficiency by TBI defined as TBI concentration <0 mg/kg.
[5] Anaemia defined as haemoglobin <12.0 g/dL.

[eng]

Case Record FORM 1.1

(For information: If: The interviewer must fill in the right category/code)*

1. What is your current marital status?

☐ Married ☐ Partnership ☐ Cohabitant ☐ Single ☐ Divorced/separated ☐ Widow ☐ Other

2. What is your level of education?

	Completed	Attending now	No. of years
Less than 7 years' schooling	☐	☐	☐☐
Primary school (7-9 years' schooling)	☐	☐	☐☐
1-2 years' upper sec./vocational school (10-11 yrs)	☐	☐	☐☐
3-year upper sec./vocational school (12 years)	☐	☐	☐☐
District college, university, up to 4 years (Nurse, teacher, Bachelor's degree)	☐	☐	☐☐
University college, university, more than 4 years (Master's, PhD)	☐	☐	☐☐

3. What was your work situation when you became pregnant?

☐ Attending educational institution

☐ Housewife

☐ Job-seeker/laid off

☐ Rehabilitation/disabled

☐ Employed in the public sector

☐ Employed in the private sector

☐ Other If other, what?:……

4. What is your occupation? State occupation/job title* **…….**
(Answer even if you are temporarily not working due to illness/leave)

5. Which religious community/religion do you belong to?* **…….....**

6. Which country were you born in? Indicate which country*…….....
If Norway:

☐ Born in Norway of two Norwegian parents

☐ Born in Norway of two foreign-national parents

☐ Born in Norway of one Norwegian + one foreign-national parent

7. Citizenship in which country? Indicate which country*…….

8. *(If the country of birth and ethnic group do not appear to agree (e.g. "Indian" but born in Kenya, Uganda, South-Africa)* **Which ethnic group (common language, culture, history) do you feel you belong to?: ……..**

9. What is your native language? **State language*** **……**

10. How do you rate your Norwegian language skills?

☐ Very good ☐ Good ☐ Fair ☐ Not very good ☐ Poor

11. Do you normally use an interpreter for doctor's appointments?

☐ Yes, professional ☐ Yes, family/friend ☐ No

12. Have you been pregnant before? (Also consider pregnancies that ended in miscarriage/ abortion or with a stillbirth)

☐ No ☐ Yes If yes:

Number born alive: ☐☐ Number stillborn: ☐☐

Number of spontaneous miscarriages: ☐☐ Number of induced abortions: ☐☐

Number of ectopic pregnancies (outside the uterus): ☐☐

13. I am now going to ask you about earlier pregnancies that have lasted more than 22 weeks.
(If more than 1 child per pregnancy, count twin 1, twin 2.)

(For each child)

Year of birth: ☐☐☐☐ Pregnancy week for birth ☐☐

Baby's weight in grams ☐☐☐☐ Gender: Boy ☐ Girl ☐

Place of birth: ☐ Norway ☐ Own native country ☐ Other

Method of delivery: ☐ Normal vaginal ☐ Forceps ☐ Vacuum ☐ Caesarean section

If multiple birth: ☐ Twins ☐ Triplets

Healthy the first week?: ☐ Yes ☐ No If no: ☐ Healthy now ☐ Ill now ☐ Dead

14. Do you have/have you had any of the following illnesses?
(Some diagnoses will mean that the woman cannot take part in the study)
(If yes, state the year the diagnosis was made).

		Year
Diabetes type 1	☐ Yes ☐ No	☐☐☐☐
Diabetes type 2	☐ Yes ☐ No	☐☐☐☐
Asthma	☐ Yes ☐ No	☐☐☐☐
Allergy	☐ Yes ☐ No	☐☐☐☐
Repeated urinary tract infections	☐ Yes ☐ No	☐☐☐☐
Chronic liver disease	☐ Yes ☐ No	☐☐☐☐
Prolonged high blood pressure	☐ Yes ☐ No	☐☐☐☐
Heart disease	☐ Yes ☐ No	☐☐☐☐

Arthritis/Bechterew's disease	☐ Yes ☐ No	☐☐☐☐
Epilepsy	☐ Yes ☐ No	☐☐☐☐
Disease of the uterus/operation	☐ Yes ☐ No	☐☐☐☐
Involuntary infertility more than 1 year	☐ Yes ☐ No	☐☐☐☐
Mental illness	☐ Yes ☐ No	☐☐☐☐
Abdominal/intestinal disorder	☐ Yes ☐ No	☐☐☐☐
Metabolism disorder	☐ Yes ☐ No	☐☐☐☐
Other: ……	☐ Yes ☐ No	☐☐☐☐

15. How old were you when you menstruated for the first time? State age in years: ☐☐

16. Have you had pregnancy diabetes during a previous pregnancy?
If yes - which pregnancy? In which pregnancy week were you diagnosed? Did you use insulin?

	Pregnancy week	Insulin
1st pregnancy	☐☐	☐ Yes ☐ No
2nd pregnancy	☐☐	☐ Yes ☐ No
3rd pregnancy	☐☐	☐ Yes ☐ No
4th pregnancy	☐☐	☐ Yes ☐ No
5th pregnancy	☐☐	☐ Yes ☐ No
6th pregnancy	☐☐	☐ Yes ☐ No
7th pregnancy	☐☐	☐ Yes ☐ No
8th pregnancy	☐☐	☐ Yes ☐ No

17. Are there any inheritable diseases in the family?

☐ None I know of ☐ Yes If yes, tick the appropriate box/boxes:

☐ Cardio-vascular disease ☐ Diabetes
☐ Cancer ☐ Neurological disease
☐ Mental illness ☐ Arthritis
☐ Muscular disorder ☐ Other If other, state:……..

18. Are you and the father of the child related?

☐ Yes ☐ No

If yes, is the father of the child your:

☐ Cousin ☐ 3rd cousin ☐ 4th cousin ☐ Uncle ☐ Nephew ☐ Other

19. Have you ever smoked/used snus?

Smoked: ☐ Never ☐ Sometimes ☐ Yes, daily

Snus: ☐ Never ☐ Sometimes ☐ Yes, daily

If the answer is never to both, go to question 23.

20. Did you smoke/use snus during the last 3 months before this pregnancy?

Smoking:		Snus:
☐ Never	Number of cigarettes/daily	☐ Never
☐ Yes, sometimes	☐☐	☐ Yes, sometimes
☐ Yes, daily	☐☐	☐ Yes, daily

21. Do you smoke/use snus now?

Smoking:		Snus:
☐ Never	Number of cigarettes/daily	☐ Never
☐ Yes, sometimes	☐☐	☐ Yes, sometimes
☐ Yes, daily	☐☐	☐ Yes, daily

22. How old were you when you started to smoke?

State age: ☐☐

If you have smoked previously, but do not smoke now, how old were you when you quit?

State age: ☐☐

23. Your alcohol consumption:

Last 3 months before pregnancy:

☐ Never ☐ Sometimes ☐ Yes, daily Amount of alcohol units, normally: ☐☐

Now: ☐ Never ☐ Sometimes ☐ Yes, daily Amount of alcohol units, normally ☐☐

(Number of alcohol units – 1 unit is: 1 glass of wine, 0.33 litres of beer, 1 glass of liquor)

24. Last menstruation's 1st day of bleeding:

Date:….. ….

25. Term before ultrasound:

Date:……. ☐ Certain ☐ Uncertain

26. Estimate your weight in kilos:

Right before you became pregnant: ☐☐☐ 25 years old: ☐☐☐ 18 years old: ☐☐☐

27. Estimate your highest and lowest weight (in kilos), not including pregnancies, after you turned 18 years of age.

Highest: ☐☐☐ Lowest: ☐☐☐

Comment if the difference as greater than 20 kilos ……

THANKS FOR TAKING THE TIME TO ANSWER THESE QUESTIONS!

[eng]

Case Record FORM 1.2

31. If you are in paid employment – how large a percentage of fulltime employment did you have during the last three months before you became pregnant? What percentage do you have now? (Applies regardless of any sick leave)

Before pregnancy: ☐☐☐ % Now: ☐☐☐ %

32. If you are in paid employment – are you currently absent from your normal job?

☐ Yes ☐ No ☐ Partly

33. (If your answer to question 32 was "Yes" or "Partly") What is the reason for your absence?

☐ Sick leave ☐ Leave ☐ Sick child ☐ Other

34. If you are in paid employment – have you been on sick leave for more than two weeks during this pregnancy?

Full sick leave: **Partial sick leave:**

If yes, state the approx. number of weeks: ☐☐ If yes, state the approx. number of weeks: ☐☐

36. Think back to when you were 10 years old. What occupation did your mother/father have?

MOTHER…… FATHER……..

37. Think back to when you were 10 years old. How many rooms did your flat/dwelling have?

(Don't count kitchen and bathroom). State number of rooms: ☐☐

How many people lived in the flat/dwelling? State number of people: ☐☐

Did your mother/father/guardian own a car? ☐ Yes ☐ No

38. How old was your mother when you were born? ☐☐ years of age

39. How many brothers and sisters (siblings) do you have? ☐☐ (With the same mother)

40. Which number were you among your siblings? ☐☐ (With the same mother)

Any half-siblings? State number, if any ☐☐

41. How long have you lived in: (State the number of years)

The city district you currently live in: ☐☐ Oslo: ☐☐

42. Where did you live for most of the time before you turned 16 years of age?

☐ In the same city district as now ☐ In another city district/area of Oslo

☐ In another county in Norway ☐ Outside Norway

State any previous city districts:……..

If outside Norway: ☐ In own country of origin ☐ Other

43. Who do you share your household with?

☐ Spouse/cohabitant ☐ Parents ☐ Parents-in-law ☐ Child/children ☐ No one

☐ Other(s), describe:…….

44. How many persons are there in your household? Count yourself as well

Number of persons 18 or older: ☐☐ Number of persons 12-17 years of age: ☐☐

Number of persons 6-11 years of age: ☐☐ Number of persons under 6 years of age: ☐☐

45. How many rooms are there (don't count kitchen and bathroom) in the flat/dwelling where you live?

State number of rooms: ☐☐

Type of dwelling:

☐ Flat in a block of flats/house with several housing units, e.g. quadruplex (four units)

☐ Terrace/row house ☐ Detached house ☐ Other

Do you own or rent your dwelling? ☐ Own ☐ Rent

46. If you are a first generation immigrant: How long have you lived in Norway?

State number of years: ☐☐

47. Are you the descendant of immigrant parents/parents who were not born in Norway?

☐ Yes ☐ No

If yes:

☐ Born in Norway, but both parents born abroad ☐ Born abroad with one parent born in Norway

☐ Born in Norway with one parent born abroad ☐ Born abroad of foreign-national parents

If you were born in Norway, with both parents born abroad, state the country of origin of your parents:

Country of origin for: your mother:…… your father:……

48. On what grounds did you come to Norway?

☐ Work ☐ Married a Norwegian ☐ Family reunification

☐ Refugee ☐ Residence on humanitarian grounds ☐ Other

49. How often in the course of the last year have you:

Read a newspaper in your own language/parents' native language:

☐ Daily ☐ Weekly ☐ Less than weekly ☐ Never

Been visited by at least one Norwegian:

Read a Norwegian newspaper/watched Norwegian TV:

Received help/support from at least one Norwegian:

Participated in a meeting arranged by your own/parents' countrymen:

50. Have you here in Norway experienced being denied a chance to rent or buy a dwelling because of your immigrant background?

☐ Yes, definitely ☐ Yes, I suspect so ☐ No ☐ Don't know

51. During the last five years in Norway have you experienced being denied a job you applied for due to your immigrant background?

☐ Yes, definitely ☐ Yes, I suspect so ☐ No ☐ Don't know

52. What was your state of health the last three months before your pregnancy?

☐ Poor ☐ Not too good ☐ Good ☐ Very good

53. Was this pregnancy planned?

☐ Yes ☐ No ☐ Partially Any comments:…….

54. If planned, how long have you been trying to get pregnant?

State number of months: ☐☐

55. Have you had any pain in any of the following parts of your body during your pregnancy?

In the lower back not radiating to the leg(s)	☐ No pain	☐ Some pain	☐ Much pain
In the lower back with it radiating to the leg(s)	☐	☐	☐
In the front of the pelvic bone, over the pubic bone	☐	☐	☐
Back, over one pelvic joint	☐	☐	☐
Back, over both pelvic joints	☐	☐	☐
Front and back of one side of the pelvic bone	☐	☐	☐
Front and back of both sides of the pelvic bone	☐	☐	☐

56. Think back over the last 14 days. Have you taken cod-liver oil/cod-liver oil capsules/pills (*tran*) and/or other dietary supplements during this time? If yes, state the number of capsules/pills/spoons per day and the correct frequency.

Cod-liver oil/Cod-liver oil capsules: ☐ Never ☐ <Once a week ☐ 1-2 times a week ☐ 3-4 times a week ☐ 5-6 times a week ☐ Every day

Fish oil capsules:
Seal oil capsules:
Folate (vitamin B):
Iron supplement:
Multi-vitamins with minerals (e.g. *Vitamineral, Kostpluss, Solaray Spektro* etc.):
Multi-vitamins without minerals: (e.g. *Sanasol, BioVit, Vitaplex* etc.)
Other dietary supplement:

State the name of the dietary supplement:…….
State the name of any iron supplements:…….

57. Have you taken medication regularly, including birth-control, the last three months before your pregnancy?

State the name of the medication……. – and the illness/disorder, if any……..

☐ The pill ☐ Mini-pill ☐ IUD/coil Brand/name:…….

58. Have you taken medication regularly during this pregnancy?

State the name of the medication……. – and the illness/disorder, if any……..

59. Have you experienced any of the following events or problems in your life during the last six months?

You have been stricken with a serious illness, been injured or assaulted ☐ Yes ☐ No

One of your closest family members (mother or father, spouse/cohabitant, children or brothers/sisters) has been seriously ill, injured or the victim of an assault

☐ Yes ☐ No

One of your closest family members (mother or father, spouse/cohabitant, children or brothers/sisters) has died

☐ Yes ☐ No

You have separated/divorced, or have broken off a long-term relationship ☐ Yes ☐ No

You have had problems/major concerns about your children (upbringing, school, discipline)

☐ Yes ☐ No

You have become unemployed or been searching in vain for a job for more than one month

☐ Yes ☐ No

You have experienced other difficult circumstances, e.g. a serious problem with a close friend, neighbour, relative or partner, serious financial concerns, something you valued dearly has been lost or stolen, death of someone close to you, or have major problems at work

☐ Yes ☐ No

ANY IMPORTANT SUPPLEMENTAL COMMENTS ON YOUR ANSWERS TO THE QUESTIONS:

Question number: ☐☐ Comment……

You can also add more detailed comments here: ……

THANKS FOR TAKING THE TIME TO ANSWER THESE QUESTIONS!

Initialer intervjuer: | | | | |

Svangerskapsuke: ☐☐

Undersøkelsesdato: ☐☐☐☐☐☐

Uker etter fødsel: ☐☐

Kvinnens fødselsdato: ☐☐☐☐☐☐

Us bydel:

Intervjuers kode: ☐☐

[engelsk – kosthold]

STORK Groruddalen

DIET

1. **Think back over the last 14 days. Now I'm going to ask you some questions about what you have drunk or poured on your cereal (such as cornflakes, muesli etc.) during this time.**
Example: Have you drunk any cola with sugar during this period? If yes, how often have you drunk this? How much did you drink each time?

Tick (X) for how often (frequency) and how much each time (in litres) where applicable.

							Amount per time **1/5l, 1/3 l, ½ l and 1l or over**			
	Have not drunk	< Once a week	1-2 t/week	3-4 t/week	5-6 t/week	Daily	[1/5 l]	[1/3 l]	[1/2 l]	1 l]
Cola with sugar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other soda pop/fizzy drink with sugar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sugar-free cola	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other sugar-free soda pop/fizzy drink	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Squash/juice and other drinks with sugar (including nectar)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sugar-free squash/juice and other drinks	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fruit juice	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Whole milk, kefir, sour milk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2% milk, Cultura, Biola, chocolate milk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

0.7% milk (green)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Skimmed milk, skimmed sour milk, Biola berry	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tea	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coffeemaker coffee, instant coffee	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coffee press, percolated coffee	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other coffee	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other drinks _____	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Comments _____

2. If you drink tea or coffee, how many teaspoons of sugar and/or honey do you use per cup?

	Don't use sugar/honey	1 tsp	2 tsp	3 tsp	4 tsp	≥ 5 tsp
			Tick box			
How many tsp sugar/-honey in tea	☐	☐	☐	☐	☐	☐
How many tsp sugar/-honey in coffee	☐	☐	☐	☐	☐	☐

3. Think back over the last 14 days. How often have you eaten/used yoghurt (from cups, with cereal

and/or in cooking)?

	Have not eaten	< Once a week	1-2 t/week	3-4 t/week	5-6 t/week	Daily
Natural yoghurt	☐	☐	☐	☐	☐	☐
Greek/Turkish yoghurt	☐	☐	☐	☐	☐	☐
Yoghurt with fruit/berries	☐	☐	☐	☐	☐	☐
Light yoghurt with fruit/berries	☐	☐	☐	☐	☐	☐

4. Think back over the last 14 days. How often have you eaten fruit and/or berries?

	Never or < once a week	1-2 t/week	3-4 t/week	5-6 t/week	Once a day	2 t/day	≥ 3 t/day
Fresh fruit, berries, fruit-salad/fruit chart etc.	☐	☐	☐	☐	☐	☐	☐

5. Think back over the last 14 days. How often have you eaten vegetables?

	Never or < once a week	1-2 t/week	3-4 t/week	5-6 t/week	Once a day	2 t/day	≥ 3 t/day
Raw vegetables, mixed vegetables/vegetable chart, salads	☐	☐	☐	☐	☐	☐	☐

Fried/wok vegetables, boiled/steamed/baked vegetables, vegetables in stews (e.g. curry)	☐	☐	☐	☐	☐	☐	☐

6. Think back over the last 14 days. How often have you eaten potatoes?

	Never or < once a week	1-2 t/week	3-4 t/week	5-6 t/week	Once a day	2 t/day	≥ 3 t/day
Potato (boiled, baked, roasted in the oven)	☐	☐	☐	☐	☐	☐	☐
Gratinated potatoes	☐	☐	☐	☐	☐	☐	☐
French fries/chips (deep-fried, fast-food)	☐	☐	☐	☐	☐	☐	☐

7. Think back over the last 14 days. How often have you eaten beans, lentils, peas, chickpeas etc?

	Never or < once a week	1-2 t/week	3-4 t/week	5-6 t/week	Once a day	2 t/day	≥ 3 t/day
Baked beans, other beans, creamed peas/beans, dahl, lentil/pea soup, chickpeas, lentil cakes, falafel (etc.), hummus	☐	☐	☐	☐	☐	☐	☐
Other _____	☐	☐	☐	☐	☐	☐	☐

8. Think back over the last 14 days. How often have you eaten meat and/or sausage products (does not refer to sandwich meats)?

	Have not eaten	< Once a week	1-2 t/week	3-4 t/week	5-6 t/week	Daily
Chicken, turkey, other lean meat such as trimmed roast beef, pork (fried, boiled, grilled, in stews etc.)	☐	☐	☐	☐	☐	☐
Dishes with lean minced beef, hamburger, hot dog/sausage meat of chicken/turkey, light hot dogs, chops trimmed of fat	☐	☐	☐	☐	☐	☐
Chops not trimmed of fat, lamb, hot dogs, fricadelles, kebab, other dishes with minced meat/other forcemeat products	☐	☐	☐	☐	☐	☐
Pizza, fast-food (purchased outside the home)	☐	☐	☐	☐	☐	☐
Other _____	☐	☐	☐	☐	☐	☐

9. Think back over the last 14 days. How often have you eaten fish and/or fish products (does not refer to sandwich spread etc.)?

	Have not eaten	< Once a week	1-2 t/week	3-4 t/week	5-6 t/week	Daily
Lean fish (fillets, whole fish) of cod, saithe/pollock, flounder, haddock, tuna, other lean fish (fried, steamed, boiled, grilled etc.)	☐	☐	☐	☐	☐	☐
Fat fish such as salmon, trout, halibut, mackerel, herring, other fat fish (fried, steamed, boiled, grilled etc.)	☐	☐	☐	☐	☐	☐

Fish products (fish cakes, fish pudding etc.)	☐	☐	☐	☐	☐	☐
Fish fingers or similar products (deep-fried or fried)	☐	☐	☐	☐	☐	☐
Other _____	☐	☐	☐	☐	☐	☐

10. Think back over the last 14 days. How often have you eaten food that has been:

	Have not eaten	Once a week	1-2 t/week	3-4 t/week	5-6 t/week	Dail
Pan-fried (with butter, margarine, oil etc.), fried in a wok/haandi	☐	☐	☐	☐	☐	☐
Deep-fried	☐	☐	☐	☐	☐	☐

11. Think back over the last 14 days.

What type of fat have you used on bread?

What type of fat have you used for frying?

What type of fat have you used for deep-frying?

What type of fat have you used for other types of cooking, for example baking?

After each question tick the box for one or more correct alternatives. First ask about fat used on bread, then for frying, deep-frying and other cooking. Use chart/pictures

	On bread	For frying	For deep-frying	For other types of cooking
Not used fat	☐	☐	☐	☐
Butter (dairy butter)	☐	☐	☐	☐

Melange (margarine), Bremyk	☐	☐	☐	☐
Brelett	☐	☐	☐	☐
Soft margarine (Soft Flora, Soft Ekstra, Soft Oliven, Vita, Soya etc.)	☐	☐	☐	☐
Light vegetable margarine (Soft light, Vita Lett etc.), ProVita/ProActiv (Becel)	☐	☐	☐	☐
Liquid margarine (Melange, Olivero, Vita, Bremyk)	☐	☐	☐	☐
Vegetable oils (sunflower/corn oil, soya oil, olive oil, rape-seed oil etc.)	☐	☐	☐	☐
Coconut/palm oil	☐	☐	☐	☐
Other margarine _____	☐	☐	☐	☐
Other oil _____	☐	☐	☐	☐
Ghee/nej/purified butter	☐	☐	☐	☐

12. Think back over the last 14 days. How often have you eaten the following food?

	Have not eaten	< Once a week	1-2 t/week	3-4 t/week	5-6 t/week	Daily
White bread (French bread, nan, breakfast crispbread etc.) and/or semi-whole-wheat bread (*kneipp*/rolls/buns/crispbread)	☐	☐	☐	☐	☐	☐

Whole-wheat and dark-brown bread/buns/crispbread, chapati	☐	☐	☐	☐	☐	☐
Cereals/muesli with no or little sugar added (oatmeal, four-grain etc) *Use chart/pictures*	☐	☐	☐	☐	☐	☐
Cereals/muesli with much sugar added *Use chart/pictures*	☐	☐	☐	☐	☐	☐
White rice, macaroni, pasta/spaghetti, couscous	☐	☐	☐	☐	☐	☐
Whole-wheat pasta or natural rice/long-grain rice/whole-grain rice, millet	☐	☐	☐	☐	☐	☐

13. Think back over the last 14 days. How often have you used the following on sandwiches?

	Did not use	< Once a week	1-2 t/week	3-4 t/week	5-6 t/week	Daily
White cheese (Norvegia, Gulost, Nøkkelost, smøreost) brown cheese, other fat cheese	☐	☐	☐	☐	☐	☐
Lighter/lean white cheese/cheese spread, light brown cheese, whey cheese spread, other light/lean cheese	☐	☐	☐	☐	☐	☐
Liver pâté, salami, saveloy/baloney, mutton sausage etc.	☐	☐	☐	☐	☐	☐
Liver pâté with less fat, liver pâté with ”healthy” fat, ham, turkey slices, light saveloy/baloney	☐	☐	☐	☐	☐	☐
Fish (mackerel in tomato sauce, other mackerel, salmon, trout, sardines, anchovies, herring, caviar etc.)	☐	☐	☐	☐	☐	☐
Jam, marmalade	☐	☐	☐	☐	☐	☐

Light jam	☐	☐	☐	☐	☐	☐
Chocolate or nut spread, Sunda, syrup, honey etc.	☐	☐	☐	☐	☐	☐
Mayonnaise salads (Italian salad, shrimp salad etc.)	☐	☐	☐	☐	☐	☐
Egg	☐	☐	☐	☐	☐	☐
Other _____	☐	☐	☐	☐	☐	☐

14. Think back over the last 14 days. How often have you eaten the following food?

	Have not eaten	< Once a week	1-2 t/week	3-4 t/week	5-6 t/week	Daily
Cakes, angel-food cake, muffins, doughnuts, Danish pastries	☐	☐	☐	☐	☐	☐
Sweet cookies (cream cookies, chocolate cookies; Balerina, Bixit, wafer cookies, Mariekjeks, Kornmo (graham wafers) etc.)	☐	☐	☐	☐	☐	☐
Sweet buns, *skolebrød* (bun with custard and icing), other sweet yeast-baked products	☐	☐	☐	☐	☐	☐
Waffles, pancakes, sweet pancakes etc.	☐	☐	☐	☐	☐	☐
Chocolate/sweets/boiled sweets, snacks with sugar (Jell-O, Turkish delight)	☐	☐	☐	☐	☐	☐
Foreign sweet snacks (mithai, jalebi, halwa, zarda, la'du, baklava etc.)	☐	☐	☐	☐	☐	☐

Ice-cream	☐	☐	☐	☐	☐	☐
Desserts/pudding/creamed rice	☐	☐	☐	☐	☐	☐
Dried fruit	☐	☐	☐	☐	☐	☐
Other sweet food/snacks	☐	☐	☐	☐	☐	☐

15. Think back over the last 14 days. How often have you eaten the following food?

	Have not eaten	< Once a week	1-2 t/week	3-4 t/week	5-6 t/week	Daily
Salt snacks (crisps/potato chips with various flavours, tortilla chips), other fattening snacks, Bombay mix etc.	☐	☐	☐	☐	☐	☐
”Light” snacks (pretzels, popcorn etc.)	☐	☐	☐	☐	☐	☐
Nuts	☐	☐	☐	☐	☐	☐

16. Think back over the last 14 days. How often do you eat the following meals during one week?

Tick a box for each meal

	Never/ rarely	Once a week	Twice a week	Three times a week	Four times a week	Five times a week	Six times a week	Every day
Breakfast	☐	☐	☐	☐	☐	☐	☐	☐
Lunch	☐	☐	☐	☐	☐	☐	☐	☐
Dinner	☐	☐	☐	☐	☐	☐	☐	☐
Supper	☐	☐	☐	☐	☐	☐	☐	☐
Midnight snack	☐	☐	☐	☐	☐	☐	☐	☐

17. Think back over the last 14 days. How often do you eat or drink one or more of the following in-between meals during the course of the day?

	Rarely	Once a day	Twice a day	Three times a day	Four times a day	More than four times a day
Chocolate, sweets, snacks, soda pop/fizzy drink etc.	☐	☐	☐	☐	☐	☐
Fruit, slice of bread/crispbread etc.	☐	☐	☐	☐	☐	☐

18. Think back over the last 14 days. Have you had a special diet during this period? ☐ No ☐ Yes

If **yes**, what has been special about it?

_____

_____

_____

_____

19. Have you changed your diet after you became pregnant/after you gave birth?
(cross out the alternative that does not fit) ☐ No ☐ Yes

If **yes**, what changes have you made and when did you make these changes?

_____

_____

_____

_____

20. How would you describe your diet? (tick the box for the closest alternative)

My diet includes meat and fish ☐

I avoid meat, but eat fish ☐

I avoid fish, but eat meat ☐

I am a vegetarian and include milk products and eggs in my diet (ovo-lacto vegetarian) ☐

I am a vegetarian and include milk products but not eggs in my diet (lacto vegetarian) ☐

I am a vegetarian and exclude all milk products and eggs from my diet (vegan) ☐

THANKS FOR TAKING THE TIME TO ANSWER THESE QUESTIONS

ERRATA

Name of candidate: Marthe-Lise Næss-Andresen

Dissertation title: IRON STATUS AND PREGNANCY. Gestational and postpartum iron deficiency and anaemia, and associations with ethnicity and clinical factors: a multi-ethnic population-based cohort study

Abbreviations for type of corrections:
Cor - correction
Celtf - change of page layout or text format

Page	Line	Footnote	Original text	Type of correction	Corrected text
22	6		Additionally a pre-post design	Cor	Additionally, a pre-post design
24	13		This study visit, is referred		This study visit is referred
26	13		The changes was calculated by subtracting	Cor	The changes were calculated by subtracting
42	5		In this thesis, one paper use a	Cor	In this thesis, one paper uses a
46	2		thus no selection bias are expected	Cor	thus no selection bias is expected
47	15		the plasma volume expansion increase	Cor	the plasma volume expansion increases
47	24		The crude prevalence rates of gestational ID	Cor	The crude prevalence rate of gestational ID
51				Celtf	Headline moved to next page
53	19		The calculations of dose x frequency	Cor	The calculation of dose x frequency
53	22		Nevertheless, use of supplements were	Cor	Nevertheless, use of supplements was
69	31		would r result in three participants	Cor	would result in three participants

STORK
Groruddalen

Oslo .....

TIL DEG SOM DELTAR I STORK - PROSJEKTET

Blodprøvene som ble tatt av deg nylig, viste:

Hemoglobin: ..... g/dl.
Ferritin: ..... µg/l.

Dette betyr at du har lav blodprosent. Dette er ikke farlig, men du bør kontakte fastlegen din for å bli fulgt opp for dette.

I mellomtiden bør du ta tilskudd av jern.

Bruk
1.....tabletter ..... daglig til du kommer til lege.

Du får kjøpt jerntilskuddet på apoteket uten resept. Mer informasjon om jernmangel kan du finne på baksiden av dette brevet.

Vennlig hilsen

.....
Jordmor i STORK prosjektet

Anemi (A) - 4.11.08.

STORK
Groruddalen

INFORMASJON OM JERN OG JERNLAGRE

Jern er et mineral alle mennesker trenger. Jern brukes i blodet til å frakte oksygen (surstoff) rundt til cellene i kroppen og jern brukes i musklene, for at de skal fungere normalt. Jern er også viktig for immunsystemet. Man kan bli trett, slapp og blek ved jernmangel. Man kan få et godt inntrykk av jerninnholdet i kroppen ved en blodprøve.

Hvordan får vi nok jern?

Jern får vi gjennom maten vi spiser. Det fins særlig i kjøtt og kjøttprodukter, men også i fisk, grønnsaker, korn og frukt. Vitamin C, som vi blant annet får gjennom frukt, øker opptaket av jern fra tarmen, mens melk, te og kaffe reduserer opptaket. Et normalt/sunt kosthold der også kjøtt inngår, dekker vanligvis jernbehovet. Man taper imidlertid jern ved menstruasjon eller annen blødning.

Hvem er utsatt for jernmangel i graviditeten?

I et normalt svangerskap trenger kvinnen ekstra jern. Dette jernet går til barnet og morkaken, samt til kvinnen selv. Jernmangel i svangerskapet oppstår når kvinnen får for lite jern gjennom kosten eller hadde lave jernlagre før graviditeten. Kun de som har jernmangel skal ta ekstra jerntilskudd i graviditeten.

Hvordan behandles jernmangel?

Jernmangel behandles ved å ta jerntilskudd. Det finnes i mange utgaver. Dosen avhenger av hvor alvorlig jernmangelen er. Når man tar jerntilskudd, kan man få problemer med kvalme, treg mage eller diaré og vondt i maven. Dette går vanligvis over etter litt tid.

Jern kan være farlig i for stor dose. Gjem esken, slik at barn ikke kan få tak i den ved et uhell! Husk å si til legen din at du tar jern, dersom du er syk og må behandles med antibiotika eller får medisin mot sure oppstøt mens du går på jerntilskudd.

4.11.08.

STORK
Groruddalen

Oslo .....

TIL DEG SOM DELTAR I STORK - PROSJEKTET

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Ferritin: ..... µg/l.

Dette betyr at du har lav blodprosent. Det er ikke farlig, men du bør kontakte fastlegen din for å få en mer nøyaktig undersøkelse og rett behandling innen 1 - 2 uker. Ta med dette brevet til legen.

I mellomtiden bør du begynne med jerntilskudd. Du får kjøpt jerntilskudd på apoteket uten resept. Spør betjeningen om veiledning.

Mer informasjon om jernmangel kan du finne på baksiden av dette brevet.

Vennlig hilsen

.....
Jordmor i STORK prosjektet

STORK
Groruddalen

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4.11.08.

STORK
Groruddalen

Oslo……..

TIL DEG SOM DELTAR I STORK – PROSJEKTET

Blodprøvene som ble tatt av deg nylig, viste:

Hemoglobin: …… g/dl
Ferritin: …… µg/l

Dette betyr at du har lite jern i kroppen, men blodprosenten er fin. Dette er ikke farlig, men du bør begynne med jerntilskudd 30 mg daglig i resten av svangerskapet.

Gjør fastlegen din oppmerksom på dette.

Du får kjøpt jerntilskudd på apoteket uten resept. Spør betjeningen om veiledning.

Mer informasjon om jernmangel kan du finne på baksiden av dette brevet.

Vennlig hilsen

……..
Jordmor i STORK prosjektet

STORK
Groruddalen

INFORMASJON OM JERN OG JERNLAGRE

Jern er et mineral alle mennesker trenger. Jern brukes i blodet til å frakte oksygen (surstoff) rundt til cellene i kroppen og jern brukes i musklene, for at de skal fungere normalt. Jern er også viktig for immunsystemet. Man kan bli trett, slapp og blek ved jernmangel. Man kan få et godt inntrykk av jerninnholdet i kroppen ved en blodprøve.

Hvordan får vi nok jern?

Jern får vi gjennom maten vi spiser. Det fins særlig i kjøtt og kjøttprodukter, men også i fisk, grønnsaker, korn og frukt. Vitamin C, som vi blant annet får gjennom frukt, øker opptaket av jern fra tarmen, mens melk, te og kaffe reduserer opptaket. Et normalt/sunt kosthold der også kjøtt inngår, dekker vanligvis jernbehovet. Man taper imidlertid jern ved menstruasjon eller annen blødning.

Hvem er utsatt for jernmangel i graviditeten?

I et normalt svangerskap trenger kvinnen ekstra jern. Dette jernet går til barnet og morkaken, samt til kvinnen selv. Jernmangel i svangerskapet oppstår når kvinnen får for lite jern gjennom kosten eller hadde lave jernlagre før graviditeten. Kun de som har jernmangel skal ta ekstra jerntilskudd i graviditeten.

Hvordan behandles jernmangel?

Jernmangel behandles ved å ta jerntilskudd. Det finnes i mange utgaver. Dosen avhenger av hvor alvorlig jernmangelen er. Når man tar jerntilskudd, kan man få problemer med kvalme, treg mage eller diaré og vondt i maven. Dette går vanligvis over etter litt tid.

Jern kan være farlig i for stor dose. Gjem esken, slik at barn ikke kan få tak i den ved et uhell! Husk å si til legen din at du tar jern, dersom du er syk og må behandles med antibiotika eller får medisin mot sure oppstøt mens du går på jerntilskudd.

Papers I - III

I

Serum ferritin, soluble transferrin receptor, and total body iron for the detection of iron deficiency in early pregnancy: a multiethnic population-based study with low use of iron supplements

Marthe-Lise Næss-Andresen,[1] Åse Ruth Eggemoen,[1] Jens Petter Berg,[3] Ragnhild Sørum Falk,[4] and Anne Karen Jenum[2]

[1]Department of General Practice, Institute of Health and Society, University of Oslo, Oslo, Norway, [2]General Practice Research Unit (AFE), Department of General Practice, Institute of Health and Society, University of Oslo, Oslo, Norway, [3]Department of Medical Biochemistry, Institute of Clinical Medicine, University of Oslo, Oslo, Norway; and [4]Oslo Centre for Biostatistics and Epidemiology, Oslo University Hospital, Oslo, Norway

ABSTRACT

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

Objective: We aimed 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.

Methods: This was a population-based cross-sectional study from primary antenatal care of 792 healthy women in early pregnancy in Oslo, Norway. We categorized the women into 6 ethnic groups: Western European, South Asian, Middle Eastern, Sub-Saharan African, East Asian, and Eastern European.

Results: Anemia was found in 5.9% of women (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 concentrations by all measures for all hemoglobin intervals. Anemia related to ID varied from 35% (sTfR) to 46% (total body iron) and 72% (ferritin) depending on the iron indicator used.

Conclusions: Women at the 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. *Am J Clin Nutr* 2019;:1–10.

Keywords: iron deficiency, anemia, pregnancy, ethnicity, serum ferritin, serum sTfR, total body iron

Introduction

The WHO report on anemia (1) states that the prevalence in pregnant women is ∼38% globally and ∼25% in Europe and, 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 with 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 birth weight, 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 birth weight 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).

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Supplemental Figure 1 and Supplemental Table 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Address correspondence to M-LN-A (e-mail: m.l.nass-andresen@medisin.uio.no).

Abbreviations used: CRP, C-reactive protein; Hb, hemoglobin; ID, iron deficiency; sTfR, soluble transferrin receptor.

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To meet the increased iron requirements, most low-income countries follow recommendations endorsed by the WHO of 30–60 mg Fe/d to all pregnant women throughout the pregnancy (7); however, the recommendations differ in high-income countries, and even within Scandinavian countries (9–13).

As in clinical practice, most studies use serum ferritin as the indicator of ID in pregnancy. However, the validity of this measure can be questioned because 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 cutoff 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 multiethnic 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.

Methods

Study population and sample selection

Data from the STORK-Groruddalen Cohort study are from the multiethnic pregnant population in 3 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, Somali, Sorani, Tamil, Turkish, Urdu, and Vietnamese and quality checked by bilingual health professionals. Women were eligible if they *1*) lived in the district, *2*) planned to give birth at 1 of the 2 study hospitals, *3*) were at <20 weeks of gestation calculated from the self-reported first day on their last menstrual period, *4*) were not suffering from diseases necessitating intensive hospital follow-up during pregnancy, *5*) could communicate in Norwegian or any of the specified languages, and *6*) 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 that 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 (Hb) measurements ($n = 31$). Our final sample included 792 pregnant women. See **Supplemental Figure 1** for the flowchart of participant recruitment. Missing values of serum sTfR ($n = 11$) were equally distributed across the ethnic groups and resulted in a subsample of 781 women.

Laboratory analysis and variable definitions

Measures of ID and anemia.

For this study, serum ferritin (micrograms per liter) and Hb (grams per deciliter) 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 method (Unicel DxI 800, Beckman Coulter) with an interassay CV of <7%. Hb was measured using a sodium lauryl sulfate method (XE 5000, Sysmex) with an interassay 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, Sentinel; on Vitros 5.1 FS) with an interassay CV of <5%. We measured serum sTfR via ELISA (Modular P800, Roche) with an interassay 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 × Roche sTfR + 0.35 mg/L.

Anemia was defined as Hb concentrations <11.0 g/dL in the first trimester and Hb concentrations <10.5 g/dL in the second trimester (1, 22). In addition, 3 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 glycated Hb, 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 Philippines), Sub-Saharan Africans (primarily from Somalia), and Eastern Europeans (primarily from Poland, Kosovo, and Russia). Parity was dichotomized into no children (nulliparous) and ≥1 children (parity ≥1). Education level was dichotomized into <10 y and ≥10 y. All participants

were asked about their intake of iron supplements during the past 2 wk and intake of iron supplements was dichotomized into "yes" and "no."

All participants were asked about conditions increasing the risk of anemia or iron status from their medical history, use of medication, and contraception use before pregnancy. This variable was categorized into 3 groups: *1*) no chronic illness/medication, *2*) chronic illness and/or medication associated with normochromic anemia (rheumatism or kidney disease, regular medication for which anemia is listed as a possible side effect in their profile), and *3*) chronic illness/medication associated with hypochromic anemia (gastrointestinal disease or use of a copper intrauterine device before the current pregnancy).

Prepregnancy 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 nonsmoking/not regular smoking 3 mo before pregnancy. Other variables of interest were age and gestational week at inclusion, calculated from women's self-reported first day on their last menstrual period.

Data from a food-frequency questionnaire, developed to capture dietary components in a multiethnic sample, were collected at the participants' second visit (gestational week 28) and self-reported dietary patterns were extracted through cluster analysis using Ward's method (23). We further dichotomized these 4 clusters into "healthy" and "unhealthy." The healthy diet contained a more frequent intake of meat, vegetables, wholegrain bread with pâté, and meat spread.

Statistical analyses

Descriptive statistics were presented as frequencies with proportions for categorical variables and means with SDs or medians with IQRs for continuous variables. The distributions of sTfR, total body iron, and Hb 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 Hb (<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); *1*) >12 mg/L in gestational weeks 8–16, *2*) >14 mg/L in gestational weeks 17–23, *3*) >20 mg/L in gestational weeks 24–27, and *4*) >37 mg/L in gestational weeks 28–31.

To examine associations between ethnic groups and ID and anemia, we performed linear regression analyses with sTfR, total body iron, and Hb 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, whereas gestational week, age, parity, education, iron supplements, chronic illness/medication affecting iron status and anemia, prepregnant 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, 1 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 ORs, both with accompanying 95% CIs. Model fit is presented by adjusted R^2 or Nagelkerke R^2, as appropriate. P values <0.05 were considered statistically significant. The SPSS (Statistical Package for the Social Sciences) version 24 (IBM SPSS statistics) and Stata (Statistics and data) version 15.0 (Stata Corp LLC) software packages were used for statistical analysis.

Results

A total of 792 women, 59% of other ethnic origin than Western European, were included (**Table 1**). The mean ± SD age was 29.8 ± 4.8 y, 46% were nulliparous, prepregnant BMI was 24.5 ± 4.8, and 11% smoked regularly 3 mo before conception. At the time of examination, mean ± SD gestational week was 15.4 ± 3.5. The non-Western women were younger, had higher parity, and lower education than the Western European women, and 86% of the non-Western women reported an unhealthy dietary pattern. In total, 18% of the women used iron supplements (14% of Western European and 21% of non-Western women). There were no significant differences between the study sample and the 31 excluded women for ethnicity, age, gestational week, parity, prepregnant BMI, and education (data not shown).

The mean ± SD Hb concentration was 12.5 ± 1.0 g/dL (**Table 2**) and the prevalence of anemia (abnormal value of Hb; Hb <11.0 g/dL in the first and <10.5 g/dL in the second trimester) was 5.9% in the whole sample. Women of Sub-Saharan African, South Asian, Middle Eastern, and East Asian ethnic origin had significantly higher prevalence of anemia (abnormal value of Hb, $P < 0.01$–0.05) than Western Europeans. In total, 65 women had low red blood cell mean corpuscular volume values (<80 fL), of whom 63 were of non-Western origin (data not shown) and 3 had hemoglobinopathy (Table 2).

The prevalence of ID (abnormal value of serum ferritin, sTfR, and total body iron; Table 2) differed by iron indicator, and was significantly higher by ferritin than by sTfR and total body iron (chi-square, $P < 0.01$). Further, the prevalence of ID by total body iron was significantly higher than that derived from sTfR (chi-square, $P < 0.01$). Of the women with ID from total body iron, 98% were also iron deficient from ferritin, and 52% were iron deficient by sTfR. Further, in women with normal total body iron, 25% were iron deficient from ferritin and 1% from sTfR. Of the anemic women in the total sample, 72% had ID defined by ferritin, compared with only 35% and 46% from sTfR and total body iron, respectively (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 than in Western European women (Table 2). To study the distribution of total body iron, we categorized the values into 9 equally wide groups, presented as grouped at the midpoint of each. **Figure 1** illustrates that the total body iron concentration shifted to the left for all non-Western groups compared with Western Europeans.

Further, we categorized Hb concentration into 4 groups, presented as grouped at the midpoint, to explore the distribution

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

	Total ($n = 792$)	Western Europe ($n = 326$) (41%)	Non-Western ($n = 466$) (59%)	South Asia ($n = 198$) (25%)	Middle East ($n = 123$) (16%)	Sub-Saharan Africa ($n = 58$) (7%)	East Asia ($n = 44$) (6%)	Eastern Europe ($n = 43$) (5%)
Gestational week	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	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, *n* (%)								
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)
Prepregnant BMI, kg/m^2	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] *n* (%)								
≥10 y	658 (84)	314 (97)	344 (74)	162 (82)	77 (64)	32 (55)	36 (82)	37 (88)
<10 y	128 (16)	10 (3)	118 (26)	35 (18)	44 (36)	26 (45)	8 (18)	5 (12)
Smoking,[3] *n* (%)								
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] *n* (%)								
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, *n* (%)								
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, *n* (%)								
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* (%) or means ± SDs. Group designation—Western Europe: participants with origin from Norway, Sweden, Denmark, other Western European countries, and North America; Non-Western: participants from South Asia, the Middle East, Sub-Saharan Africa, East Asia, and Eastern 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 Philippines; Sub-Saharan Africa: participants with origin primarily from Somalia; Eastern Europe: participants with origin primarily from Poland, Kosovo, and Russia.

[2]Missing information in 6 women.

[3]Self-reported smoking 3 mo 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 for which 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 a copper intrauterine device before the current pregnancy).

TABLE 2 Serum ferritin, serum sTfR, total body iron (calculated from serum ferritin and sTfR concentrations), and serum hemoglobin concentrations, and prevalence of abnormal values (iron deficiency and anemia) in pregnant women in the STORK-Groruddalen study[1]

	n	Ferritin, μg/L	sTfR, mg/L	Total body iron, mg/kg	Hemoglobin, g/dL
Mean ± SD[2]		32.5 ± 33.0	2.5 ± 1.2	4.8 ± 4.0	12.5 ± 1.0
Median (IQR)[2]		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[3]					
Total sample[2]		33 (30, 36)	6.5 (5.0, 8.5)	11 (8.9, 13)	5.9 (4.5, 7.8)[4]
Ethnic group[5]					
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]Group designation—Western Europe: participants with origin from Norway, Sweden, Denmark, other Western European countries, and North America; Non-Western: participants from South Asia, the Middle East, Sub-Saharan Africa, East Asia, and Eastern 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 Philippines; Sub-Saharan Africa: participants with origin primarily from Somalia; Eastern Europe: participants with origin primarily from Poland, Kosovo, and Russia. sTfR, soluble transferrin receptor.

[2]$n = 792$ for serum ferritin and hemoglobin, $n = 781$ for sTfR and total body iron (calculated from serum ferritin and soluble transferrin receptor concentrations).

[3]Abnormal values presented as percentage (95% CI), defined as serum ferritin <15 μg/L, serum sTfR >4.4 mg/L, total body iron <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.

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

[5]The differences in prevalence of abnormal values between Western Europeans and each non-Western group were tested by chi-square test: $^{*}P < 0.05$, $^{**}P < 0.01$.

of the 3 different iron indicators within the 4 Hb intervals by ethnicity. **Figure 2** shows that by increasing Hb 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 Hb concentration was lower, and the iron status was poorer by all 3 iron indicators (i.e., serum sTfR was higher, total body iron was lower, and the risk of ID by ferritin was higher) in women of South Asian, Middle Eastern, and Sub-Saharan African origin, compared with Western European women (**Tables 3** and **4**). These ethnic differences persisted after adjustment for possible confounders (gestational week, age, parity, education, prepregnant 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 of ID from ferritin, compared with Western European women. High gestational week was associated with low Hb and poor iron status by all 3 iron indicators (i.e., serum sTfR was higher, total body iron was lower, and the risk of ID by ferritin was higher), whereas multiparity (≥1) and low education (<10 y) were associated with poorer iron status (i.e., serum sTfR was higher, total body iron was lower, and the risk of ID by ferritin was higher) only. In addition, low prepregnant BMI, low age, and use of iron supplements were independently associated with low total body iron. Lastly, low prepregnant BMI and use of iron supplements were also independently associated with low Hb, as well as chronic illness and medication affecting iron status and hypochromic anemia. The ethnic groups with highest prevalence of anemia and ID 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**).

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 3 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, the Middle East, and Sub-Saharan Africa were more prone to anemia and ID by all iron indicators and these ethnic differences persisted after adjustment for confounders. Further, the iron concentrations remained lower for these ethnic minority groups regardless of the Hb 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 epidemiologic European studies in pregnant women report a prevalence of anemia ≤30% (29). The low prevalence of anemia in Western Europeans (1.8%) in our study is in accordance with the prevalence in Caucasians in other studies from Europe and

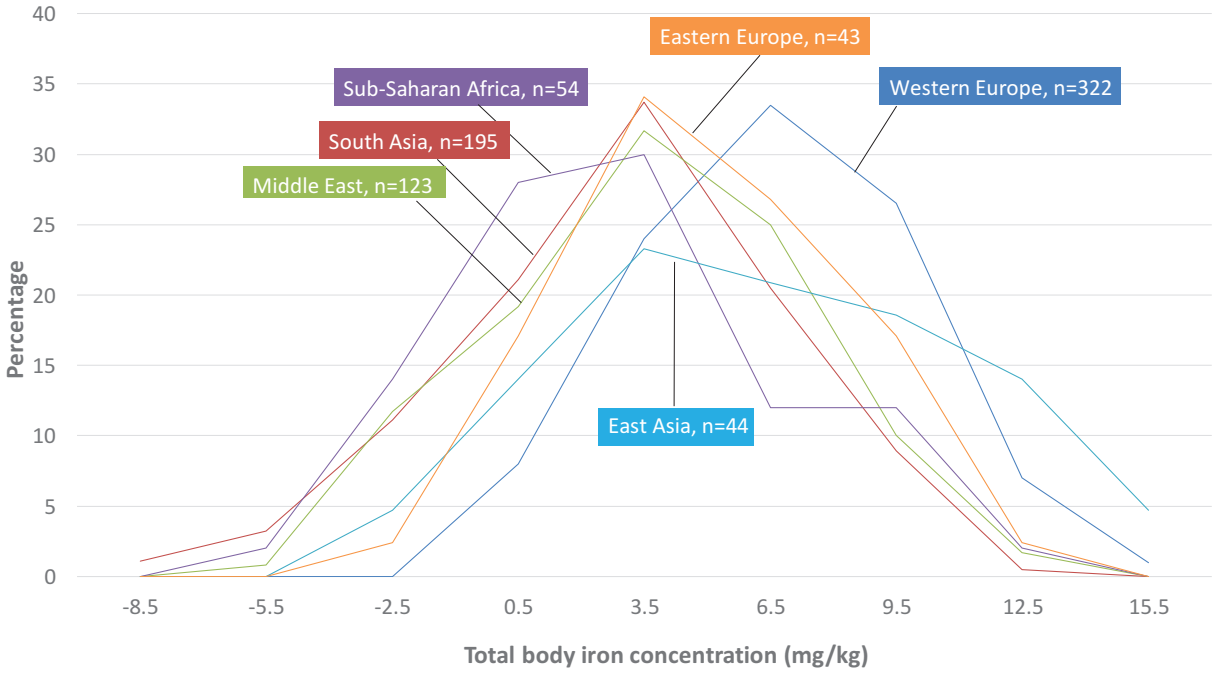

FIGURE 1 Distribution of total body iron (calculated from serum ferritin and soluble transferrin receptor concentrations) in pregnant women by ethnic group from the STORK-Groruddalen study. Nine total-body iron concentration intervals presented as grouped at the midpoint of each. Group designation—Western 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 Philippines; Sub-Saharan Africa: participants with origin primarily from Somalia; Eastern Europe: participants with origin primarily from Poland, Kosovo, and Russia.

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 have 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 2 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 women the prevalence was higher (45%) than in results from NHANES (23–39%) (18), although the composition of ethnic groups differed.

The overall prevalences of ID by sTfR (6.5%) and total body iron (11%) in our study were similar to findings for the first trimester in NHANES (18), but lower than in an Australian study (32). However, in contrast to findings in NHANES, in which 12–13% of the Caucasians were iron deficient by sTfR and total body iron, <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 for non-Caucasians in NHANES (18).

Generally, comparisons between studies are hampered by different sampling methods, assays, or cutoff values. Without information from the "gold standard" method, iron staining of bone marrow, 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 ours (27). The low prevalence of 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, ~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 in phytates, inhibiting 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% of our sample reported a chronic disease or used relevant medication that could induce anemia or ID, with no difference between ethnic groups. We cannot, however, rule out the possibility that iron loss might be

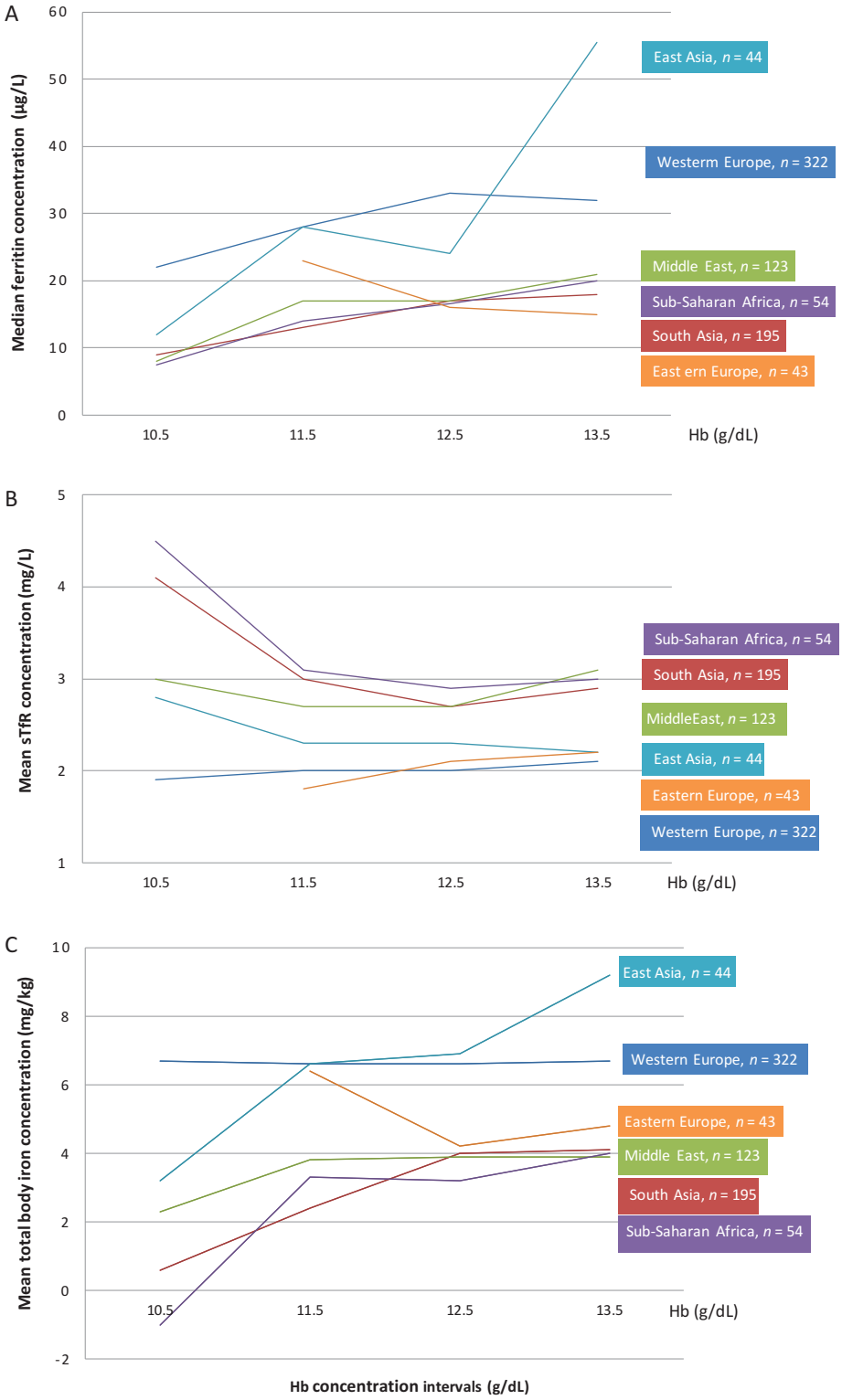

FIGURE 2 Median ferritin concentration (A), mean serum sTfR (B), and mean total body iron (C) in 4 Hb concentration intervals. Group designation—Western Europe: participants with origin from Norway, Sweden, Denmark, other Western European countries, and North America; South Asia: participants with origin 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 Philippines; Sub-Saharan Africa: participants with origin primarily from Somalia; Eastern Europe: participants with origin primarily from Poland, Kosovo, and Russia. Hb, hemoglobin; sTfR, soluble transferrin receptor.

TABLE 3 Linear regression analyses of the iron indicators serum 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]

	sTfR, mg/L		Total body iron, mg/kg		Hemoglobin, g/dL	
	β, 95% CI	adj β^2 (95% CI); $R^2 = 0.19$	β (95% CI)	adj β^3 (95% CI); $R^2 = 0.30$	β (95% CI)	adj β^4 (95% CI); $R^2 = 0.20$
Ethnicity (Western European = reference)						
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. Group designation—Western Europe: participants with origin from Norway, Sweden, Denmark, other Western European countries, and North America; Non-Western: participants from South Asia, the Middle East, Sub-Saharan Africa, East Asia, and Eastern 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 Philippines; Sub-Saharan Africa: participants with origin primarily from Somalia; Eastern Europe: participants with origin primarily from Poland, Kosovo, and Russia. adj, adjusted; sTfR, soluble transferrin receptor.

[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, prepregnant 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, prepregnant BMI, iron supplement, chronic illness/medication, smoking, and dietary pattern. *$P < 0.05$, **$P < 0.01$.

aggravated in some ethnic minority women owing 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 ID and anemia (14, 34).

In total, we found indications of hemoglobinopathy in only 3 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 Hb samples with mean corpuscular volume <70 fL were tested for hemoglobinopathies (35).

Strengths of the present study include its population-based sample, and several adaptations to reduce barriers to the 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 iron status in early pregnancy and helps to detect high-risk women. We also added sensitivity analyses in which 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, because 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 therefore adjusted for gestational week (36). Further, the collected food-frequency data were used to identify dietary patterns, not iron intake directly (23). In addition, the usefulness of serum sTfR as a marker of ID is

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)	Adjusted OR (95% CI) ($R^2 = 0.26$)
Ethnicity (Western European = reference)		
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)
Eastern 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, prepregnant BMI, iron supplement, smoking, and dietary pattern. Group designation—Western Europe: participants with origin from Norway, Sweden, Denmark, other Western European countries, and North America; Non-Western: participants from South Asia, the Middle East, Sub-Saharan Africa, East Asia, and Eastern 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 Philippines; Sub-Saharan Africa: participants with origin primarily from Somalia; Eastern Europe: participants with origin primarily from Poland, Kosovo, and Russia. *$P < 0.01$. R^2, Nagelkerke R-square.

limited by the 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 when they have normal Hb 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 3 different blood-based indicators in a multiethnic sample of healthy pregnant women in which 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 3 iron indicators, and the lower iron concentrations by all measures for all Hb intervals, in pregnant women with ethnic origin from South Asia, the Middle East, and Sub-Saharan Africa than in women with ethnic origin from Western Europe.

The authors' responsibilities were as follows—M-LN-A: had full access to all the data in this study and is responsible for the integrity of the data and accuracy of the data analysis; M-LN-A, ÅRE, JPB, and AKJ: contributed to the study concept and design, to analysis, tables, and interpretation of the data; RSF: guided the statistical analysis and revised the tables; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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RESEARCH ARTICLE

Prevalence of postpartum anaemia and iron deficiency by serum ferritin, soluble transferrin receptor and total body iron, and associations with ethnicity and clinical factors: a Norwegian population-based cohort study

Marthe-Lise Næss-Andresen[1]*, Anne Karen Jenum[2], Jens Petter Berg[3], Ragnhild Sørum Falk[4] and Line Sletner[5,6]

[1]*Department of General Practice, Institute of Health and Society, University of Oslo, Oslo, Norway*
[2]*General Practice Research Unit, Department of General Practice, Institute of Health and Society, University of Oslo, Oslo, Norway*
[3]*Department of Medical Biochemistry, Institute of Clinical Medicine, University of Oslo, Oslo, Norway*
[4]*Oslo Centre for Biostatistics and Epidemiology, Oslo University Hospital, Oslo, Norway*
[5]*Department of Paediatric and Adolescents Medicine, Akershus University Hospital, Lørenskog, Norway*
[6]*Institute of Clinical Medicine, University of Oslo, Oslo, Norway*

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Abstract

Worldwide, there are limited data on the prevalence of postpartum anaemia and iron status. The aims of the present study were to assess the prevalence of anaemia and iron deficiency (ID) by three iron indicators 14 weeks postpartum, their relations to haemoglobin (Hb) and associations with ethnicity and clinical factors in a multi-ethnic population. We conducted a population-based cohort study of 573 women followed from early pregnancy. The prevalence of postpartum anaemia (Hb <12·0 g/dl) was 25 %. ID prevalence varied from 39 % by serum ferritin (SF <15 μg/l), to 19 % by soluble transferrin receptor (sTfR >4·4 mg/l) and 22 % by total body iron (TBI < 0 mg/kg). The mean Hb concentration was 12·8 g/dl in women with no ID, 12·6 g/dl in those with ID by SF only and 11·6 g/dl in those with ID by SF, sTfR and TBI. ID by sTfR and TBI defined by the current threshold values probably identified a more severe iron-deficient population compared with ID assessed by SF. Compared with Western Europeans, the prevalence of anaemia was at least the double in ethnic minorities (26–40 % *v.* 14 %; $P < 0{\cdot}01$–$0{\cdot}05$), and the prevalence of ID by sTfR and TBI, but not of ID by SF < 15 μg/l, was significantly higher in some minority groups. After adjustment for covariates, only South Asians had lower Hb and higher sTfR concentration. Insufficient iron intake, gestational anaemia or ID, and postpartum haemorrhage were associated with lower postpartum Hb concentration and poorer iron status.

Key words: **Anaemia: Cohort: Ethnic minorities: Iron deficiency: Postpartum iron status**

Introduction

The prevalence of postpartum anaemia in high-income countries is estimated to 10–30 %, but is generally higher in low- and middle-income countries[1]. Iron deficiency (ID) is considered the main cause of anaemia, due to bleeding during childbirth or inadequate dietary iron intake/uptake[2–4]. Women who have emigrated from low- and middle-income countries to Europe are considered a vulnerable group concerning several nutritional insufficiencies in pregnancy, e.g. ID, vitamin D deficiency and low use of folic acid[5–9], but their postpartum iron status has scarcely been assessed.

ID occurs through a gradually reduction of iron stores, from being replete to being depleted and eventually absent, which consequently results in ID anaemia. ID can be measured by a variety of biomarkers. Serum ferritin (SF) concentration <15 or <12 μg/ml is widely used in the diagnosis of ID,

* **Corresponding author:** Marthe-Lise Næss-Andresen, email m.l.nass-andresen@medisin.uio.no

and is considered a sensitive indicator of body iron stores in the absence of pregnancy, infection and inflammatory processes. The SF concentration below the threshold reflects depleted body iron stores, but cannot determine the severity of the ID[10]. Soluble transferrin receptor (sTfR) is an alternative iron marker suggested used when SF interpretations are hampered, and better reflects the cellular iron demand. In contrast to SF, sTfR is increased in ID. Elevated sTfR concentration reflects increasing cellular iron demand and falling Hb-synthesis, and the circulating sTfR and cellular iron demand are found to be proportional, thus reflecting early functional ID[11]. Together, SF and sTfR cover the full range of iron status, and combining them in a new model, called total body iron (TBI) is considered to better predict the absence of bone marrow iron[10]. While this marker is now widely used in the United States, it has not been implemented in Europe[12]. A positive value of TBI represents iron storage, while negative values indicate a deficient iron supply to peripheral tissues[12,13].

We have previously shown that in early pregnancy ethnic minority women had more ID for all three iron indicators, also when adjusting for covariates[5]. Postpartum anaemia and ID may have adverse short – and long-term health implications for the mother and her child, such as fatigue, lower work capacity and increased risk of postpartum depression and poorer mother–child interaction[4]. Furthermore, maternal mortality increases with severe anaemia, as well as the risk of infections in the puerperium and poorer wound healing[4].

There are limited data on the prevalence of postpartum anaemia and iron status. The aims of the present study were to assess the prevalence of anaemia and ID by three iron indicators 14 weeks after delivery, including the relations between the ID indicators and their relations to haemoglobin (Hb), and their associations with ethnicity and clinical factors.

Subjects and methods

Study population and data selection

Our data is from the STORK-Groruddalen study of multiethnic pregnancies in Oslo, Norway, collected at public Child Health Clinics for primary antenatal care in the period 2008–10 in three administrative districts in Oslo. The study methods have been described in detail elsewhere[14]. 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. Pregnant 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 (GW) (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 informed consent.

In total, 823 pregnant healthy women from 65 countries were included in early pregnancy (mean GW 8–20), with planned follow-up visits in GW 28 and about 3 months after delivery. The time point for the postpartum visit was chosen to ensure a high attendance rate, as at this point, most women have recovered from birth, and have established daily routines. Furthermore, physiological pregnancy-related changes, including haemodilution, will have returned to normal. At all three study visits, questionnaire data were collected through interviews by authorised study personnel, assisted by professional interpreters when needed[14]. The questionnaire data covered a wide range of health issues, and clinical measurements were collected according to the study protocol. Participating women were found representative for the main ethnic groups of pregnant women attending the Child Health Clinics[14]. Ethical approval was obtained from The Regional Ethics Committee.

Outcome measures

Postpartum anaemia was defined as Hb concentrations <12·0 g/dl[3,15] measured 14 weeks post-delivery. In addition, we used three established definitions for ID; SF concentration <15 μg/l, the primary indicator used by the WHO[16], sTfR concentration >4·4 mg/l according to the manufacturer's guidelines, and TBI<0 mg/kg[13]. SF concentration below the threshold indicates depleted body iron stores, while an increased sTfR concentration reflects early functional ID and TBI is meant to be a quantitative estimate of the iron status[10–13,17]. Iron-deficiency anaemia (IDA) was defined as anaemia in the presence of ID by any iron indicator.

Measurements of iron indicators and anaemia

Blood samples were drawn at all visits[5,14]. Hb (g/dl) and SF (μg/l) were analysed consecutively at the Department of Multidisciplinary Laboratory Medicine and Medical Biochemistry at Akershus University Hospital, Oslo, Norway. Hb was measured using an SLS method (XE 5000 from Sysmex; inter-assay coefficient of variation (CV) 0·7 %). SF was measured using an electro-chemiluminescence immunoassay (ECLIA) method (Unicel DxI 800 from Beckman Coulter; inter-assay CV <7 %). Blood samples were frozen and biobanked at −80 °C. In 2016, sTfR and high sensitivity C-reactive protein (CRP) were analysed from biobanked serum samples at the Department of Medical Biochemistry at Oslo University Hospital, Oslo, Norway. CRP was measured by a particle-enhanced turbidimetric immunoassay (CRP Vario from Sentinel on Vitros 5.1 FS; inter-assay CV <5 %), and sTfR by ELISA (Modular P800 from Roche; inter-assay CV <5 %[18]). We calculated TBI according to Cook[13] from the ratio of sTfR concentration (by Flowers assay) to SF concentration: $-[\log_{10}(\text{sTfR} \times 1000 \div \text{SF}) - 2{\cdot}8229] \div 0{\cdot}1207$. To convert our Roche sTfR concentration to Flowers sTfR concentrations, we used the conversion equation Flowers sTfR 1·5 × Roche sTfR + 0·35 mg/l[13].

Socio-demographic variables

Ethnicity was defined as each participant's country of birth, or the participant's mother's country of birth if the participants'

mother was born outside Europe or North America[14,19], and grouped as Western Europeans (Norway, other Western European countries and North America), South Asians (primarily Pakistan and Sri Lanka), Middle Easterners (primarily Iraq, Morocco and Turkey), East Asians (primarily Vietnam and The Philippines), Sub-Saharan Africans (primarily Somalia) and Eastern Europeans (primarily Poland, Kosovo and Russia). Maternal age was calculated from date of birth and date at enrolment in the present study. Parity was dichotomised into primiparous (first pregnancy lasting >22 weeks) and parous (one or more previous births) women. Pre-pregnancy body mass index (BMI, kg/m^2) was calculated from self-reported weight before pregnancy and height measured at inclusion[14]. Gestational age was primarily derived from the first day of the mother's last menstrual period (LMP), but ultrasound-derived gestational age was used in 7 % of pregnancies where there were reasons to believe that the LMP-derived GA was uncertain[20].

Most variables reflecting socioeconomic position (SEP) and level of integration are strongly correlated, although representing different dimensions of societal and contextual factors. Individual and household markers of maternal present SEP and variables related to the level of integration were therefore entered into a principal components analysis (PCA). Two separate, uncorrelated components were extracted[19]. The first component was strongly correlated with predefined markers reflecting integration such as language skills, time of residence, social interaction with ethnic Norwegians and use of Norwegian media, and was skewed to the right, as all ethnic Norwegians had high scores. Due to the skewed distribution, and as we were mainly interested in the potential effect of low integration, and to ease the interpretation of our results, the integration score was further dichotomised as the 40 % with the lowest scores *v.* the 60 % with highest scores. This pragmatic cut-point had a sufficient number in the lowest category and still carried important information. The second component had strong correlations with the predefined individual and household markers of SEP such as educational level, occupational class, employment status, renting tenure and rooms per person in the household, and was normally distributed, with a higher score reflecting higher SEP. Maternal early life SEP was derived from a separate PCA of three childhood socio-demographic variables (family occupational class (highest of mother and father), rooms per person in household and family ownership of car, all referring to maternal age of 10 years), and was also normally distributed. Time of residence was calculated from the participants' self-reported year of arrival to Norway, and the participants' Norwegian language skills were categorised as good and poor, derived from the their need of interpreter at the study visits[19].

Variables potentially associated with iron metabolism

Of ethical reasons, all participants with Hb <10·0 g/dl during pregnancy were informed by letter, and encouraged to seek their family doctor. Women with SF <20 µg/l and Hb >10 g/dl were recommended to use 30–50 mg iron supplementation per day. All participants were asked about their intake of iron supplements during the past 2 weeks at all three visits. Iron supplementation was dichotomised into 'yes', covering daily or intermittent iron supplements, and 'no'. Data from a food frequency questionnaire, developed to capture dietary patterns in a multi-ethnic sample, were collected in GW 28. Four clusters were extracted using the Ward's method. Clusters were referred to as 'a healthier dietary pattern' *v.* three 'less healthy dietary patterns'[21], here dichotomised into 'healthy' and 'unhealthy'. The 'healthy dietary pattern' represented more frequent intake of fruit, vegetables, wholegrain bread with pate and meat spread, and meat, i.e. food items representing relatively high intake of iron. From questions about their medical history, we categorised three groups: (1) no medical conditions associated with anaemia or ID, (2) self-reported chronic illness or medication associated with ID or normochromic anaemia (i.e. kidney or rheumatic disease, use of carbamazepine or infliximab) and (3) self-reported chronic illness or medication associated with ID or hypochromic anaemia (i.e. gastrointestinal disease or Copper IUD use before conception (associated with heavier menstrual bleeding and a poor iron status prior to their pregnancy))[5]. Haemoglobinopathy was either self-reported, identified from the HPLC (Tosoh G8, Tosoh Corporation) analysis of HbA1c (glycated Hb) or based on microcytic anaemia in the absence of low SF.

Birth-related variables potentially associated with postpartum iron status

We have detailed data on birth complications extracted from hospital birth records. We categorised delivery mode into (1) normal vaginal delivery, (2) instrumental vaginal delivery (i.e. forceps or vacuum-assisted vaginal delivery), (3) elective caesarean section and (4) emergency caesarean section. Postpartum blood loss after delivery was extracted from the hospital's birth record (mainly reflecting blood loss directly related to birth); and further dichotomised into <500 ml and ≥500 ml – the last category defined as postpartum haemorrhage. Due to small numbers of each type of birth complications, we also constructed a composite variable reflecting the presence of at least one of the following complications; episiotomy, third- or fourth-degree perineal tear, obstructed labour and manual removal of placenta as an outcome.

Sample size

Of the 823 (74 % of invited participants) women included in early pregnancy, 644 (78 %) attended at the postpartum visit in mean postpartum week 13·9 (SD ± 2·5) (flowchart, Supplementary Figure S1). For the present study, we included participants with no missing values for SF, sTfR and TBI at the postpartum visit, resulting in a total sample of 573 women (89 % of those attending the postpartum visit). There were no significant differences between the study sample and the 250 excluded women regarding age, parity, pre-pregnant BMI and SEP (data not shown). However, the study sample consisted of a slightly larger proportion of ethnic minority women as they were prioritised for fasting blood

samples at the postpartum visit due to resource limitations, compared with the excluded women[22].

Statistical analyses

The STORK-Groruddalen study was originally designed to identify ethnic differences in the prevalence of gestational diabetes and aimed at including 800 women. In the present study, we take advantages of the collected date and performed regression models based on the large sample size available. Descriptive statistics are presented as frequencies with proportions for categorical variables and mean with standard deviations (SD) or medians with interquartile range for continuous variables. The sTfR, TBI and Hb values were approximately normally distributed (Table 1). We calculated percentages of abnormal values for SF (<15 μg/l), sTfR (>4·4 mg/l), TBI (<0 mg/kg) and Hb (<12·0 g/dl) for the total sample, and for each ethnic group. The differences in prevalence between Western Europeans and each non-Western group were tested by χ^2 tests (Table 2). We used a scaled Venn diagram to illustrate the degree of overlap between measures of ID, and further to illustrate their relations to Hb by measuring mean Hb concentration in the groups with ID defined by the different iron indicators (Fig. 1). We also categorised Hb concentration into four groups, presented at the group midpoint, to explore the distribution of the three different iron indicators by ethnicity (Fig. 2). Furthermore, we performed sensitivity analysis, using the threshold of SF <12 μg/l for the prevalence of ID to ease comparison to other studies using this threshold and to the prevalence rates between the different iron indicators (Supplementary Table S1).

To examine associations between ethnicity, maternal factors before and during pregnancy, birth complications, and postpartum anaemia and ID, we performed linear regression analyses with Hb, sTfR and TBI as continuous outcome variables, and logistic regression analyses with SF <15 μg/l as a dichotomous outcome variable due to its skewed distribution. Factors of particular clinical relevance, such as ethnicity, gestational anaemia and ID, postpartum haemorrhage, parity, dietary pattern and iron supplementation were hence forced into the models. However, other potentially relevant factors with *P*-value <0·2 in the univariate analysis were also included into the multiple regression analyses, but only included in the final model if still significantly associated with the outcome after a stepwise backward elimination process (Table 3). Interactions with ethnicity were examined graphically and by entering cross-product terms, one-by-one, into the model. We *a priori* defined an interaction to be significant if the *P*-value was <0·01 and consistent for Hb and all three iron indicators. No significant interactions were observed.

As ethnicity is a broader concept than geographical ancestry, we also explored the impact of level of social integration, as alternative explanatory variables. First, we performed multivariable regression analyses replacing the ethnicity variable with the dichotomous variable low and high social integration (Supplementary Table S2). Second, we conducted sensitivity analyses in a sub-sample of ethnic minority women (*n* 332), dichotomised into 'South Asian' or 'other' ethnic origin, and explored if social integration could explain the differences observed between ethnic minority groups (Supplementary Table S3).

Results from linear regressions are presented as β-coefficients and results from logistic regression as odds ratios (ORs), both with accompanied 95 % confidence intervals (CIs). Model fit is presented by adjusted R^2 or Nagelkerke R^2, as appropriate. *P*-values <0·05 were considered statistically significant. SPSS version 25 and Stata version 15 were used for statistical analysis.

Results

Sample characteristics

Of the 573 women who constitute the sample 14 weeks postpartum sample, 62 % had ethnic origin outside Western Europe, mean age at inclusion was 29·7 (SD ± 4·8) years, mean pre-pregnant BMI was 24·6 (±4·8) kg/m^2, and 46 % were primiparous (Table 1). Non-Western women were younger, and more often reported a dietary pattern categorised as less healthy compared with Western European women. They also had a lower socioeconomic position, both in childhood and as adults, represented by lower SEP-scores generated from the PCA analyses of several individual and household socio-demographic variables.

Prevalence of anaemia and ID, relations between ID indicators and relations with anaemia

The mean Hb concentration was 12·5 ± 1·0 g/dl at the postpartum visit, and the overall prevalence of anaemia was 25 %, but the prevalence of ID differed by iron indicator, and was significantly higher by SF than by sTfR and TBI (Table 2). Only four women with haemoglobinopathy were identified. The Venn diagram (Fig. 1) illustrates that among the 298 women with ID by any indicator, 35 % had ID by all three indicators, 38 % by SF only, 6·3 % by sTfR only, while none had ID identified by TBI only. The mean Hb concentration was highest in those with no ID by any iron indicator (12·8 g/dl), lowest in those with ID by all indicators (11·6 g/dl), while 12·6 g/dl in those with ID by SF only (Fig. 1).

The prevalence of anaemia differed significantly between ethnic groups and was at least the double in all ethnic minority women compared with Western European women (26–40 % *v.* 14 %; $P < 0{\cdot}01$–$0{\cdot}05$) (Table 2). Regarding ID, the prevalence by TBI was twice as high in South Asians, Middle Easterners and Sub-Saharan Africans compared with Western Europeans. We found no significant ethnic differences for ID using SF <15 μg/l. East Asians had an overall better iron status by all indicators compared with the other ethnic groups in all four Hb concentration intervals, including those with anaemia (Hb concentration interval 8·0–11·9 g/dl) (Fig. 2).

After having excluded women with elevated CRP concentration >5 mg/l (9–26 % by ethnic groups), only minor changes in the mean and median values and in the prevalence

Table 1. Socio-demographic characteristics of the total sample in the STORK-Groruddalen study stratified into Western Europeans and non-Western women, and further into ethnic minority groups[a]

		Total		Western Europe		Non-Western		South Asia		Middle East		Sub-Saharan Africa		East Asia		Eastern Europe	
		n or means	SD or %	*n* or means	SD or %	*n* or means	SD or %	*n* or means	SD or %	*n* or means	SD or %	*n* or means	SD or %	*n* or means	SD or %	*n* or means	SD or %
	n	573	100	217	38	356	62	157	27	94	16	38	7	33	6	34	6
Postpartum week	566	13·9	2·5	13·6	2·2	14·1	2·5	14·0	2·5	14·0	2·7	14·4	2·2	14·7	3·3	13·7	1·9
Age at inclusion, years	573	29·7	4·8	30·9	4·6	29·2	4·8	28·7	4·4	29·7	5·6	29·0	5·1	30·9	4·4	28·4	4·3
Parity	564																
Primiparous		260	46	113	53	147	42	65	42	30	32	17	47	13	39	22	65
Multiparous		304	54	99	47	205	58	91	58	63	68	19	53	20	61	12	35
Pre-pregnant Body Mass Index, kg/m^2	564	24·6	4·8	25·0	4·7	24·2	4·5	23·8	4·1	25·5	2·9	25·0	4·7	22·0	3·2	23·9	4·8
Maternal adult socioeconomic position[b]	570	0·01	1·0	0·5	0·8	−0·3	0·9	−0·2	0·8	−0·5	0·9	−1·0	1·2	0·1	0·7	0·1	1·1
Low social integration[c]	573	239	42	12	5·5	227	64	100	64	62	66	20	53	20	61	25	74
Early life socioeconomic position[d]	565	− 0·01	1·0	0·8	0·7	−0·5	0·8	−0·5	0·8	−0·5	0·8	−0·4	0·9	−0·6	0·9	−0·3	0·8
Gestational iron deficiency or anaemia[e]																	
Gestational ID by SF	572	178	31	26	12	152	43	75	48	37	39	20	54	9	27	11	32
Gestational ID by sTfR	562	32	6	0	0	32	9	19	12	9	10	4	12	0	0	0	0
Gestational ID by TBI	562	63	11	1	1	62	18	33	21	15	16	9	27	2	6	3	9
Gestational anaemia	564	32	6	3	1	29	8	17	11	6	7	4	11	2	6	0	0
Self-reported iron supplement use[f]																	
In early pregnancy	564	101	18	25	12	76	22	38	24	16	17	9	25	10	30	3	9
In gestational week 28	539	232	43	65	32	167	50	84	56	38	44	18	51	11	34	16	50
Postpartum	555	124	22	46	22	78	23	40	27	20	22	6	16	4	13	8	24
Dietary pattern[g]	555																
Healthy		164	30	122	58	42	12	14	9	6	7	5	15	7	22	10	29
Unhealthy		391	70	89	42	302	88	140	91	84	93	29	85	25	78	24	71
Chronic illness/medication	564																
Yes, associated with normochromic anaemia[h]		14	3	5	2	9	3	2	1	4	4	2	6	1	3	0	0
Yes, associated with hypochromic anaemia[i]		55	10	25	12	30	9	13	8	7	8	2	6	5	15	3	9
Delivery mode	573																
Normal vaginal delivery		473	83	175	81	298	84	134	85	79	86	30	79	27	82	28	82
Instrumental vaginal delivery[j]		58	10	21	10	37	10	17	11	8	9	6	16	3	9	3	9
Elective Caesarean section		29	5	16	7	13	4	5	3	1	1	1	3	3	9	3	9
Emergency Caesarean section		68	12	25	12	43	12	18	12	12	13	7	18	3	9	3	9
Birth complications	573																
Episiotomy		65	11	27	13	38	11	15	10	11	12	3	8	4	12	5	15
Third- or fourth-degree perineal tear		15	3	7	3	8	2	4	3	0	0	2	5	2	6	0	0
Obstructed labour		33	6	15	7	18	5	5	3	6	6	1	3	1	3	5	15
Manual removal of placenta		25	4	13	6	12	3	3	2	8	9	1	3	0	0	0	0

Continued

Table 1. Continued

	Total		Western Europe		Non-Western		South Asia		Middle East		Sub-Saharan Africa		East Asia		Eastern Europe	
	n or means	SD or %	*n* or means	SD or %	*n* or means	SD or %	*n* or means	SD or %	*n* or means	SD or %	*n* or means	SD or %	*n* or means	SD or %	*n* or means	SD or %
Postpartum haemorrhage[k]	33	6	15	7	18	5	5	3	6	6	1	6	1	3	5	15

Hb, haemoglobin; ID, iron deficiency; SEP, socioeconomic position; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI; total body iron.
[a] The STORK-Groruddalen multi-ethnic pregnancy cohort from Oslo, Norway, 2008–10.
[b] Variable derived from a principal components analysis of predefined individual and household markers SEP, with a higher score reflect higher SEP.
[c] Variable derived from a principal components analysis of predefined markers reflecting integration such as language skills, time of residence, social interaction with ethnic Norwegians and use of Norwegian media, with a higher score reflect higher social integration. 'Low social integration' represents participants belonging to the 40 % with the lowest scores.
[d] Variable derived from a principal components analysis of three childhood socio-demographic variables representing maternal SEP at age 10 years, with a higher score reflecting higher SEP.
[e] Gestational iron deficiency by (1) SF <15 μg/l; (2) sTfR >4·4 mg/l or (3) TBI <0 mg/kg; and gestational anaemia by trimester-specific haemoglobin <10·5 or 11·0 g/dl, analysed in mean gestational week 15-1.
[f] Self-reported intake of iron supplements during the past 2 weeks at all three study visits dichotomised into 'yes', covering daily or intermittent iron supplements, and 'no'.
[g] Data from a food frequency questionnaires collected in GW 28; four clusters were extracted using the Ward's method. Clusters were referred to as 'a healthier dietary pattern' *v.* three 'less healthy dietary patterns'; here dichotomised into 'healthy' and 'unhealthy' dietary pattern.
[h] Self-reported chronic illness or medication associated with normochromic anaemia (i.e. kidney or rheumatic disease, use of carbamazepine or infliximab).
[i] Self-reported chronic illness or medication associated with ID and hypochromic anaemia (i.e. gastrointestinal disease or Copper intrauterine device use before conception).
[j] Assisted vaginal delivery through forceps or vacuum.
[k] Excessive blood loss (≥500 ml) after delivery.

of ID and anaemia across ethnic groups were observed (data not shown). Furthermore, using SF concentration <12 μg/l as the threshold for ID, the overall prevalence of ID declined from 39 to 29 % in the total sample, approaching the prevalence levels for sTfR and TBI (Supplementary Table S1).

Associations between ethnicity and clinical factors with Hb, SF, sTfR and TBI

In unadjusted analyses, the Hb concentration was significantly lower in South Asians, Sub-Saharan Africans and East Asians compared with Western Europeans (Table 3). After adjusting for covariates, the ethnic differences were reduced and only South Asians had lower Hb concentration compared with Western European women. Gestational anaemia, other chronic illnesses/medication and postpartum haemorrhage were associated with lower Hb concentration, and higher age, self-reported intake of iron supplement in GW 28, and higher SEP in childhood were associated with higher Hb concentration.

Also for the ID indicators, ethnic differences were reduced after adjusting for covariates, as only South Asians had higher sTfR concentrations compared with Western Europeans (Table 3). Gestational ID and an 'unhealthy' dietary pattern were consistently associated with poorer iron status postpartum by all iron indicators. Postpartum haemorrhage was associated with higher OR of SF <15 μg/l and lower TBI concentration, and multiparous women and women with self-reported intake of iron supplement in the second trimester had better iron stores by all iron indicators.

Associations between level of integration and clinical factors with Hb, SF, sTfR and TBI

Adjusting for SEP had minimal effect on the effect estimates (data not shown), and when exploring relations with variables reflecting the level of social integration, as alternatives to ethnicity, we found no or only weak associations with anaemia or the ID measures (Supplementary Table S2). In the ethnic minority sub-sample, we observed that the level of social integration could not explain the higher sTfR – and lower Hb concentrations found in South Asians compared with other ethnic minority groups (Supplementary Table S3).

Discussion

To the best of our knowledge, this is one of very few studies from Europe to estimate the prevalence of postpartum anaemia and ID in a multi-ethnic population, and the only one comparing three indicators of ID, their relations and their relations to anaemia. One-fourth of the women had anaemia 14 weeks postpartum, two in five had ID by SF and about one in five had ID by sTfR or TBI. The mean Hb concentration was higher in those with ID by SF only (12·6 g/dl) than in those with ID by all indicators (11·6 g/dl). Women with ethnic origin outside Europe had a crude prevalence of anaemia and ID by sTfR and TBI that was about the double compared with European women. However, after adjusting for clinically

Table 2. Values for serum ferritin, soluble transferrin receptor (sTfR), total body iron (calculated from ferritin and sTfR concentrations) and haemoglobin concentration, and prevalence of abnormal values (iron deficiency and anaemia) 14 weeks postpartum in the STORK-Groruddalen study[a]

	n	SF, μg/l	SD or IQR	sTfR, mg/l	SD or IQR	TBI, mg/kg	SD or IQR	Hb, g/dl	SD or IQR
Mean in total sample[a]	573	23	18	3·7	1·7	2·7	3·7	12·5	1·0
Median in total sample[a]	573	18	10, 32	3·3	2·7, 4·1	3·1	0·4, 5·4	12·6	11·9, 13·2
Prevalence of abnormal values[b,c]	*n*	%	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI
Total sample[a]	573	39	35, 43	19	16, 22	22	19, 26	25	22, 29
Ethnic group[d]									
Western Europe	217	35	29, 42	12	8, 17	15	11, 20	14	10, 19
South Asia	157	44	36, 52	30	23, 38**	30	23, 37**	40	33, 48**
Middle East	94	39	30, 50	22	15, 32*	29	20, 39**	27	19, 37**
Sub-Saharan Africa	38	50	34, 66	21	11, 38	32	18, 49**	26	14, 43*
East Asia	33	36	21, 55	12	4, 29	15	6, 33	33	19, 52**
Eastern Europe	34	32	8, 50	6	1, 22	18	8, 35	18	8, 36

Hb, haemoglobin; ID, iron deficiency; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI; total body iron.
[a] The STORK-Groruddalen multi-ethnic pregnancy cohort from Oslo, Norway, 2008–10. *N* 573 for serum ferritin, *n* 568 for soluble transferrin receptor (sTfR) and total body iron and *n* 569 for haemoglobin.
[b] Abnormal values are presented as percentage (95 % CI), defined as serum ferritin <15 μg/l, soluble transferrin receptor (sTfR) >4·4 mg/l, total body iron <0 mg/kg and haemoglobin <12·0 g/dl.
[c] Haemoglobinopathy (*n* 4) was either self-reported, identified from the HPLC (Tosoh G8, Tosoh Corporation) analysis of glycated haemoglobin, or from a combination of microcytic anaemia and high ferritin.
[d] The difference in the prevalence of abnormal values between Western Europeans and each non-Western group were tested by χ^2 test.
*$P < 0·05$, **$P < 0·01$.

relevant covariates, these ethnic differences mostly disappeared, with only South Asians having lower Hb concentration and higher sTfR concentration. The level of social integration into the Norwegian mainstream society did not explain these differences.

Different biomarkers are used to measure ID in clinical practice, SF being the most commonly used[23]. A Nordic study compared ID defined by SF <15 μg/l to bone marrow staining, and found SF to have a 75 % sensitivity and 98 % specificity[24]. The comparison of the different iron indicators revealed a larger dispersion for median SF concentration than mean sTfR concentration between the ethnic groups for the same Hb concentration interval, which could suggest that sTfR has less random variation and is a reliable iron indicator. Furthermore, Fig. 1 shows that the mean Hb concentration in those with ID by SF is only slightly influenced (mean Hb 0·2 g/dl lower compared with those with no ID by any marker), while it is significantly decreased in those with ID by sTfR and TBI (mean Hb 1·2 g/dl lower). This supports findings from others that SF covers an earlier stage of ID, called 'depleted body iron stores', while sTfR and TBI reflect a later stage of ID where the synthesis of Hb is affected[11]. This is also supported by a higher proportion of anaemia among those with ID by sTfR or TBI compared with those with ID by SF. To achieve an even higher concordance between ID assessed by SF and TBI, assuming TBI being a

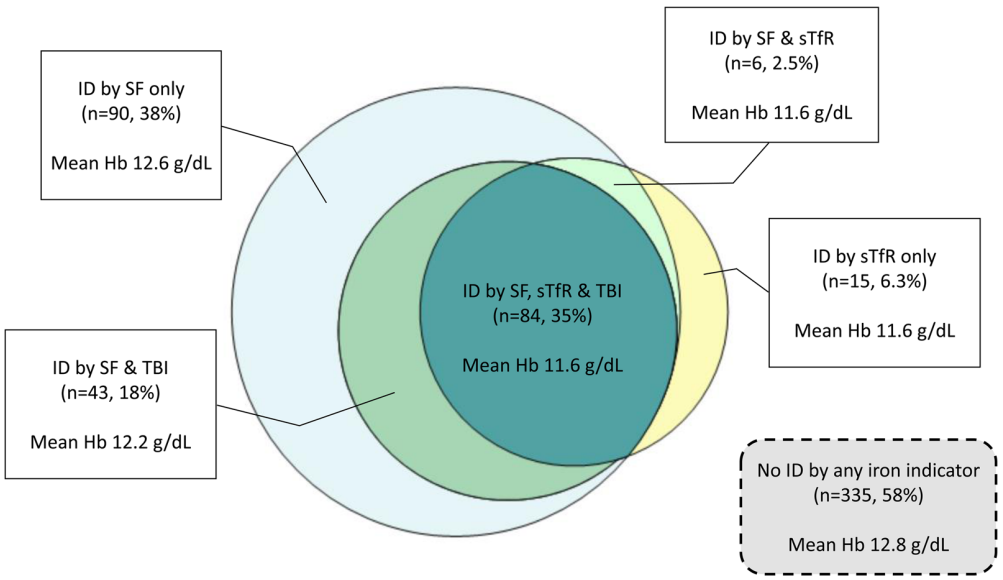

Fig. 1. Venn diagram for postpartum women with iron deficiency by ≥1 of the three iron indicators serum ferritin, soluble transferrin receptor and total body iron (*n* 238) 14 weeks postpartum in the STORK-Groruddalen study[a]. Hb, haemoglobin; ID by SF, iron deficiency by serum ferritin concentration <15 μg/l; ID by sTfR, iron deficiency by soluble transferrin receptor concentration >4·4 mg/l; ID by TBI, iron deficiency by total body iron concentration <0 mg/kg. [a]The STORK-Groruddalen multi-ethnic pregnancy cohort from Oslo, Norway, 2008–10.

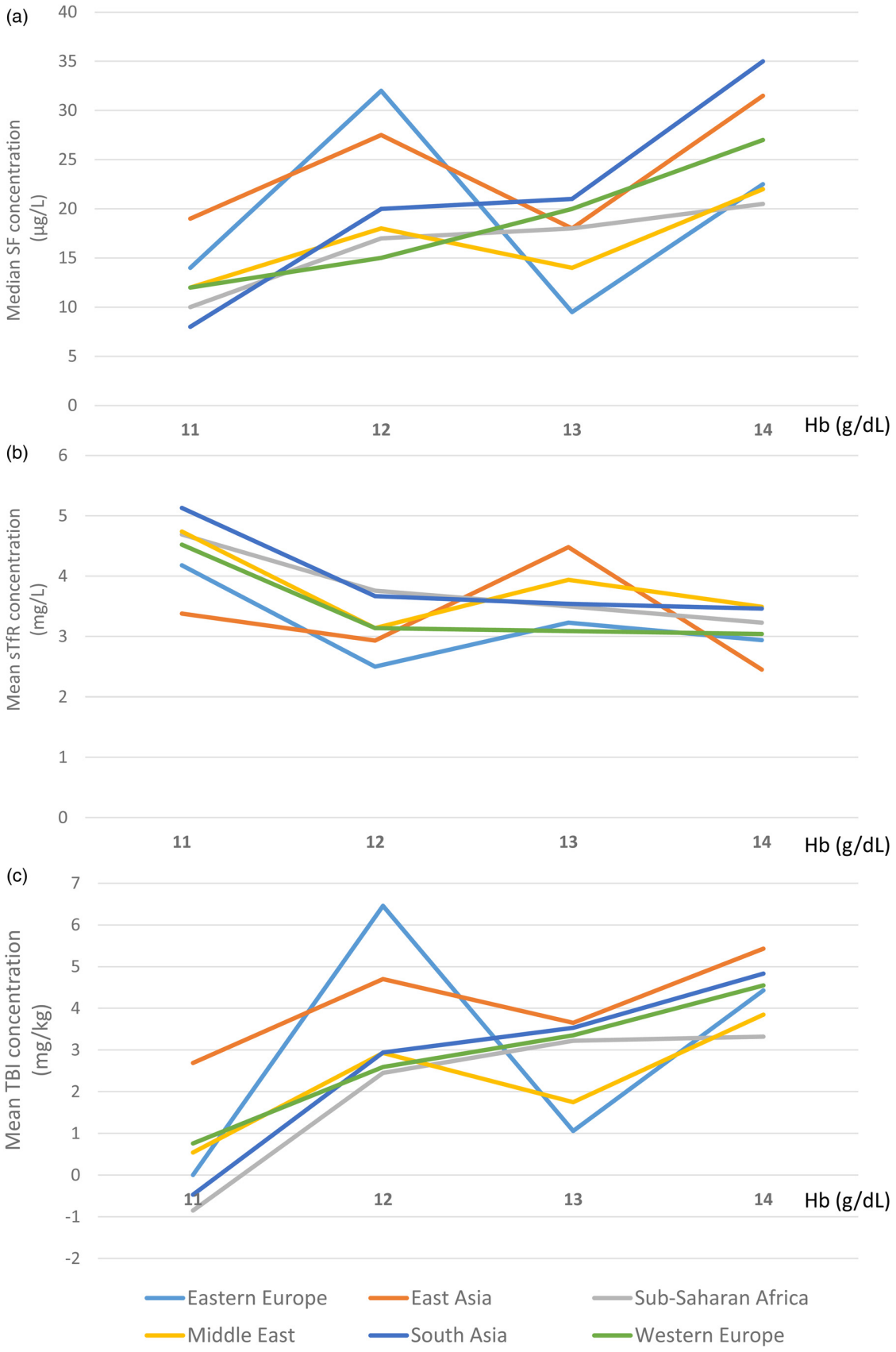

Fig. 2. Median serum ferritin concentration (µg/l), mean soluble transferrin receptor concentration (mg/l) and mean total body iron concentration (mg/kg) in four haemoglobin concentration intervals (g/dl)[a] at the postpartum visit in the STORK-Groruddalen multi-ethnic pregnancy cohort from Oslo, Norway, 2008–10. Hb, haemoglobin; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI, total body iron. [a]Haemoglobin as grouped midpoint; 11 (8·0–11·9); 12 (12·0–12·5); 13 (12·6–13·0) and 14 (13·1–15·0).

Table 3. Logistic regression analysis of serum ferritin <15 μg/l, and linear regression analyses of soluble transferrin receptor, total body iron and haemoglobin concentration 14 weeks postpartum in the STORK-Groruddalen study[a]

	SF <15 μg/dl				sTfR, mg/l				TBI, mg/kg				Hb, g/dl			
	OR	95 % CI	adj OR	95 % CI	β	95 % CI	adj β	95 % CI	β	95 % CI	adj β	95 % CI	β	95 % CI	adj β	95 % CI
		R^2 0·13				R^2 0·14				R^2 0·15				R^2 0·17		
Ethnicity (Western European = reference)																
South Asia	1·4	0·9, 2·2	1·1	0·6, 1·7	0·9	0·6, 1·3**	0·8	0·4, 1·2**	−1·2	−2·0, −0·05**	−0·4	−1·2, 0·5	−0·6	−0·8, −0·4**	−0·4	−0·7, −0·2**
Middle East	1·2	0·7, 1·9	0·8	0·4, 1·5	0·6	0·1, 1·0**	0·4	−0·1, 0·9	−0·9	−1·8, −0·01*	−0·1	−1·1, 0·9	−0·2	−0·8, 0·01	0·03	−0·3, 0·3
Sub-Saharan Africa	1·8	0·9, 3·6	1·0	0·4, 2·3	0·5	−0·1, 1·1	0·4	−0·3, 1·0	−1·2	−2·5, 0·1	−0·1	−1·5, 1·3	−0·4	−0·8, −0·03*	−0·1	−0·5, 0·3
East Asia	1·0	0·5, 2·2	0·8	0·3, 1·8	0·2	−0·4, 0·8	0·2	−0·4, 0·9	0·6	−0·7, 1·9	0·9	−0·5, 2·2	−0·4	−0·8, −0·1*	−0·2	−0·6, 0·2
East Europe	0·9	0·4, 1·9	0·5	0·2, 1·3	−0·1	−0·7, 0·5	−0·1	−0·7, 0·5	0·01	−1·3, 1·3	0·8	−0·5, 2·2	0·3	−0·02, 0·7	0·5	0·1, 0·9*
Postpartum week	1·0	0·9, 1·0			−0·1	−0·1, −0·01*	−0·3	−0·1, −0·03**	0·1	−0·04, 0·2			0·02	−0·01, 0·05		
Age, per 5 year	0·8	0·7, 1·0*			−0·2	−0·4, −0·1**			0·7	0·4, 1·0**	0·5	0·2, 0·9**	0·1	−0·01, 0·02	0·1	0·01, 0·2*
Multiparous (primiparous = reference)	0·6	0·4, 0·8**	0·6	0·4, 0·8**	−0·2	−0·5, 0·1	−0·3	−0·6, −0·04*	−0·1	−0·4, 0·2	0·8	0·1, 1·4*	−0·03	−0·1, 0·1	−0·2	−0·3, 0·02
Pre-pregnant Body Mass Index, per 5 kg/m^2	1·0	0·8, 1·2			0·2	0·03, 0·3*			−0,1	−0·4, 0·4			0·03	−0·1, 0·1		
Adult socioeconomic position[b]	0·8	0·7, 0·9*			−0·3	−0·4, 0·1**			0·6	0·3, 0·9**			0·2	0·1, 0·2**		
Early life socioeconomic position[c]	0·9	0·7, 1·0			−0·2	−0·4, −0·1**			0·3	0·01, 0·6*			0·2	0·1, 0·3**	0·1	0·03, 0·2*
Gestational ID or anaemia (no = reference)[d]	1·3	0·9, 1·8	1·6	1·0, 2·5*	2·0	1·4, 2·6**	1·7	1·1, 2·4**	−2·5	−3·4, −1·5**	−2·5	−3·5, −1·5**	−0·9	−1·3, −0·6**	−0·9	−1·3, −0·6**
Iron supplementation use in GW 28 (no = reference)[e]	0·6	0·4, 0·9*	0·5	0·3, 0·7**	−0·3	−0·6, −0·004*	−0·6	−0·9, −0·3**	0·8	0·1, 1·4*	1·3	0·7, 1·9**	−0·002	−0·2, 0·2	0·2	0·004, 0·3*
Unhealthy dietary pattern (healthy = reference)[f]	2·2	1·5, 3·3**	2·8	1·7, 4·5**	0·6	0·3, 1·0**	0·4	0·1, 0·8*	−1·5	−2·2, −0·8**	−1·3	−2·1, −0·5**	−0·2	−0·4, −0·1**	0·04	−0·2, 0·2
Chronic illness/medication associated with normochromic anaemia (no = reference)[g]	1·5	0·5, 4·4			0·8	−0·1, 1·7			−0·1	−2·0, 1·9			−0·8	−1·3, −0·2**	−0·8	−1·4, −0·3**
Chronic illness/medication associated with hypochromic anaemia (no = reference)[h]	0·7	0·4, 1·3			−0·2	−0·7, 0·3			0·4	−0·7, 1·4			−0·1	−0·3, 0·2	−0·1	−0·3, 0·2
Operative delivery (no = reference)[i]	1·3	0·9, 1·9			−0·03	−0·3, 0·3			−0·5	−1·2, 0·1			0·01	−0·2, 0·2		
Postpartum haemorrhage (<500 mL = reference)	2·9	1·4, 6·0**	3·3	1·5, 7·4**	0·3	−0·3, 0·9	0·3	−0·3, 0·9	−1·6	−2·9, −0·3*	−2·0	−3·3, −0·7**	−0·2	−0·5, 0·2	−0·4	−0·8, −0·1*
Birth complications (no = reference)[j]	1·4	0·9, 2·2			0·07	−0·3, 0·5			−0·8	−1·6, −0·04*			−0·1	−0·3, 0·2		

Adj, adjusted; ID, iron deficiency; GW, gestational week; Hb, haemoglobin; SEP, socioeconomic position; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI; total body iron.

[a] The STORK-Groruddalen multi-ethnic pregnancy cohort from Oslo, Norway, 2008–10. Multivariable regression analyses with stepwise backward elimination; ethnicity and clinical relevant variables were forced into the model.

[b] Variable derived from a principal components analysis of predefined individual and household markers of SEP, with a higher score reflect higher SEP.

[c] Variable derived from a separate principal components analysis of three childhood socio-demographic variables representing maternal SEP at age 10 years, with a higher score reflecting higher SEP.

[d] Gestational iron deficiency by (1) SF <15 μg/l; (2) sTfR >4·4 mg/l or (3) TBI <0 mg/kg; and gestational anaemia by trimester-specific haemoglobin < 10·5 or 11·0 g/dl, analysed in mean gestational week 15·1.

[e] Self-reported intake of iron supplements during the past 2 weeks at all three study visits dichotomised into 'yes', covering daily or intermittent iron supplements, and 'no'.

[f] Data from a food frequency questionnaires collected in GW 28; four clusters were extracted using the Ward's method. Clusters were referred to as 'a healthier dietary pattern' *v.* three 'less healthy dietary patterns'; here dichotomised into 'healthy' and 'unhealthy' dietary pattern.

[g] Self-reported chronic illness or medication associated with normochromic anaemia (i.e. kidney or rheumatic disease, use of carbamazepine or infliximab).

[h] Self-reported chronic illness or medication associated with ID and hypochromic anaemia (i.e. gastrointestinal disease or Copper intrauterine device use before conception).

[i] Operative delivery: Caesarean section (elective and emergency) or assisted vaginal delivery (forceps or vacuum), with normal vaginal delivery as a reference.

[j] A composite variable created by combining following four birth complication; episiotomy, third- and fourth-degree perineal tear, obstructed labour and manual removal of placenta.

* $P < 0·05$, ** $P < 0·01$.

better predictor of ID, a lower threshold for ID by SF might perform better. This is supported by our findings that the prevalence of ID by SF <12 μg/l, also a widely used definition[16], provided more comparable prevalence rates to those for sTfR and TBI (29 % *v.* 19 and 22 %, respectively). SF is an acute phase protein and the concentration is known to increase with infection and inflammatory processes, and can lead to an underestimate of ID[17]. sTfR and TBI are believed to better assess the severity of ID, as the sTfR is proportional to the cellular iron demand, but recent studies show that also these biomarkers may be affected by these processes, more specifically low-grade chronic inflammation that can result in an overestimate of ID[11,12]. We therefore ran the analyses in women without inflammatory response (CRP < 5), and found only minor changes in the mean/median concentration and prevalence rates of ID and anaemia across ethnic groups and conclude that inflammation could not explain the differences observed in our population. Therefore, we chose not to adjust SF, sTfR or TBI values for CRP to correct for inflammation, as suggested by some others[11,12,17].

The crude total prevalence estimates for postpartum anaemia are in accordance with other studies from Europe[1,25], the US[26,27] and estimates published by the WHO[2,28] when measured at least >8 weeks postpartum. Although ethnic minority background is recognised as a risk factor[25,26,29–31], we did not find any studies from Europe reporting prevalence rates stratified by ethnic groups. The prevalence of postpartum anaemia in minority groups in our study was slightly lower than in the US[32], but generally similar to those reported from their country of origin[2], although prevalence rates reported from South Asian countries differ considerably (23–62 %)[28].

We have only identified two studies reporting postpartum iron status[26,33], and no studies comparing three different iron indicators or different ethnicities in postpartum women. The prevalence of postpartum ID defined by SF <12 μg/l in the NHANES study[26] was about half of the prevalence in our study (13 % *v.* 29 %) and the mean SF concentration in postpartum women attending the special supplemental nutrition programme for women, infants and children (the WIC-programme) was higher than in our study (37 μg/l *v.* 23 μg/l)[33]. Of note, few women in our cohort used oral iron supplementation at postpartum. Studies among women in reproductive age, however, consistently indicate that ethnic minority and low SEP groups are at higher risk for the condition than the majority population[6,34–38]. In our study, East Asians had lower prevalence of ID (Fig. 2) and generally better iron status than the other ethnic minority groups. Although caution is needed in the interpretation of these findings due to the low number, East Asian women also had higher vitamin D concentrations compared with the other ethnic minority groups[8], indicating that they may have a generally better nutritional status. Lastly, in line with others, we found that gestational anaemia and ID, an inadequate iron intake, and also postpartum haemorrhage were strongly associated with Hb concentration and poor postpartum iron status[25–27,30,39,40].

Our study did not suggest that socioeconomic position or level of social integration played an important role in explaining ethnic differences in postpartum anaemia and ID. Women with South Asian origin had higher sTfR and lower Hb concentrations, both when compared with Western Europeans and with other ethnic minority groups, also after adjusting for covariates, including different measures of social integration, dietary pattern and life course SEP. We can, however, only speculate if this could be related to specific dietary factors among women with South Asian origin, such as Chapatti-based meals which contains a high level of phytates, a well-known inhibitor of iron absorption.

Strength and limitations

The present study's major strength is its population-based cohort design with a high proportion of ethnic minorities, found to be fairly representative for the main ethnic groups of pregnant women living in Oslo, Norway. We present more robust data for anaemia than in our previous study from early pregnancy[5], and were therefore able to compare the relations between three iron indicators and this clinical outcome. We have a broad, high-quality data set that enabled us to explore the relations between simultaneously measured Hb, and three indicators of ID and adjust for relevant covariates, and including socioeconomic conditions across the woman's life course, and we performed additional analyses to explore the impact of integration. There is, however, also limitations to report, including the possibility of heterogeneity within relatively broad ethnic groups. Furthermore, the number in some ethnic groups was low. We had some loss to follow-up at the postpartum visit, but we prioritised ethnic minority women for blood sampling. Low SF concentrations indicate ID, but different thresholds (<15 or <12 μg/ml) are used in the diagnosis of ID. To ease comparison with other studies, we primarily used the definition used by WHO when estimating ID[16,23]. We lack detailed information on iron intake, and postpartum haemorrhage was not measured exactly, but based on clinical judgement. We may also have underestimated the prevalence of haemoglobinopathy.

Clinical implications

Hb measurements are often performed shortly after delivery, and only in women with postpartum haemorrhage or in women presenting symptoms of anaemia. In view of the clinical consequences of postpartum anaemia, a more active case-finding among high-risk women, such as most ethnic minority women, women with gestational anaemia and ID, and women with excessive postpartum bleeding seems needed – and could be implemented in clinical guidelines for later postpartum follow-up visit. This is also supported by the WHO target to reduce anaemia with 25 % by 2025[41]. Laboratory measurements are essential for a proper diagnosis of ID. Although more expensive, sTfR and TBI seem to assess the severity of ID better than SF. As it is considered clinically important to prevent the later stages of ID associated with IDA, our findings suggest that each iron indicator offers a slightly different interpretation of the physiological processes involved in the body's response to low iron stores. Further research is

needed to disentangle the different stages and pathways in more detail.

Conclusion

We present the first population-based study from Europe on postpartum anaemia and ID using three different iron indicators in a multi-ethnic sample of women. In total, 25 % of the women had postpartum anaemia measured 14 weeks after delivery. The prevalence of ID varied between 20 and 40 % by the different iron indicators. The current threshold values used to define ID by sTfR and TBI probably identified a more severe iron-deficient population compared with ID assessed by SF threshold values. Gestational anaemia or ID, insufficient iron intake in pregnancy and postpartum haemorrhage were independent risk factors of postpartum anaemia and ID, but women with South Asian origin had more anaemia and ID by sTfR, even when adjusting for covariates. To improve women's postpartum health status, clearer recommendation about measuring Hb and iron status in women at risk should be implemented in clinical guidelines in Norway and internationally.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/jns.2022.45.

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M. N. A. and L. S. had full access to all data in this study. M. N. A. is responsible for the integrity of data and accuracy of the data analysis; M. N. A., L. S., J. P. B. and A. K. J. revised the study concept and design, contributed to analysis, tables and interpretation of data and critical revision of the manuscript. R. S. F. guided the statistical analysis and revised the tables; and all authors read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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RESEARCH

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The impact of recommending iron supplements to women with depleted iron stores in early pregnancy on use of supplements, and factors associated with changes in iron status from early pregnancy to postpartum in a multi-ethnic population-based cohort

Marthe-Lise Næss-Andresen[1*], Anne Karen Jenum[2], Jens Petter Berg[3], Ragnhild Sørum Falk[4] and Line Sletner[5,6]

Abstract

Background We aimed to evaluate the impact of recommending supplementation to pregnant women with serum ferritin (SF) < 20 µg/L in early pregnancy on use of supplements, and to explore which factors were associated with changes in iron status by different iron indicators to 14 weeks postpartum.

Methods A multi-ethnic population-based cohort study of 573 pregnant women examined at mean gestational week (GW) 15 (enrolment), at mean GW 28 and at the postpartum visit (mean 14 weeks after delivery). Women with SF < 20 µg/L at enrolment were recommended 30-50 mg iron supplementation and supplement use was assessed at all visits. Change of SF, soluble transferrin receptor and total body iron from enrolment to postpartum were calculated by subtracting the concentrations at the postpartum visit from that at enrolment. Linear and logistic regression analyses were performed to assess associations between use of supplements in GW 28 and changes in iron status and postpartum iron deficiency/anaemia. Change of iron status was categorized into 'steady low', 'improvement', 'deterioration', and 'steady high' based on SF status at enrolment and postpartum. Multinomial logistic regression analyses were performed to identify factors associated with change of iron status.

Results At enrolment, 44% had SF < 20 µg/L. Among these women (78% non-Western European origin), use of supplements increased from 25% (enrolment) to 65% (GW 28). Use of supplements in GW 28 was associated with improved iron levels by all three indicators ($p < 0.05$) and with haemoglobin concentration ($p < 0.001$) from enrolment to postpartum, and with lower odds of postpartum iron deficiency by SF and TBI ($p < 0.05$). Factors positively associated with 'steady low' were: use of supplements, postpartum haemorrhage, an unhealthy dietary pattern and South Asian ethnicity ($p \leq 0.01$ for all); with 'deterioration': postpartum haemorrhage, an unhealthy dietary pattern,

*Correspondence:
Marthe-Lise Næss-Andresen
m.l.nass-andresen@medisin.uio.no
Full list of author information is available at the end of the article

primiparity and no use of supplements ($p < 0.01$ for all), and with 'improvement': use of supplements, multiparity and South Asian ethnicity ($p < 0.03$ for all).

Conclusions Both supplement use and iron status improved from enrolment to the postpartum visit among women recommended supplementation. Dietary pattern, use of supplements, ethnicity, parity and postpartum haemorrhage were identified as factors associated with change in iron status.

Keywords Nutrition, Iron deficiency, Anaemia, Supplementation, Pregnancy, Ethnic minority, Maternal and Child health

Background

Iron is crucial for numerous physiological and cellular processes, and iron deficiency (ID) has diverse health consequences [1]. Persistent ID will lead to depleted iron stores, usually defined by a low serum ferritin (SF) concentration [1], and to iron deficiency anaemia (IDA). Iron needs are tripled during pregnancy due to expansion of maternal red cell mass and growth of the fetus and placenta [2]. ID is associated with maternal fatigue, potentially poorer quality of life, increased risk of postpartum depression, and a higher risk to develop IDA [3]. Gestational anaemia is associated with maternal, perinatal and neonatal mortality, low birth weight and preterm birth [1, 4, 5]. In 2019, the global anaemia prevalence in pregnant women was 36.5%, a slight decrease from 2000 [6], and ID is estimated to account for half of the cases of anaemia [7]. In pregnancy, interpretations of SF values can be hampered by infections, inflammation and pregnancy related changes [3, 8], and soluble transferrin receptor (sTfR) and total body iron (TBI) have been suggested as more valid iron indicators [9, 10].

Iron supplementation programs are implemented in low-income countries to meet the WHO Global Nutrition Target from 2012 to reduce the prevalence of anaemia with 25% by 2025 [7]. There is international consensus to prevent gestational anaemia, and WHO recommends daily or intermittent iron supplementation, depending on the populations' risk of maternal anaemia [11]. In high-income countries the guidelines differ, some recommend universal supplements from early pregnancy [12], while others recommend supplements only to women with ID or anaemia [5, 13–15]. The Norwegian recommendations for antenatal care have changed several times over the last decades. From 1995, women in early pregnancy were recommended to be screened by SF and haemoglobin (Hb) concentration, and iron supplementation was recommended if the SF concentration was below 60 µg/L [16]. In 2005, the guidelines were revised, and recommended to screen women by Hb concentration only, and further to recommend supplementation if anaemia was detected [17]. These were the guidelines during the data collection period of the present study. In 2018 guidelines were revised again, now recommending women to be screened by SF and Hb concentration at their first antenatal visit, and to recommend supplementation if SF concentration is below 70 µg/L [14]. The current guideline aims to prevent gestational and postpartum ID and IDA, and is supported by a Nordic systematic review concluding that 40 mg iron supplementations from gestational week (GW) 18-20 is an effective strategy to prevent ID in more than 90% and IDA in more than 95% of women at delivery and at 6-8 weeks postpartum [1]. How to ensure that women follow the recommendations and increase their use of iron supplements is less clear. Previous studies have shown that maternal age, socioeconomic status, parity, body mass index (BMI) and ethnic origin are factors affecting the use of iron supplements during pregnancy [18, 19].

The STORK-G study included hard-to-reach-women, as all information, material and questionnaires were translated into eight languages, and about 20% of women with non-Western European origin were assisted by professional interpreters. We have previously described the prevalence of gestational and postpartum ID by different indicators and anaemia in our cohort [8, 20]. We found significantly higher prevalence of ID and anaemia at GW 15 in ethnic minority women compared to Western European women [8], while at the postpartum visit the ethnic disparities had almost disappeared [20]. We therefore wanted to assess factors associated with the change in iron status. Women with SF concentration < 20 µg/L at enrolment (GW 15), were provided written information describing their iron status and recommended 30-50 mg iron supplementation. The aims of this paper were therefore (1) to evaluate the impact of this simple recommendation of supplementation to pregnant women with depleted iron stores (SF < 20 µg/L) in early pregnancy on the self-reported use of supplements later in pregnancy in a population with diverse ethnic origin and socioeconomic status, and (2) to explore which factors were associated with changes in iron status by different iron indicators from enrolment to 14 weeks postpartum in women recommended supplementation or not.

Subjects and methods

Study population and data selection

Data from the population-based multi-ethnic STORK-G cohort were collected at public Child Health Clinics for primary antenatal care in three administrative districts in Oslo, Norway between 2008 and 2010. The study methods have been described in detail elsewhere [21]. 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. Pregnant 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 GW, (IV) were not suffering from diseases necessitating intensive hospital follow-up during pregnancy, (V) could communicate in Norwegian or any of the eight specified languages and (VI) were able to provide written informed consent.

In total, 823 healthy women were enrolled at mean GW 15 ± standard deviation (SD) 3.4 in this cohort study; referred to as 'at enrolment' with planned follow-up visits in GW 28 (GW 28 ± 1.3) and approximately three months after delivery (13.9 ± 2.4 weeks after delivery), referred to as 'postpartum visit'. Questionnaire data covering a wide range of health issues were collected through interviews by authorized study personnel, assisted by professional interpreters when needed at all three study visits [21]. In addition, clinical measurements were collected according to the study protocol. Participating women were found representative for the main ethnic groups of pregnant women attending the Child Health Clinics [21]. The study protocol and the consent-forms were approved by The Regional Committee for Medical and Health Research Ethics for South Eastern Norway and The Norwegian Data Inspectorate.

Measurements of iron indicators (SF, sTfR and TBI) and Hb

Blood samples were drawn at all visits (2008-2011) [21] and sent the same day for analyses of SF and Hb at the Department of Multidisciplinary Laboratory Medicine and Medical Biochemistry at Akershus University Hospital, Lørenskog, Norway. SF concentration was measured using an electro-chemiluminescence immunoassay (ECLIA) method (Unicel DxI 800 from Beckman Coulter; inter-assay CV < 7%). Hb was measured using an SLS method (XE 5000 from Sysmex; inter-assay CV < 0.7%).

In 2016, sTfR was analysed by ELISA (Modular P800 from Roche; inter-assay CV < 5%), at the Department of Medical Biochemistry at Oslo University Hospital, Oslo, Norway, using biobanked serum samples. We calculated TBI according to Cook [22] from the ratio of sTfR concentration (by Flowers assay) to SF concentration: $-[\log_{10}(\text{sTfR} \times 1000 \div \text{SF}) - 2.8229] \div 0.1207$). To convert our Roche sTfR concentration to Flowers sTfR concentrations, we used the conversion equation Flowers sTfR = 1.5 × Roche sTfR + 0.35 mg/L [22].

Recommendation of supplementation

For ethical reasons, and according to the protocol, women with depleted iron stores (SF < 20 µg/L) at enrolment were provided written information describing their SF concentration, recommended to start iron supplementation 30-50 mg/day, and to consult their General Practitioner (GP) for follow-up. At enrolment, in GW 28, and postpartum, all participants were asked about their intake of iron supplements during the past two weeks and to specify the name of the compounds used, and the number of tablets per day and per week. Of those reporting type of iron supplements, 57% used ferrous gluconate, 39% ferrous sulphate, and 4% ferrous fumarate. However, we were only able to calculate the exact iron intake in GW 28 in about 40% of cases. Iron supplementation was therefore dichotomized as 'no intake' and 'intake of iron supplementation', covering daily or intermittent iron supplement use.

Outcome measures

Our outcome measures were postpartum ID (defined as SF concentration < 15 µg/L, sTfR concentration > 4.4 mg/L or TBI < 0 mg/kg) and anaemia (defined as Hb < 12.0 g/dL), and changes in SF, sTfR, TBI and Hb concentrations, calculated by subtracting the concentrations at the postpartum visit from that at enrolment for each indicator. Further, according to the SF concentration at enrolment the sample was first dichotomized, as either 'recommended supplements' (SF < 20 µg/L) or 'not recommended supplements' (SF ≥ 20 µg/L). Second, we further categorized women with SF > 20 µg/L into four groups (20-29, 30-49, 50-69, > 70 µg/L). Third, the sample was categorized based on the postpartum iron status (SF < 15 µg/L or ≥ 15 µg/L), resulting in four distinct groups called 'steady low', 'improvement', 'deterioration', and 'steady high', reflecting their change in iron status by SF from enrolment to postpartum.

Sociodemographic variables

Maternal age was calculated from date of birth and date at enrolment in the present study. Parity was dichotomized into primiparous (first pregnancy lasting > 22 weeks) and parous (one or more previous births) women. Pre-pregnancy BMI (kg/m^2) was calculated from self-reported weight before pregnancy and height measured at enrolment [21]. GW was primarily derived from the first day of the mother's last menstrual period (LMP), but ultrasound-derived gestational age was used in 7% of pregnancies where there were reasons to believe the LMP-derived gestational age was uncertain [23]. Ethnicity was defined

as the participant's country of birth, or the participant's mother's country of birth if the participant's mother was born outside Europe or North America [21], and grouped as Western Europeans (Norway, other Western European countries and North America), South Asians (primarily Pakistan and Sri Lanka), Middle Easterners (primarily Iraq, Morocco, and Turkey), East Asians (includes East- and South-East Asian countries, primarily Vietnam and The Philippines), Sub-Saharan Africans (primarily Somalia) and Eastern Europeans (primarily Poland, Kosovo, and Russia). Maternal present socioeconomic position (SEP) was a score derived from a principal component analysis (PCA) of 11 different demographic variables [24]. The variables contributing most to the score were individual level data about education, occupational class and employment status, and household variables as own or renting tenure and rooms per person in the household. The score was standard normally distributed, with a higher score reflecting higher SEP.

Variables potentially associated with iron metabolism

From questions about the women's medical history, we categorized three groups; (I) no medical conditions associated with anaemia or ID, (II) self-reported chronic illness or medication associated with ID or normochromic, and (III) self-reported chronic illness or medication associated with ID or hypochromic anaemia [8]. Data from a food frequency questionnaire, developed to capture dietary patterns in a multi-ethnic sample, were collected in GW 28. Four clusters were extracted using the Ward's method [25]. Clusters were referred to as 'a healthier dietary pattern' vs. three 'less healthy dietary patterns' [25], here dichotomized into 'healthy' and 'unhealthy'. The 'healthy dietary pattern' represented more frequent intake of fruit, vegetables, wholegrain bread with pate and meat spread, and meat, i.e. food items representing relatively high iron content (heme and non-heme) and foods rich in vitamin C which again improves the bioavailability of iron compared to the other dietary pattern. After conducting sensitivity analyses excluding women with possible inflammation (elevated C-reactive protein (CRP)) in our previous published papers [8, 20], we found only minor changes in the mean and median values and in the prevalence of ID and anaemia across ethnic groups and concluded that inflammation did not explain the differences observed in our population. We therefore chose not to adjust SF, sTfR or TBI values for CRP to correct for inflammation, as suggested by some others [26–28].

Birth-related variables potentially associated with postpartum iron status

We have detailed data on birth complications extracted from hospital birth records. Delivery mode was categorized as normal vaginal delivery, instrumental vaginal delivery (i.e. forceps or vacuum assisted vaginal delivery), elective caesarean section and emergency caesarean section. Blood loss after delivery was dichotomized into <500 mL and ≥ 500 mL, where the latter was defined as postpartum haemorrhage. We constructed a composite index for birth complications reflecting the presence of at least one of the following complications; episiotomy, third- or fourth-degree perineal tear, obstructed labour and manual removal of placenta, due to small numbers in each of the categories.

Sample size

Of the 823 women enrolled in mean GW 15, 644 (78%) women gave birth to a live and singleton baby and attended the postpartum visit. For this study, we included participants with values for SF from both visits, resulting in a total sample of 573 women (Flow chart, Additional file 1, Figure S1). There were no significant differences between the study sample and the 250 excluded women for age, parity, pre-pregnant BMI, and SEP (data not shown). However, the study sample consisted of a slightly larger proportion of ethnic minority women compared to the excluded women, as they were prioritised for fasting blood samples at the postpartum visit due to resource limitations [20].

Statistical analyses

Descriptive statistics are presented as frequencies with proportions for categorical variables and mean with SD or medians with interquartile range for continuous variables. The sTfR and TBI values were approximately normally distributed, and SF skewed to the left. Difference in SF from enrolment to postpartum within the five SF categories at enrolment was assessed by Wilcoxon signed rank test.

We performed multinomial logistic regression analyses to investigate factors associated with belonging to one of the following three groups 'steady low', 'improvement' and 'deterioration', reflecting the women's iron status at enrolment and postpartum, using 'steady high' as the reference. Both unadjusted and adjusted odds ratios (OR) are presented. GW, age, parity, SEP, diet, ethnicity, use of iron supplementation in GW 28 and postpartum, postpartum haemorrhage and birth complications were included in the fully adjusted model.

Furthermore, we stratified the cohort in two subsamples based on whether the women were recommended use of iron supplements at enrolment or not. In each stratum, we performed two sets of regression analyses. Linear regression analyses were performed to assess the association between self-reported use of iron supplementation in GW 28 and changes in SF, sTfR, TBI, and Hb

concentrations from enrolment to the postpartum visit. Logistic regression analyses were performed when postpartum ID (defined by SF, sTfR and TBI) and anaemia were the outcome. Adjustments were made for GW, age, parity, SEP, diet, ethnicity, and use of iron supplements in pregnancy. In addition, SF concentration at enrolment was included to reduce dilution of regression to the mean. In a final model we also adjusted for use of supplements postpartum, postpartum haemorrhage, and birth complications although these were not true confounders, but potentially strongly associated with the outcome. The estimates changed marginally by including the last covariates. Results are presented as β-coefficients and ORs with accompanied 95% confidence intervals (CI). SPSS version 28 and Stata version 16 were used for statistical analysis.

Results

At enrolment (mean GW 15.1 (SD ± 3.4), maternal age was 29.7 ± 4.8 years, pre-pregnant BMI was 24.6 ± 4.8 kg/m^2, 45% were primiparous and 62% had ethnic origin from countries outside Western Europe (Table 1). Iron supplementation was recommended to 252 (44%) women with depleted iron status (SF < 20 µg/L) at enrolment in early pregnancy, of whom 204 (78%) were of non-Western European origin.

From enrolment in early pregnancy to GW 28, the reported use of iron supplementation increased from 25 to 65% in women recommended supplements, and from 13 to 26% in the group not exposed to our recommendations. Further, the proportion of women reporting use of iron supplements at GW 28 was higher (68%) in the group with 'improvement' in iron status from enrolment in early pregnancy to postpartum (mean postpartum week 13.9 (SD ± 2.5), compared to the group with 'steady low' iron status (55%). It was also higher in women with 'steady high' (29%) compared to the group with 'deterioration' (15%) in iron status (Table 1**).**

The effect of supplemental use

The change in SF concentration from enrolment in early pregnancy to the postpartum visit, stratified into five groups by SF concentration at enrolment, is illustrated in Fig. 1. A moderate increase in median SF concentration was observed in women recommended iron supplementation ($p < 0.001$), while a reduction was seen in all other groups ($p < 0.001$ in all). As a consequence, the prevalence of postpartum ID by SF (defined as SF < 15 µg/L) was approximately 40% in all groups, except in women with SF concentration ≥ 70 µg/L at enrolment (24%) (Additional file 2, Table S1). The prevalence of postpartum ID in the group recommended iron supplementation (SF < 20 µg/L at enrolment) was 44% by SF, 25% by sTfR and 28% by TBI, while 31% had postpartum anaemia (Additional file 2, Table S1). The change in SF from enrolment in early pregnancy to the postpartum visit shown in Fig. 1 were similar across the largest ethnic groups (data not shown).

Results from the multinomial logistic analysis showed that compared to 'steady high' iron status by SF, use of supplements in GW 28 was positively associated with having 'steady low' (OR 2.7, 95% CI 1.5-4.8, $p < 0.001$), but even more strongly with 'improvement' (OR 5.2, 95% CI 3.0-9.0, $p < 0.001$) in iron status by SF, while negatively associated with having 'deterioration' in iron status by SF (OR 0.3, 95% CI 0.2-0.6, $p < 0.001$) (Table 2).

The stratified linear and logistic regression analyses showed that use of iron supplements in GW 28 was associated with improvement in iron status from enrolment in early pregnancy to 14 weeks postpartum by SF, sTfR, TBI, and in Hb concentration ($p < 0.05$—0.001) (Table 3**).** Use of supplements were associated with lower odds of postpartum ID by SF and TBI ($p < 0.05$—0.001) both in women exposed to recommendations and not. However, supplement use in iron depleted women (SF < 20 µg/L at enrolment) was not significantly associated with lower odds of postpartum ID by sTfR, and supplement use was in neither groups significantly associated with lower odds of postpartum anaemia (Table 3). Both SF change and TBI change were consistently associated with supplementation use.

Factors associated with change in iron status

In addition to no use of supplements, an unhealthy dietary pattern (ORs 4.0 and 2.8, $p < 0.01$) and postpartum haemorrhage (ORs 5.4 and 4.7, $p \leq 0.01$) were associated with higher odds of having 'steady low' and 'deterioration' of iron status by SF (Table 2). Primiparity was associated with lower odds of 'improvement' (OR 0.5, $p = 0.03$) and higher odds of 'deterioration' (OR 2.7, $p < 0.01$) in iron status by SF. Further, South Asian ethnic origin was associated with having high odds of 'improvement' in iron status by SF, but higher odds of having 'steady low" in iron status by SF (ORs 2.7 and 3.7, *p<0.02*) (Table 2).

Discussion

Main findings

In this cohort, iron supplementation was recommended to 44% of the pregnant women due to depleted iron status (SF concentration < 20 µg/L) at enrolment in mean GW 15, of whom the majority (78%) were of non-Western European origin. In the group recommended supplementation, the use of iron supplements increased from 25% at enrolment to 65% at the postpartum visit. Further, in women recommended supplements, median SF concentration increased from 11 to 16 µg/L, while the median

Table 1 Characteristics of participants by serum ferritin concentration at enrolment and postpartum [1]

	n	Total	SF < 20 µg/L in early pregnancy (depleted iron stores and recommended supplements)			SF ≥ 20 µg/L in early pregnancy (not exposed to recommendations)		
			Total	SF < 15 µg/l postpartum	SF ≥ 15 µg/l postpartum	Total	SF < 15 µg/L postpartum	SF ≥ 15 µg/L postpartum
				Steady low	Improvement		Deterioration	Steady high
		n = 573	*n* = 252 (44)	*n* = 111	*n* = 141	*n* = 321 (56)	*n* = 114	*n* = 207
Mean baseline SF (95% CI)		33 (30, 35)	11 (11, 12)	11 (10, 12)	12 (11, 12)	50 (46, 54)	45 (40, 50)	53 (47, 58)
Median baseline SF (IQR)		23 (12, 41)	11 (7,15)	11 (7, 15)	12 (8, 15)	38 (27, 60)	34 (26, 55)	40 (27, 62)
Gestational week at enrolment	573	15.1 ± 3.4	16.1 ± 3.9	15.7 ± 3.8	16.5 ± 4.0	14.2 ± 2.6	14.2 ± 2.8	14.3 ± 2.6
Postpartum week	565	13.9 ± 2.4	14.0 ± 2.5	14.0 ± 2.7	14.0 ± 2.4	13.9 ± 2.4	13.6 ± 2.4	14.0 ± 2.4
Parous women (≥ 1 previous births)	573	313 (55)	163 (65)	69 (63)	94 (67)	150 (47)	36 (32)	114 (55)
Age at inclusion, *years*	573	29.7 ± 4.8	29.4 ± 4.9	29.1 ± 5.3	29.6 ± 4.6	30.0 ± 4.8	29.3 ± 4.9	30.3 ± 4.6
Pre-pregnant Body Mass Index, kg/m2	563	24.6 ± 4.8	23.9 ± 4.5	23.9 ± 4.3	23.8 ± 4.7	25.3 ± 4.9	25.4 ± 5.6	25.2 ± 4.5
Western European ethnicity	217	217 (38)	48 (22)	17 (15)	31 (22)	170 (78)	61 (54)	109 (53)
South Asian ethnicity	157	157 (27)	102 (65)	46 (42)	56 (40)	55 (35)	23 (20)	32 (15)
Middle Eastern ethnicity	94	94 (16)	51 (54)	11 (10)	12 (9)	43 (46)	7 (6)	7 (3)
Sub-Saharan African ethnicity	37	37 (7)	23 (62)	5 (5)	5 (4)	14 (38)	7 (6)	16 (8)
East Asian Ethnicity	33	33 (6)	10 (30)	5 (5)	5 (4)	23 (70)	7 (6)	16 (8)
Eastern European ethnicity	34	34 (6)	18 (53)	7 (6)	11 (8)	16 (47)	4 (4)	12 (33)
Socioeconomic position [2]	569	0.02 (1.0)	-0.2 (1.0)	-0.4 (1.0)	-0.1 (1.0)	0.2 (1.0)	0.2 (0.9)	0.3 (1.0)
Unhealthy dietary pattern [3]	555	391 (70)	202 (82)	95 (86)	107 (76)	189 (61)	80 (70)	109 (53)
Chronic illness / medication associated with normochromic anaemia [4]	564	14 (2.5)	4 (1.6)	3 (3)	1 (1)	10 (3.2)	4 (4)	6 (3)
Chronic illness / medication associated with hypochromic anaemia [5]	564	55 (10)	24 (10)	10 (90)	14 (10)	31 (10)	8 (7)	23 (11)
Self-reported use of iron supplement in early pregnancy	564	101 (18)	61 (25)	24 (22)	37 (26)	40 (13)	16 (14)	24 (12)
Self-reported use of iron supplement in gestational week 28	538	232 (43)	156 (65)	60 (55)	96 (68)	76 (26)	17 (15)	59 (29)
Self-reported use of iron supplement 14 weeks postpartum	554	124 (22)	69 (28)	25 (23)	44 (31)	55 (18)	20 (18)	35 (17)
Normal vaginal delivery	414	414 (72)	188 (75)	78 (71)	110 (78)	226 (70)	76 (67)	150 (73)
Instrumental vaginal delivery	58	58 (10)	29 (12)	16 (15)	13 (9)	29 (9)	11 (10)	18 (9)
Elective caesarean section	68	68 (12)	25 (10	12 (11)	13 (9)	43 (13)	18 (16)	25 (12)
Emergency caesarean section	29	29 (5)	8 (3)	5 (4)	4 (3)	21 (7)	7 (6)	14 (7)

Table 1 (continued)

	n	Total	SF < 20 µg/L in early pregnancy (depleted iron stores and recommended supplements)			SF ≥ 20 µg/L in early pregnancy (not exposed to recommendations)		
			Total	SF < 15 µg/l postpartum	SF ≥ 15 µg/l postpartum	Total	SF < 15 µg/L postpartum	SF ≥ 15 µg/L postpartum
				Steady low	Improvement		Deterioration	Steady high
		n = 573	*n* = 252 (44)	*n* = 111	*n* = 141	*n* = 321 (56)	*n* = 114	*n* = 207
Birth complications [7]	573	97 (17)	35 (14)	15 (14)	20 (14)	62 (19)	30 (26)	32 (15)
Postpartum haemorrhage (≥ 500 mL)	573	33 (5.7)	12 (4.8)	8 (7)	4 (3)	21 (6.5)	13 (11)	8 (4)

Enrolment mean gestational week 15; *GW* Gestational week, *Hb* Haemoglobin, *Postpartum* mean 14 weeks after delivery, *SF* Serum ferritin

[1] The STORK-G multi ethnic pregnancy cohort from Oslo, Norway, 2008-2010. Values are n (%) or mean ± standard deviation. Group designation: Steady low, SF < 20 µg/L at enrolment and < 15 µg/l postpartum; Improvement, SF < 20 µg/L at enrolment and SF ≥ 15 µg/l postpartum; Deterioration, SF ≥ 20 µg/L at enrolment and SF < 15 µg/l postpartum, and Steady high, SF ≥ 20 µg/L at enrolment and SF ≥ 15 µg/l postpartum

[2] Maternal present socioeconomic position (SEP) was a score derived from a principal component analysis (PCA) of 11 different demographic variables. The variables contributing most to the score, were individual level data about education, occupational class and employment status, and household variables as own or renting tenure and rooms per person in the household. The score was normally distributed, had mean = 0 and SD = 1 (mean and range), with a higher score reflecting higher SEP

[3] Self-reported dietary pattern in gestational week 28 extracted from a food frequency questionnaire

[4] Self-reported chronic illness or medication associated with normochromic anaemia (i.e. kidney or rheumatic disease, use of carbamazepine or infliximab)

[5] Self-reported chronic illness or medication associated with ID and hypochromic anaemia (i.e. gastrointestinal disease or Copper intrauterine device use before conception)

[6] Forceps or vacuum assisted vaginal delivery

[7] A composite variable created by combining following 4 birth complication; episiotomy, third—and fourth degree perineal tear, obstructed labour, and manual removal of placenta

SF concentration was substantially reduced in women not exposed to our recommendations. Use of iron supplements in GW 28 was associated with lower odds of 'deterioration' and higher odds of 'improvement' in iron status by SF, and with lower odds of postpartum ID by SF and TBI, both in women recommended iron supplement and those not exposed to our recommendations. Use of supplements in GW 28 did however not reduce the odds of postpartum anaemia or postpartum ID by sTfR in women with depleted iron stores (SF concentration < 20 µg/L) at enrolment. Primiparity, unhealthy dietary pattern and postpartum haemorrhage were independently associated with 'steady low' and 'deterioration' in iron status by SF. South Asian ethnic origin was associated with higher odds of both 'steady low' and 'improvement' of iron status by SF.

This study confirms the widespread prevalence of depleted iron stores in pregnancy and the early postpartum period [29]. Even though the prevalence of gestational anaemia in the total sample was low (5.9%) in a global context [8], as many as 89% had SF < 70 µg/L at enrolment, and would be recommended supplementation according to current Norwegian antenatal guidelines, implemented after this study was performed. We found that women not exposed to our recommendations showed a more adverse iron status development, and that women having SF between 20-70 µg/L at enrolment had a high prevalence of postpartum ID supports the current guidelines [14].

In our multi-ethnic cohort, we observed that 65% of the women recommended supplementation seemed to follow our recommendations and reported use in GW 28, and the median SF and the mean TBI concentration in this group increased from enrolment to 14 weeks postpartum. The compliance in randomized, controlled trials (RCT's) has been reported to be around 85-90% [29–31], however strict follow-ups to encourage and ensure compliance might not reflect real-life settings. Previous studies have found a higher proportion of ID in populations with low socioeconomic status [32] and that pregnant women with ethnic minority background are less likely to follow recommendations regarding supplemental use [18]. Despite our recommendations, the proportion of postpartum ID in women recommended supplementation in our study was still high (33% by SF < 12 µg/L and 44% by SF < 15 µg/L). This is higher than observed in two RCT's with low-dose iron supplementations, where the prevalence of ID (SF < 15 µg/L) 2-6 months after delivery was 7-16% in the intervention and 29% in the placebo group [29, 30]. Moreover, in an US cross-sectional study, 13% had ID (SF < 12 µg/L) 0-6 months after delivery [33]. There may be several reasons for such discrepancies. Our study was population-based, and it is well-known that participants in RCTs often are highly selected and motivated for the intervention. Furthermore, our cohort

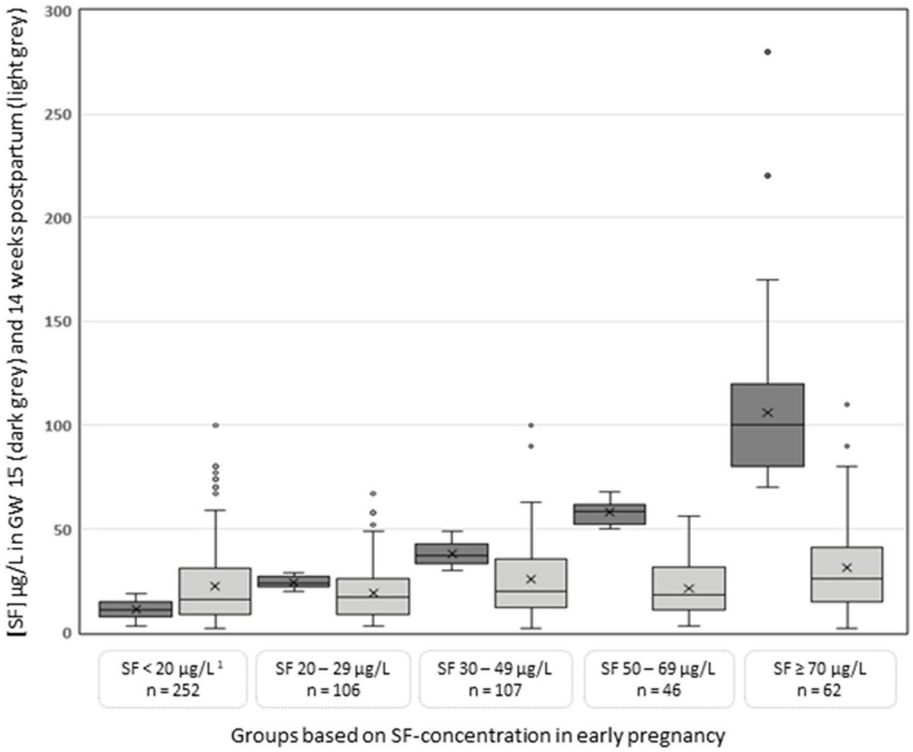

Fig. 1 Change in serum ferritin concentration from enrolment in early pregnancy to postpartum in participants with different value at enrolment [1]. [1] Data from the STORK-G multi-ethnic pregnancy cohort from Oslo, Norway, 2008-2010. The group with SF < 20 µg/L (depleted iron stores) at enrolment, were recommended supplementation. *Enrolment* mean gestational week 15.1 ± standard deviation 3.4. *Postpartum visit* mean postpartum week 13.9 ± 2.4. SF, serum ferritin

consists of many women from ethnic minority- and hard-to-reach groups, and we observed significant ethnic differences in iron status, including Hb levels at enrolment [8]. However, at the postpartum visit, only women of South Asian origin had significantly higher sTfR and lower Hb concentration [20] compared to Western Europeans, suggesting that the simple intervention recommending supplementation to iron depleted women, of whom 78% were ethnic minority women, had an effect on decreasing ethnic differences. The effects of iron supplements on change in SF from enrolment to the postpartum visit showed in Fig. 1 were similar across the largest ethnic groups (data not shown), indicating that these are universal effects.

In line with others, we found that use of supplements reduced the risk of postpartum ID and that women reporting use of iron supplements were more likely to improve their iron status by SF and mean Hb concentration in the postpartum period [1, 5]. We also found that supplementation was associated with improvements of iron status in mean sTfR and TBI. Nevertheless, while use of supplements in GW 28 was strongly associated with postpartum ID by TBI, it did not reduce the odds of postpartum anaemia, nor of postpartum ID by sTfR, in women with depleted iron stores (SF concentration < 20 µg/L) at enrolment. This could possibly suggest that TBI is a better indicator of the effect of supplementation use on ID. However, the test is more expensive, which restricts its general availability, and more data on its use in pregnancy are needed. Furthermore, SF in pregnancy is affected by haemodilution. We can hence not rule out the possibility that some of the increase in SF from enrolment to postpartum in women with depleted iron stores at baseline could be related to changes in blood volume. However, the observed decrease in SF observed in women that were not exposed to our recommendations, does not support this. Further, sTfR and TBI, which are less affected by pregnancy-related factors, show similar pattern, which suggests that changes in iron status measured by SF cannot be explained by pregnancy related changes such as haemodilution alone. Although associated with higher Hb concentration postpartum, use of supplements was in our cohort not associated with reduced risk of anaemia. A study from Burkina Faso, Africa, in a population with a high rate of anaemia while unknown iron status, found that iron supplementation was only associated with increasing Hb from early to late pregnancy if the woman was anaemic [34]. We did

Table 2 Factors associated with change in serum ferritin from enrolment in early pregnancy to postpartum visit [1]

Candidate factors	SF < 20 µg/L at enrolment (depleted iron stores and recommended supplements)												SF ≥ 20 µg/L at enrolment (not exposed to recommendations)					
	Steady low						Improvement						Deterioration					
	n = 111						*n* = 141						*n* = 114					
	Postpartum iron deficiency (SF < 15 µg/L)						Postpartum *no* iron deficiency SF ≥ 15 µg/L						Postpartum iron deficiency (SF < 15 µg/L)					
	Unadjusted model			Final model			Unadjusted model			Final model			Unadjusted model			Final model		
	OR	95% CI	*p*	Adj OR	95% CI	*p*	OR	95% CI	*p*	Adj OR	95% CI	*p*	OR	95% CI	*p*	Adj OR	95% CI	*p*
Self-reported use of iron supplement in GW 28	3.0	1.8–4.9	< 0.001	2.7	1.5–4.8	< 0.001	5.2	3.2–8.3	< 0.001	5.2	3.0–9.0	< 0.001	0.4	0.2–0.8	0.004	0.3	0.2–0.6	0.001
Unhealthy dietary pattern [2]	7.0	3.5–13.8	< 0.001	4.0	1.7–9.5	0.001	2.7	1.7–4.4	< 0.001	1.5	0.8–3.0	0.212	2.0	1.2–3.2	0.008	2.8	1.5–5.2	0.002
Primiparous women	0.7	0.4–1.1	0.160	0.8	0.4–1.5	0.447	0.6	0.4–1.0	0.031	0.5	0.3–0.9	0.033	2.7	1.6–4.3	< 0.001	2.7	1.5–5.1	0.002
Postpartum haemorrhage (≥ 500 mL)	2.0	0.7–5.3	0.197	5.4	1.5–19.2	0.010	0.7	0.2–2.5	0.607	1.7	0.4–6.9	0.486	3.2	1.3–8.0	0.012	4.7	1.6–14.0	0.006
Western European ethnicity (reference)																		
South Asian ethnicity	9.8	4.9–19.6	< 0.001	3.7	1.6–8.7	0.003	6.2	3.4–11.1	< 0.001	2.7	1.2–5.9	0.014	1.3	0.7–2.3	0.429	0.7	0.1–0.8	0.295
Middle Eastern ethnicity	5.5	2.6–11.6	< 0.001	2.4	0.9–6.1	0.072	2.9	1.5–5.7	0.001	1.8	0.7–4.3	0.796	0.7	0.3–1.4	0.327	0.3	0.1–0.8	0.021
Sub-Saharan African ethnicity	10.7	3.6–31.6	< 0.001	2.6	0.7–10.4	0.173	6.0	2.2–16.6	< 0.001	2.7	0.7–4.3	0.146	1.8	0.6–5.3	0.298	1.1	0.3–3.9	0.927
East Asian Ethnicity	2.1	0.7–6.6	0.191	1.0	0.3–3.7	0.965	1.1	0.4–3.2	0.864	0.6	0.2-.2.3	0.477	0.8	0.3–2.0	0.608	0.6	0.2–1.8	0.361
Eastern European ethnicity	4.0	1.3–11.6	0.011	1.8	0.5–6.5	0.326	3.2	1.3–8.0	0.012	2.2	0.8–6.6	0.145	0.6	0.2–1.9	0.387	0.3	0.1–1.2	0.091

Adj Adjusted, *enrolment* mean gestational week 15, *GW* Gestational week, *OR* Odds ratio, *postpartum* mean 14 weeks after delivery, *postpartum ID by SF* serum ferritin < 15 µg/L, *SF* Serum ferritin, *95% CI* 95% confidence interval

[1] Multinomial logistic regression analyses to investigate factors associated with belonging to one of the following three groups 'steady low', 'improvement' and 'deterioration', reflecting the women's iron status by serum ferritin at enrolment and postpartum, using 'steady high' (n = 207) as the reference in the STORK-G study, a multi-ethnic pregnancy cohort from Oslo, Norway, 2008-2010

[2] Both unadjusted and adjusted odds ratios are presented. The fully adjusted model included gestational week, age, parity, socioeconomic position, diet, ethnicity, use of iron supplementation in gestational week 28 and postpartum, postpartum haemorrhage and birth complications

[3] Self-reported dietary pattern in gestational week 28 extracted from a food frequency questionnaire

Table 3 Use of supplementation on changes in iron status and odds of postpartum iron deficiency and anaemia [1]

Linear regression: change in SF, sTfR, TBI, and Hb concentration							Logistic regression: postpartum iron deficiency or anaemia						
	Unadjusted model			Final linear model [a]				Unadjusted model			Final logistic model [a]		
	β	95% CI	*p*-value	Adj β	95% CI	*p*-value		OR	95% CI	*p*-value	Adj OR	95% CI	*p*-value
Recommended supplements (SF < 20 µg/L at enrolment in early pregnancy)							Recommended supplements (SF < 20 µg/L at enrolment in early pregnancy)						
Self-reported use of supplementation in GW 28							Self-reported use of supplementation in GW 28						
Change in SF	5.7	1.0, 10	0.018	5.1	0.2, 10.0	0.040	Postpartum ID by SF	0.6	0.3, 1.0	0.047	0.5	0.3, 1.0	0.044
Change in sTfR	-0.7	-1.2, -0.3	0.003	-0.7	-1.2, -0.2	0.009	Postpartum ID by sTfR	0.9	0.5, 1.6	0.665	0.6	0.3, 1.3	0.221
Change in TBI	2.1	1.1, 3.2	< 0.001	1.8	0.7, 2.9	0.001	Postpartum ID by TBI	0.4	0.2, 0.8	0.005	0.3	0.1, 0.6	0.001
Change in Hb	0.5	0.3, 0.8	< 0.001	0.6	0.4, 0.9	< 0.001	Postpartum anaemia	0.8	0,5, 1.5	0.560	0.7	0.4, 1.4	0.348
SF ≥ 20 µg/L at enrolment in early pregnancy (not exposed to recommendations)							SF ≥ 20 µg/L at enrolment in early pregnancy (not exposed to recommendations)						
Self-reported use of iron supplementation in GW 28							Self-reported use of supplementation in GW 28						
Change in SF	18.6	10, 21	< 0.001	5.1	0.5, 9.6	0.029	Postpartum ID by SF	0.4	0.2, 0.8	0.004	0.4	0.2, 0.7	0.004
Change in sTfR	-0.5	-0.9, -0.1	0.011	-0.6	-1.0, -0.2	0.007	Postpartum ID by sTfR	0.4	0.1, 0.9	0.039	0.3	0.91, 0.9	0.027
Change in TBI	2.2	1.3, 3.2	< 0.001	1.8	0.9, 2.7	< 0.001	Postpartum ID by TBI	0.3	0.1, 0.7	0.006	0.3	0.1, 0.6	0.001
Change in Hb	0.3	0.1, 0.6	0.010	0.4	0.2, 0.7	0.001	Postpartum anaemia	1.1	0.6, 2.0	0.854	0.9	0.5, 19	0.854

Adj Adjusted, *β* beta, *95% CI* 95% confidence interval, *enrolment*: mean gestational week 15, *GW* Gestational week, *Hb* Haemoglobin, *ID* Iron deficiency, *OR* Odds ratio, *postpartum* mean 14 weeks after delivery, *postpartum ID by SF* serum ferritin < 15 µg/L, *postpartum anaemia* Haemoglobin < 12.0 g/dL, *postpartum ID by sTfR* soluble transferrin receptor > 4.4 mg/L, *postpartum ID by TBI* total body iron < 0 mg/kg, *SF* Serum ferritin, *sTfR* Soluble transferrin receptor, *TBI* Total body iron

[a] Linear regression analyses to assess the association between use of supplementation in gestational week 28 and changes in SF, sTfR, TBI and Hb from enrolment to 14 weeks postpartum in iron depleted women (SF < 20 µg/L) recommended supplements at enrolment (top panel) or not (bottom panel) in the STORK-G, a multi-ethnic pregnancy cohort from Oslo, Norway, 2008-2010

[b] Logistic regression analyses to assess the association between use of supplementation in gestational week 28 on iron deficiency by SF, sTfR, TBI and anaemia in iron depleted women (SF < 20 µg/L) recommended supplements at enrolment (top panel) or not (bottom panel) in the STORK-G, a multi-ethnic pregnancy cohort from Oslo, Norway, 2008-2010

[c] Both unadjusted and adjusted beta and odds ratios are presented. The fully adjusted model included SF concentration at enrolment, gestational week, age, parity, socioeconomic position, diet, ethnicity, and use of supplement in gestational week 28

not have statistical power to evaluate the effect of iron supplements stratified by anaemia status, as few women were anaemic at enrolment (5.9%). Further, we were primarily interested in change in iron status by different iron indicators, and factors associated with such change.

In high-income countries, haemorrhage is recognized as the most important factor associated with postpartum ID and anaemia [1]. Our study supports that it is important to offer women with haemorrhage a postpartum follow-up to assess iron status. In line with others we found that primiparity appears to be an important factor associated with an adverse iron status development [35], also after adjusting for birth complications, haemorrhage, ethnicity and supplement use. This might suggest that there may be other, unidentified factors related to primiparous pregnancies that could affect the need for iron. We have previously reported significant ethnic differences in crude ID and anaemia prevalence. The prevalence of anaemia ranged from 7-14% in early pregnancy in women with ethnic origin outside Europe [8] and 26-40% postpartum [20]. Wheras in Europeans the prevalence was lower, both in early pregnancy (0-1.8%) [8] and postpartum (14-18%) [20]. Recent studies from the UK found a high proportion of women from ethnic minority groups to be anaemic during pregnancy, and also that severe anaemia was associated with adverse foetal and infant outcomes (stillbirth, perinatal death, small for gestational age infants, low birth weight infants and maternal postpartum haemorrhage [36]), highlighting the importance of preventing anaemia and identifying vulnerable groups, e.g. minority women, which could need extra attention. On the other hand, no existing evidence indicates that preventive iron supplementation has an effect on birth weight or other adverse infant outcomes [37]. In our study, 65% of women with South Asian ethnic origin had depleted iron stores at enrolment and were recommended supplementation. Many of these women benefitted from the simple intervention, having higher odds for being in the 'improvement' group, but some did not and developed postpartum ID ('steady low' group). This could suggest that language skills and cultural factors not accounted for in our study may also play a role in the compliance to our recommendations. Another possible explanation could be that this ethnic group have a diet richer of phytates, which inhibits iron uptake

[38]. Therefore, clinicians should strive to increase the compliance in order to ensure improvement of iron status, prevent anaemia and to prevent associated adverse maternal and neonates outcome [5, 32, 36].

Strength and limitations

The major strength of our multi-ethnic population-based study is that we followed a large number of healthy women from enrolment in early pregnancy to 14 weeks postpartum with measurements of iron status by three different indicators, analysed at the same laboratories. We collected a broad high-quality data set, which enabled us to explore relations between iron status and a wide range of explanatory factors, and to adjust for a range of possible confounders. The questionnaires were translated to eight languages and data collection methods were adapted to facilitate enrolment of ethnic minorities and even illiterate women, who often are excluded in research [21]. Professional interpreters were used to ensure the quality of the interview-administrated questionnaire data. The women were found representative for the main ethnic groups of Oslo. Lastly, this study represents close-to-practice research, addressing the gap between what works in research (RCT) and what works in practice, especially in hard-to-reach-groups.

However, there are also weaknesses to report. We evaluated the impact of giving a simple recommendation of iron supplementation by self-reported use of supplements in GW 28 through a pre-post-test design, not a RCT. We do not know to which extent the women adhered to our recommendations. However, substantially more women reported use of iron supplements in GW 28 among women undergoing this 'simple intervention', compared to those not exposed to recommendation, suggesting an effect. In Norway ferrous gluconate, sulphate and fumarate can be bought in grocery stores or pharmacies without prescription. For the majority of women we lack information of the iron dose taken, the frequency and duration of intake, the type of iron supplements as well as of other variables affecting the iron uptake. We assessed the effect of recommending iron supplementation on the women's postpartum status only, and have not evaluated the effect of iron supplementation on the neonates. Further, the number of participants in some ethnic groups was low, and the presence of heterogeneity within relatively broad ethnic groups is possible. As in most studies, we had some loss to follow-up at the postpartum visit, partly due to logistic reasons, but we prioritized ethnic minority women for blood sampling. Further, the collected food-frequency data were used to identify dietary patterns, and we were therefore not able to calculate iron intake. Postpartum haemorrhage was not exactly measured, but based on clinical judgement. On average, the plasma volume increases from about 2.4 L to about 2.8 L from the time before pregnancy to week 19 [39], and we cannot rule out that plasma volume can partly explain some of the differences found during pregnancy. There are no simple biomarkers that provide good estimates of changes in plasma volume [40, 41], however we adjusted for gestational week in our regression models, as a proxy for plasma volume. Furthermore, we have no reason to believe that our explanatory variables of interest will systematically influence the blood volume, and hence affect our results, other than by reducing the precision of our estimates.

Implications

First, our results lend support to recommend iron supplementation to women during pregnancy to prevent gestational and postpartum ID and anaemia, and indicate that there is a need for an enhanced focus on iron supplementation during pregnancy to be incorporated in high risk groups in antenatal care. Further, our findings also suggest that postpartum ID or IDA needs to be addressed, and postpartum counselling that includes measurement of Hb and SF concentrations are required in high-risk women. In addition, as prophylactic iron supplementation during pregnancy is still a subject of international disagreement, further research to examine the effect on ID and IDA in pregnant women should be undertaken.

Conclusion

We evaluated the impact of recommending supplements to pregnant women with depleted iron stores, including women in hard-to-reach-groups, and observed an increase of use of supplements and improved iron status from at enrolment to the postpartum visit. Women not exposed to our recommendations, showed a deteriorated iron status postpartum. Further, factors associated with change in iron status were dietary iron intake, use of iron supplements, ethnicity, parity and postpartum haemorrhage.

Abbreviations

BMI	Body mass index
CRP	C-reactive protein
CV	Coefficient of variation
CI	Confidence interval
ECLIA	Electro-chemiluminescence immunoassay
HbA1c	Glycated haemoglobin
Hb	Haemoglobin
ID	Iron deficiency
IDA	Iron deficiency anaemia
PCA	Principal component analysis

OR	Odds ratio
SF	Serum ferritin
SEP	Socioeconomic position
SD	Standard deviation
sTfR	Soluble transferrin receptor
TBI	Total body iron
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12884-023-05668-5.

Additional file 1. Flow chart of study participants in the STORK-G multiethnic cohort from Oslo 2008-2010.

Additional file 2. Table S1. Average SF, sTfR, TBI and Hb concentration in early pregnancy and postpartum, and prevalence of postpartum iron deficiency/anaemia.

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Authors' contributions
MNA and LS had full access to all data in this study. MNA is responsible for the integrity of data and accuracy of the data analysis. MNA, LS, JPB, and AKJ revised the study concept and design, contributed to analysis and tables. RSF guided the statistical analysis and revised the tables. All authors interpreted the data, and critical revision of the manuscript. AKJ initiated the STORK-G study and was the principal investigator until 2021. All authors read and approved the final manuscript.

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Availability of data and materials
The editors can access data in de-identified form used in the manuscript, code book, and analytical code upon request. The project manager Anja Brænd, and co-author Line Sletner, will contribute to the access being provided under appropriate conditions. However, research data for this publication include identifying health information subject to confidentiality. It is therefore not possible to share raw data.

Declarations

Ethics approval and consent to participate
The study was started after approval from The Regional Ethics Committee, Oslo (Norway). The Regional Ethics Committee (2007/894) and the Norwegian Data Inspectorate (25 October 2007; 07/01355–2/MOF) approved the study protocol. All participants provided written informed consent. The study was carried out according to the Helsinki Declaration.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of General Practice, Institute of Health and Society, University of Oslo, Blindern, P.O. Box 1130, 0318 Oslo, Norway. [2]General Practice Research Unit, Department of General Practice, Institute of Health and Society, University of Oslo, Oslo, Norway. [3]Department of Medical Biochemistry, Institute of Clinical Medicine, University of Oslo, Oslo, Norway. [4]Oslo Centre for Biostatistics and Epidemiology, Research Support Services, Oslo University Hospital, Oslo, Norway. [5]Department of Paediatric and Adolescents Medicine, Akershus University Hospital, Lørenskog, Norway. [6]Institute of Clinical Medicine, University of Oslo, Oslo, Norway.

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