Pre-conception serum ferritin concentrations are associated with metal concentrations in blood during pregnancy: A cohort study in Benin

Sarah Davies[a,b] (sdavies72@gmail.com), Valérie Briand[c] (valerie.briand@ird.fr), Manfred Accrombessi[d,e] (accrombessimanfred@yahoo.fr), Nadine Fievet[d,f] (nadine.fievet@ird.fr), Barbara Le Bot[g] (Barbara.LeBot@ehesp.fr), Séverine Durand[g] (severine.durand@ehesp.fr), Gino Agbota[d] (ginodric@yahoo.fr), Emmanuel Yovo[d] (emmanuelkoffiyovo@yahoo.fr), Bertin Vianou[d] (bertinosidase@yahoo.fr), Darius Sossou[d] (anax20032002@yahoo.fr), Yves Martin-Prevel[i] (yves.martin-prevel@ird.fr), Achille Massougbodji[h] (massougbodjiachille@yahoo.fr), Michel Cot[f] (michel.cot@ird.fr), Philippe Glorennec[g] (philippe.glorennec@ehesp.fr), Florence Bodeau-Livinec[a,b] (florence.bodeau-livinec@ehesp.fr)

[a] Département Méthodes Quantitatives en Santé Publique (METIS), Ecole des Hautes Etudes en Santé Publique (EHESP), F-35000 Rennes, France

[b] Université de Paris, Center of Research in Epidemiology and Statistics/CRESS, INSERM, INRA, Obstetrical, Perinatal and Pediatric Epidemiology Research Team (EPOPé)—UMR1153, F-75004, Paris, France

[c] Institut de Recherche Pour le Développement (IRD), University of Bordeaux, Inserm, UMR 1219, 146 rue Léo-Saignat, 33076, Bordeaux Cedex, France, Université de Paris, MERIT, IRD, 75006, Paris, France

[d] Institut de Recherche Clinique du Benin (IRCB), 04 BP1114, Abomey-Calavi, Benin

[e] Faculty of Infectious and Tropical Diseases, Disease Control Department, London School of Hygiene and Tropical Medicine, London, United Kingdom

[f] Institut de Recherche pour le Développement (IRD), Paris Descartes Université, 75006 Paris, France

[g] Ecole des Hautes Études en Santé Publique (EHESP), Institut National de la Sante et de la Recherche Medicale (Inserm), Institut de Recherche en Santé, Environnement et Travail (Irset)—UMR_S 1085, University of Rennes, F-35000 Rennes, France

[h] University of Abomey-Calavi Faculté des Sciences de la Santé, Cotonou, Bénin

[i] Nutripass Research Unit, Institut de Recherche pour le Développement (IRD), University of Montpellier, SupAgro, Montpellier, France

Corresponding Author Contact Information
Name: Sarah Davies
E-mail : sdavies72@gmail.com
Present Postal address : 24 Gow St, Hamilton North 2292, Australia

Sarah Davies: Methodology, Formal analysis, Writing – Original Draft **Valérie Briand:** Conceptualization, Supervision, Writing – Review & Editing **Manfred Accrombessi:** Investigation, Writing – Review & Editing **Nadine Fievet:** Conceptualization, Supervision **Barbara Le Bot:** Investigation, Formal Analysis **Séverine Durand:** Investigation, Formal Analysis, Writing – Review & Editing **Gino Agbota:** Investigation, Writing – Review & Editing **Emmanuel Yovo:** Investigation **Bertin Vianou:** Biological Analysis **Darius Sossou:** Biological Analysis **Yves Martin-Prevel:** Conceptualization, Supervision, Writing – Review & Editing **Achille Massougbodjii:** Supervision **Michel Cot:** Supervision **Philippe Glorennec:** Methodology, Writing – Review & Editing **Florence Bodeau-Livinec:** Conceptualization, Methodology, Supervision, Writing – Review & Editing

Title

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Abstract

Background: Iron deficiency is a common nutritional deficiency that impacts maternal health and fetal development and is also associated with increased uptake of toxic metals. Women in sub-Saharan Africa are highly exposed to both iron deficiency and metals in the environment. As research on the developmental origins of health and disease increasingly shows impacts of pre-conception maternal health on pregnancy and fetal health, these environmental exposures are of concern.

Objectives: This study investigated the association between iron status pre-pregnancy and blood metal concentrations in the first trimester of pregnancy with potential implications for iron supplementation.

Methods: Pre-conception and first trimester blood samples taken from 262 Beninese women were tested for serum ferritin, inflammation markers, manganese (Mn), cadmium (Cd), lead (Pb), copper, zinc, selenium, mercury and arsenic. Associations between serum ferritin adjusted for inflammation and metal concentrations were analyzed using multivariate linear regression.

Results: Women with iron deficiency before conception (13%) were more likely to remain iron deficient in the first trimester (4%) (adjusted OR=41.2, 95%CI 6.2; 275.0) even within the context of routine iron supplementation during pregnancy.

Lower pre-pregnancy serum ferritin concentrations were significantly related to higher concentrations of Mn, Cd and Pb in the first trimester. Every 1% increase in serum ferritin concentration was associated with a 0.13% decrease in Mn (adjusted β=-0.13, 95%CI -0.18; -0.07), a 0.22% decrease in Cd (adjusted β=-0.22, 95%CI -0.28; -0.15) and a 0.06% decrease in Pb concentration (adjusted β=-0.06, 95%CI -0.12; -0.006).

Discussion: These results suggest that increasing iron stores prior to pregnancy may prevent excessive uptake of toxic concentrations of the metals Mn, Cd and Pb and argue in favour of testing the effects of iron supplementation prior to pregnancy on metal concentrations.

Keywords: Iron deficiency, metals, environmental exposure, pregnancy, pre-conception

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Competing Interests: The authors declare they have no actual or potential competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval: The authors confirm that consent from subjects participating in the cohort study was received prior to conducting the study and that the study was approved by the Ethics Committee of the Institut des Sciences Biomédicales Appliquées (ISBA) in Benin.

1. Introduction

Iron deficiency is the most common nutritional deficiency worldwide and with or without anemia has important consequences for human health and child development. Globally, about 33% of all women of reproductive age are estimated to be anemic (WHO, 2016a), with 16-25% estimated to be due to iron deficiency (Petry et al., 2016). Women's iron requirements increase significantly during pregnancy, rising during the second trimester and continuing to increase throughout the remainder of pregnancy (Bothwell, 2000).

Iron is essential for brain development, particularly in late fetal and early postnatal life, where deficits during these critical periods can permanently affect brain function (Georgieff et al., 2018). Maternal iron deficiency also reduces iron stores in the fetus which further exposes neonates to neurodevelopmental risks as infants cannot regulate iron absorption until 6-9 months of age (Radlowski & Johnson, 2013). The WHO recommends oral iron and folic acid supplementation for pregnant women to prevent maternal anaemia, puerperal sepsis, low birth weight, and preterm birth (WHO, 2016b).

Higher iron levels, however may be detrimental in malaria endemic areas such as Benin, as some studies in children have found malaria risk increases in relation to serum ferritin concentrations and iron deficiency seems to act as a protective factor (Moya-Alvarez et al., 2017; Muriuki et al., 2019). A recent systematic review of malaria risk and iron status in pregnancy found that iron deficiency was only associated with increased risk of malaria when measured by serum ferritin and not when other biomarkers of iron deficiency were included. In the light of limited evidence however, iron supplementation is still recommended to be provided in combination with malaria prevention during pregnancy (Sangaré et al., 2014).

Iron deficiency is also associated with the increased uptake of divalent metals which can adversely affect neurodevelopment (Kim, 2018). Dietary non-heme iron is transported into intestinal cells by the divalent metal-ion transporter 1 (DMT1) and then exported into circulation via ferroportin 1 (FPN1) or stored as ferritin. Iron homeostasis is regulated by the hormone hepcidin, which is in low concentrations when tissue iron stores are low, up-regulating DMT1 and FPN1 (Anderson & Frazer, 2017). DMT1 has been demonstrated to transport divalent metal ions other than iron, notably manganese, cadmium and lead as well as to a lesser extent copper and zinc (Bressler et al.,2004; Garrick et al., 2006). There is also evidence that FPN1 can transport manganese and zinc (Loréal et al., 2014; Madejczyk & Ballatori, 2012).

Research conducted within the framework of the Developmental origins of Health and Disease (DoHAD) continues to provide evidence of the long-term consequences on health of environmental exposures from conception and including the role of maternal nutrition prior to conception (Mandy & Nyirenda, 2018; Stephenson et al., 2018). Many studies have examined the effects of iron deficiency and its relationship to a range of environmental metal exposures during pregnancy and in relation to fetal and childhood development. This study examines data from a unique cohort where women were recruited prior to conception, allowing iron status before pregnancy to be examined in relation to whole blood concentrations in early pregnancy of 8 potentially toxic metals: Manganese, Copper, Zinc, Selenium, Lead, Cadmium, Mercury and Arsenic.

Manganese is an essential nutrient but also a neurotoxicant at high exposure levels. Lead and cadmium are long-lived, toxic heavy metals that have no known beneficial function in the human body. The presence of these metals in the environment and the likelihood of increased uptake before and during pregnancy for women with low iron stores can result in a range of deleterious effects.

Cd accumulation over time can damage renal function and affect bone metabolism (ATSDR, 2012a) as well as some evidence it is toxic to endocrine systems resulting in decreased birth weights (Rahman et al., 2016). There is some evidence that prenatal Cd exposure may impact cognitive development in children (Kippler et al., 2012). Although Cd accumulates in the placenta acting as a partial barrier to foetal exposure with cord blood concentrations significantly lower than maternal blood (Esteban-Vasallo María D. et al., 2012; Truska et al., 1989), Cd also affects placental transport of calcium and zinc which are essential for foetal development (Gundacker & Hengstschläger, 2012).

The health effects of Pb are diverse, not only is Pb a known developmental neurotoxicant but exposure to Pb is associated with toxicity to every organ system (ATSDR, 2020). Lead readily crosses the placenta (Bellinger, 2005; Rossipal et al., 2000) and there is good evidence that maternal lead exposure during pregnancy can adversely affect both maternal and child health. There is no apparent threshold below which adverse effects of lead do not occur (Budtz-Jørgensen et al., 2013; CDC, 2010). Bone lead stores persist for decades and are mobilized during periods of increased bone turnover such as pregnancy and lactation. Iron status, along with intake of dietary calcium and zinc can affect absorption of Pb, potentially leading to alterations in blood lead concentrations (ATSDR, 2020). In addition, there is some evidence that combined Pb and Mn exposures in-utero and in childhood could aggravate negative impacts on neurodevelopment (Kim et al., 2009; Lin et al., 2013; Menezes-Filho et al., 2018).

Se is an essential trace element which can also be toxic and has a complex interplay of metabolic interactions with numerous other nutrients and toxic substances (Arnaud & van Dael, 2018; Sun et al., 2014). Se intake varies widely dependent on concentrations in soils transferred to crops.

The objective of this study was to investigate the association between iron status pre-pregnancy and during the first trimester with the blood concentrations of eight metals in the first trimester of pregnancy: manganese, cadmium, lead, copper, zinc, selenium, mercury and arsenic.

2. Methods

2.1 Study Population

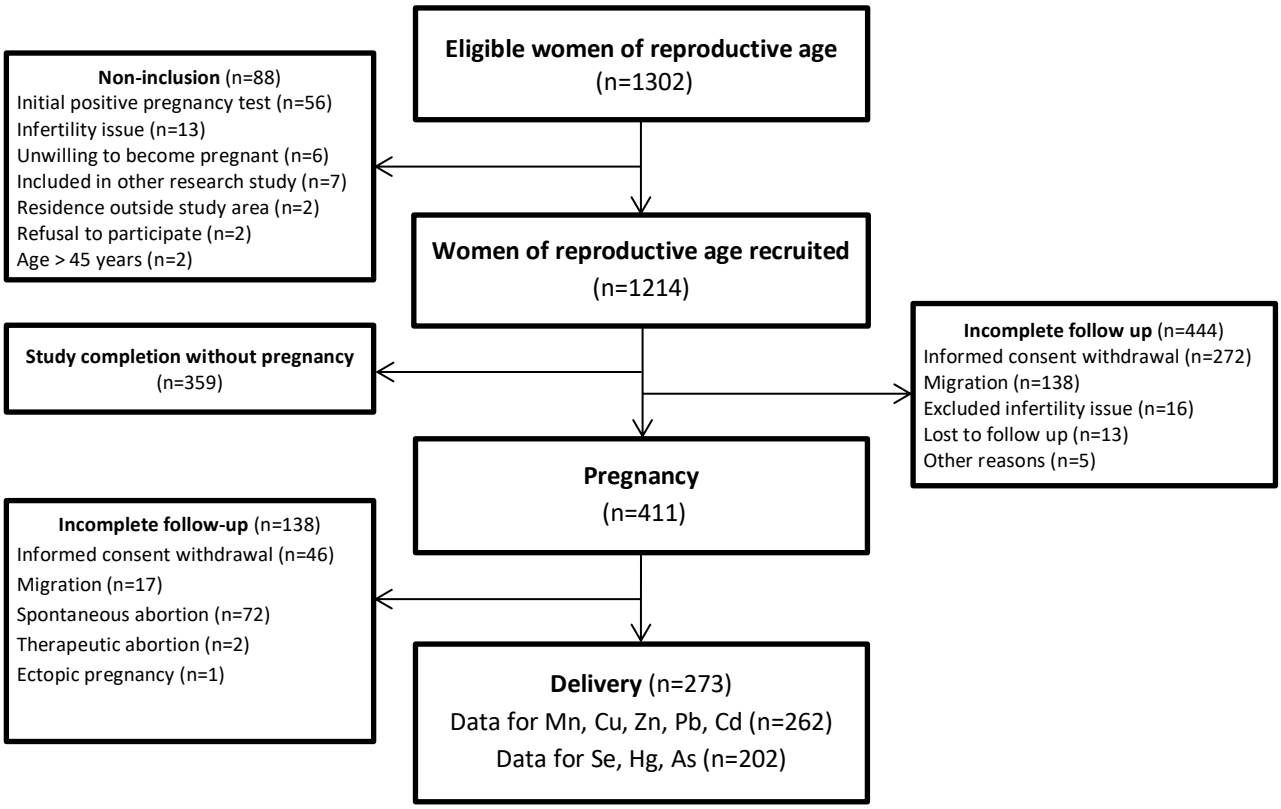
This study includes 262 women from the REtard de Croissance Intra-uterin et PALudisme (RECIPAL) cohort in southern Benin primarily designed to assess the consequences of malaria in early pregnancy for the fetus as detailed in Accrombessi et al., 2018 and summarized in Figure 1. Women in the RECIPAL cohort were recruited prior to conception between June 2014 and December 2016 and followed monthly until pregnancy and through to delivery. At inclusion demographic, household and socioeconomic characteristics were collected. In addition, blood samples were taken at

enrolment before conception and at the second antenatal care (ANC2) visit which occurred for most women during the 1st trimester of pregnancy.

2.2 Ethics Statement

The RECIPAL study was approved by the Ethics Committee of the Institut des Sciences Biomédicales Appliquées (ISBA) in Benin. Women enrolled in the cohort received regular free medical follow-up treatment as necessary during the life of the study. Blood samples were stored and assessed for metals at a later date, these results at the population level were presented to health authorities at local and national levels.

Figure 1: Flowchart diagram of the study



2.3 Gestational Follow-up

According to Beninese national recommendations, a kit including iron and folic acid tablets for 1 month was given to each woman at the first ANC visit and maternity staff were encouraged to administer iron and folic acid supplementation throughout the pregnancy (200 mg oral ferrous sulfate and 10 mg folic acid daily). As part of the study, the cost of drugs were supported by the project; however, the iron and folic acid supplementation were not observed by the study staff.

2.4 Blood Measurements

Blood samples were initially tested for parameters related to iron deficiency, inflammation markers and malaria. Blood serum samples were tested for ferritin, C-reactive protein (CRP) and α_1-acid glycoprotein (AGP). Ferritin may be measured in either serum or plasma, with both positively correlated with the size of total body iron stores (WHO, 2020). As nutrition was one of the core issues in the RECIPAL cohort, serum ferritin was used as part of the assessment of nutritional status and the micronutrient intake of women. Hb level was measured with a HemoCue. Before conception and during pregnancy, malarial screening was performed using both microscopy (thick blood smear) and PCR. Subsequently two sets of ANC2 whole blood samples of women who completed the study follow-up were tested for metals. A first group of 60 tested for Manganese (Mn), Copper (Cu), Zinc (Zn), lead (Pb) and Cadmium (Cd) with results reported previously (Guy et al., 2018). Samples from a further 202 were later tested for these five metals with the addition of tests for Selenium (Se), Mercury (Hg) and Arsenic (As).

The 262 whole blood samples stored at -18°C, were analyzed by a plasma torch coupled with tandem mass spectrometer (ICP-MSMS 8800, Agilent Technologie) after mineralization. Mineralization is performed by adding nitric acid and heating at 83°C for 4 hours using a graphite block (Hotblock Pro, model SC-189, Environmental Express). The mineralization diluted at 1:20 was then analyzed, the use of the tandem mass spectrometer and the oxygen mode made it possible to manage the interferences in particular for the determination of selenium and arsenic. An online addition of internal standards (Sc, Ge, 77Se, Rh, Re and Ir) was performed to correct matrix effects and to verify the absence of drift (signal variation less than 25%). The quantification of Se was performed by isotope dilution with 77Se to resolve persistent effects. At each analysis sequence, consumable blanks and certified blood controls were systematically analyzed (Utak level 1, Utak level 2). The results of the blood tests were validated if the concentrations of the blood controls were within the limits of the control cards. The method of analysis was validated by 2 good inter-laboratory tests over 1 year. This method is accredited by the French accreditation committee (Cofrac). The limits of quantification (LOQ) of Pb, Mn, Cu, Zn, Cd, As, Se and Hg are respectively 2, 5, 200, 200, 0.25, 1, 20 and 0.4 µg/L.

2.5 Definitions

Iron deficiency was defined as serum ferritin less than 15 µg/l after adjustment for inflammation. Serum ferritin is a reliable indicator of total body iron stores when inflammation is not present. In regions such as the study area in Benin where there is widespread infection or inflammation, defining iron deficiency using serum ferritin becomes complicated as ferritin is a positive acute phase response protein. WHO recommends in these areas to assess serum ferritin with concurrent measurement of CRP and AGP and apply one of four possible methods: raising the cut-off value that defines iron deficiency to 70 µg/l; excluding individuals with elevated CRP or AGP concentrations; use an arithmetic correction; or use a regression correction (WHO, 2020). A comparison of methods on a cohort of Kenyan pregnant women concluded that using a regression correction approach led to better estimates of iron deficiency (Mwangi et al., 2019). This regression correction approach according to the following equation was also used in this study as it allowed a correction to

continuous data and the inclusion of a correction for inflammation present due to malaria infection. (Namaste et al., 2017; WHO, 2020).

$$Ferritin_{adjusted} = Ferritin_{unadjusted} - \beta_1(CRP_{obs} - CRP_{ref}) - \beta_2(AGP_{obs} - AGP_{ref}) - \beta_3 malaria$$

where β_x are the regression coefficients, obs is the observed value and ref is the external reference value generated to define low inflammation.

Inflammation was defined as either CRP > 5 mg/l and/or AGP > 1 g/l (Namaste et al., 2017; WHO, 2020)

Anemia was defined prior to pregnancy as hemoglobin <120 g/l and during pregnancy as <110 g/l with *moderate anemia* between 70 to 110 g/l and *severe anemia* < 70 g/l (WHO, 2015)

Iron deficiency anemia was defined as those meeting the criteria both for iron deficiency and anemia.

Insufficient iron stores for pregnancy was defined as serum ferritin <32 µg/l approximately equivalent to 300 mg of total body iron stores, the estimated minimum with which a woman must enter pregnancy to meet her requirements fully assuming an optimal diet.

First trimester was defined as less than 14 weeks gestation based on both ultrasound scan and LMP

2.6 Statistical Analysis

The association between serum ferritin pre-pregnancy and at ANC2 and blood metal concentrations for Mn, Cu, Zn, Pb and Cd, Se, Hg and As were modelled separately using multivariate linear regression. Log transformations of the metal concentrations were made due to non-normal distributions except for Se which did not have a distribution significantly different from normal when using a Shapiro-Wilk test. Log transformations of serum ferritin concentrations were also used in the models for all metals except Se as this improved the linear relationships checked by visual inspection. Confounders were selected based on literature review, the consideration of directed acyclic graphs and after testing for collinearity between candidate variables. Gestational age at ANC2, gravidity and indicators for education level and wealth were included in the models for all metals. As there was little variability in education level, literacy was used as the variable to represent whether any formal education had been received. At inclusion in the cohort, detailed questions were asked on the ownership of assets, the number owned was used as an indicator of wealth. Other potential confounders were tested such as age, body mass index (BMI), the time between pre-pregnancy and ANC2 testing, being positive for malaria by microscopy at ANC2 and living lakeside or inland as a potential indicator of different environmental and dietary exposures. Univariate analysis was first conducted retaining variables with a p value less than 0.25. These variables were then removed stepwise from multivariate models if p values were greater than 0.05. Analysis was carried out using Stata version 15 for Windows.

3. Results

3.1 Population characteristics

Table 1 presents the general characteristics of the 262 women included in the study population. Women were on average 27 years old, primarily of Toffin ethinicity (74.4%) and living within 4 sub-districts of Sô-Ava district: Vekky and Houedo-Aguekon on the edge of the polluted Lake Nokoue; Sô-Ava along the River So; and Akassato where women lived within 3 to 10 km from Lake Nokoue. The majority of women were multigravidae (92.4%), were non-smokers (99.2%) and were within a healthy BMI range at the start of pregnancy (67%). Table 2 presents the blood test results related to iron deficiency at pre-pregnancy and ANC2 visits and Table 3 gestational age information at the first two ANC visits. Before pregnancy, 12.7% of women were iron deficient, 57.9% anemic and 7% tested positive for malaria. Most women became pregnant within 6 months of inclusion (51.2%) with a further 37.3% conceiving within a year. Pregnancy was confirmed at the first ANC visit which occurred at an average gestational age of 6.9 weeks at which time iron supplementation was prescribed. Gestational age at ANC2, at which blood was sampled for iron parameters and metals analysis, averaged 11.7 weeks with 84.4% considered in the first trimester. By ANC2 only 3.5% of women were still considered as iron deficient, 38.9% anemic and 5.4% tested positive for malaria.

Table 1: Study Population Descriptive Statistics

Maternal Characteristics	**No.**	**Mean ± SD, %**
Age	262	26.7 (±4.9)
Subdistrict		
Vekky	92	35.1%
Sô-Ava	91	34.7%
Akassato	65	24.8%
Houedo-Aguekon	14	5.3%
Marital Status		
Married (Monogamy)	173	66.0%
Married (Polygamy)	71	27.1%
Cohabitation	18	6.9%
Ethnicity		
Toffin	195	74.4%
Aizo	32	12.2%
Fon	20	7.6%
Other	13	5.8%
Household density[a]	262	6.1 (±3.9)
Education		
Illiterate	183	69.8%
Gravidity		
Primigravidae	20	7.6%
Multigravidae	242	92.4%
Tobacco during pregnancy		
During 1st trimester	1	0.4%
Throughout pregnancy	1	0.4%
None	254	99.2%
BMI in early pregnancy		
Underweight (<18.5)	24	9.2%
Normal (18.5-25)	175	66.8%

Overweight/Obese (≥25)	63	24.0%

[a]Number of people per household

Table 2: Maternal blood test results for iron deficiency parameters

	No.	Pre-pregnancy visit	No.	2nd Ante Natal Care (ANC2) visit
Hemoglobin (g/l)				
Mean (±SD)	261	117.6 (±13.0)	260	114.1 (±11.8)
Range (min - max)		84 - 151		84 - 146
Ferritin (µg/l)				
Mean (±SD)	260	58.1 (±34.6)	261	84.0 (±40.7)
Range		4.3 - 208		10.3 - 187
Adjusted Ferritin (µg/l)[a]				
Mean (±SD)	260	42.0 (±23.6)	261	74.1 (±35.8)
Range		4.3 - 105		9.9 - 171
Anemia[b]	261	151 (57.9%)	260	101 (38.9%)
Iron Deficiency[c]	260	33 (12.7%)	261	9 (3.5%)
Iron Deficiency Anemia[d]	260	25 (9.6%)	261	5 (1.9%)
Inflammation present[e]	260	48 (18.5%)	261	63 (24.1%)
Malaria positive by microscopy	259	18 (7.0%)	261	14 (5.4%)
Insufficient iron stores for pregnancy[f]	260	98 (37.7%)		

[a]Ferritin results adjusted for inflammation based on C-Reactive Protein (CRP) and α_1-acid-glycoprotein (AGP) biomarkers according to the BRINDA approach

[b]Anemia defined pre-pregnancy as hemoglobin <120g/l and during pregnancy as <110g/l

[c]Iron deficiency defined as adjusted serum ferritin <15 µg/L

[d]Iron deficiency anemia defined as meeting both anemia and iron deficiency criteria

[e]Inflammation defined as either CRP>5 µg/l or AGP>1 µg/l

[f]Insufficient iron stores for pregnancy defined as adjusted serum ferritin <32 µg/L

Table 3: Gestational age information and maternal blood test timing

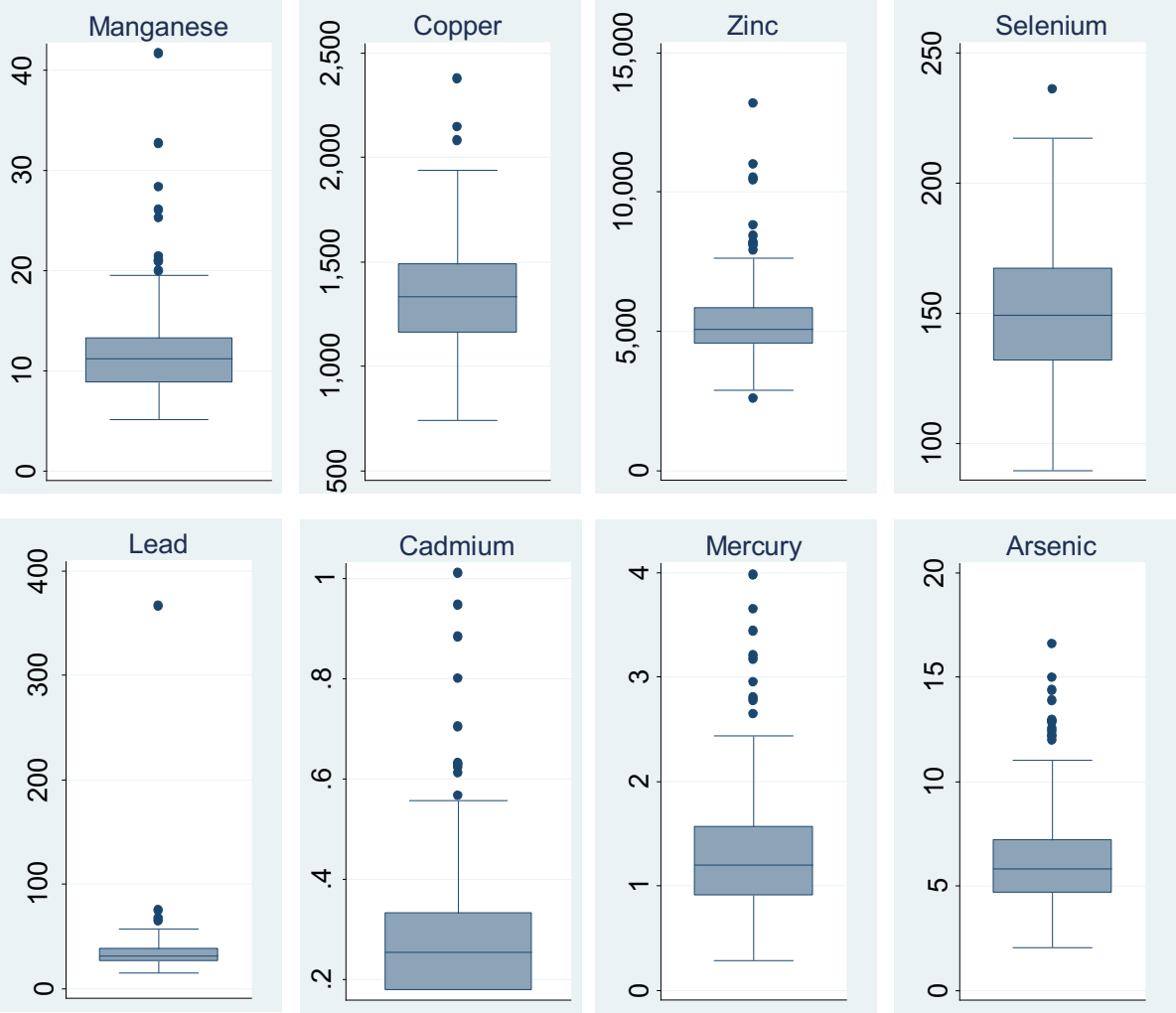
	No.	1st Ante Natal Care (ANC1) visit[g]	No.	2nd Ante Natal Care (ANC2) visit
Gestational age (weeks)				
Mean (±SD)	262	6.9 (±2.3)	261	11.7 (±2.8)
Range		3.7 – 19.3		7.0 – 24.0
1st Trimester (≤14 weeks gestation)			262	221 (84.4%)
2nd Trimester (>14 weeks gestation)			262	41 (15.6%)
Time from pre-pregnancy blood test to confirmation of pregnancy (weeks)	260			
Geometric Mean (95%CI)		17.4 (15.6; 19.4)		
Range		2.1 - 106		
n (%) > 26 weeks		97 (37.3%)		
n (%) > 52 weeks		30 (11.5%)		

[g]Pregnancy confirmed at the first ante-natal visit and iron supplements provided to the mother from this point onwards

3.2 Metal Associations

The range of concentrations in maternal blood at the ANC2 visit for the eight metals tested is shown in Figure 2. The median and interquartile range concentrations were for Mn 11.2 µg/l (IQR 8.76-13.2), Cu 1333 µg/l (IQR 1165-1490), Zn 5074 µg/l (IQR 4541-5830), Se 149.1 µg/l (IQR 132.1-167.3), Pb 31.3 µg/l (IQR 25.7-38.9), Cd 0.253 µg/l (IQR 0.177-0.331), and Hg 1.20 µg/l (IQR 0.907-1.56).

Figure 2: Maternal blood concentrations of elements (µg/l) at ANC2 for n=262 for Mn, Cu, Zn, Pb, Cd and n=202 for Se, Hg, As*



*Boxes represent median values with interquartile range (IQR) and whiskers highest and lowest points within 1.5 IQR length from the box. Dots represent outliers

The relationship between pre-pregnancy iron deficiency and iron deficiency at ANC2 is presented in Table 4. Women found to be iron deficient before conception were significantly more likely to remain iron deficient when re-tested in the first trimester (adjusted OR=41.2, 95%CI 6.18; 275). However, the numbers of iron deficient women, were small with 13% of the sample found to be iron deficient before conception and 4% in the first trimester.

Table 4: Effect of pre-pregnancy iron deficiency on iron deficiency at ANC2

	n	Crude OR (95% CI)	Adjusted OR (95% CI) [a]
		Iron deficient[b] at ANC2	
Iron deficient[b] pre-pregnancy	259	**16.5 (3.24;106)**	**41.2 (6.18;275)**
		Iron stores insufficient[c] at ANC2	
Iron stores insufficient for pregnancy[c] at pre-pregnancy blood test	259	**10.3 (4.23; 27.2)**	**12.5 (5.11;30.6)**

[a]Adjusted for G2 gestational age, gravidity, education and wealth (based on no. possessions)
[b]Iron deficiency defined as serum ferritin adjusted for inflammation <15 µg/L
[c]Insufficient iron stores for pregnancy based on an estimated requirement of at least 300mg which corresponds to a serum ferritin level of ≥32 µg/L. As iron requirements are low in the first trimester, the same cut-off was used for pre-pregnancy and ANC2 blood test results

The results for blood metal concentrations at ANC2 showed that higher pre-pregnancy serum ferritin concentrations and higher ANC2 serum ferritin concentrations were significantly related to lower concentrations of Mn, Cd and Pb in the first trimester after adjustment for potential confounders (Table 5). Every 1% increase in pre-pregnancy serum ferritin concentration was associated with a 0.13% decrease in Mn (adjusted β=-0.13, 95%CI -0.18; -0.07), a 0.22% decrease in Cd (adjusted β=-0.22, 95%CI -0.28; -0.15) and a 0.06% decrease in Pb concentration (adjusted β=-0.06, 95%CI -0.12; -0.006). Similarly, every 1% increase in ANC2 serum ferritin concentration was associated with a 0.19% decrease in Mn (adjusted β=-0.19, 95%CI -0.25; -0.13), a 0.21% decrease in Cd (adjusted β=-0.21, 95%CI -0.29; -0.13) and a 0.11% decrease in Pb concentration (adjusted β=-0.11, 95%CI -0.17; -0.04).

The only other metal to show a significant positive association was Se where every 1 microgram unit increase in pre-pregnancy serum ferritin concentration was associated with a 0.19 microgram increase in Se concentration (adjusted β=0.19, 95%CI 0.043; 0.34) and every 1 unit microgram increase in ANC2 serum ferritin concentration was associated with a 0.11 microgram increase in Se concentration (adjusted β=0.11, 95%CI 0.013; 0.22).

After adjusting the models no significant associations were found between serum ferritin concentrations and Zn, Cu, As or Hg blood concentrations in the first trimester.

Table 5: Associations between serum ferritin concentrations pre-pregnancy and ANC2 blood metal concentrations

	No.	Crude β (95% CI)	Adjusted β (95% CI)
Log Mn (µg/l)			
Log Serum ferritin pre-pregnancy (µg/l)	259	**-0.13 (-0.18, -0.07)**	**-0.13 (-0.18, -0.07)[a]**
Log Serum ferritin at ANC2 (µg/l)	259	**-0.19 (-0.25, -0.13)**	**-0.19 (-0.25, -0.13)[a]**
Log Cd (µg/l)			
Log Serum ferritin pre-pregnancy (µg/l)	259	**-0.21 (-0.28, -0.15)**	**-0.22 (-0.28, -0.15)[b]**
Log Serum ferritin at ANC2 (µg/l)	260	**-0.20 (-0.28, -0.13)**	**-0.21 (-0.29, -0.13)[b]**
Log Zn (µg/l)			
Log Serum ferritin pre-pregnancy (µg/l)	259	-0.03 (-0.07, 0.01)	-0.03 (-0.07, 0.01)[b]
Log Serum ferritin at ANC2 (µg/l)	260	-0.03 (-0.08, 0.01)	-0.04 (-0.09, 0.003)[b]
Log Cu (µg/l)			
Log Serum ferritin pre-pregnancy (µg/l)	258	-0.02 (-0.06, 0.01)	-0.03 (-0.06; 0.004)[c]
Log Serum ferritin at ANC2 (µg/l)	258	-0.03 (-0.07, 0.01)	-0.02 (-0.05; 0.02)[c]
Log Pb (µg/l)			
Log Serum ferritin pre-pregnancy (µg/l)	259	-0.04 (-0.10, 0.02)	**-0.06 (-0.12, -0.006)[d]**
Log Serum ferritin at ANC2 (µg/l)	260	-0.07 (-0.14, 0.001)	**-0.11 (-0.17, -0.04)[d]**
Log As (µg/l)			
Log Serum ferritin pre-pregnancy (µg/l)	201	-0.03 (-0.10, 0.04)	-0.02 (-0.09, 0.05)[a]
Log Serum ferritin at ANC2 (µg/l)	201	-0.07 (-0.15, 0.004)	-0.06 (-0.13, 0.02)[a]
Se (µg/l)			

Serum ferritin pre-pregnancy (µg/l)	201	**0.16 (0.004, 0.31)**	**0.19 (0.043, 0.34)**[e]
Serum ferritin at ANC2 (µg/l)	201	**0.11 (0.010, 0.22)**	**0.11 (0.013, 0.22)**[e]
Log Hg (µg/l)			
Log Serum ferritin pre-pregnancy (µg/l)	201	**0.003 (0.000, 0.01)**	-0.07 (-0.01, 0.16)[f]
Log Serum ferritin at ANC2 (µg/l)	201	0.002 (-0.000, 0.003)	0.03 (-0.06, 0.13)[f]

[a]Adjusted for G2 gestational age, gravidity, education, wealth and time from pre-pregnancy blood test to conception
[b]Adjusted for G2 gestational age, gravidity, education and wealth
[c]Adjusted for G2 gestational age, gravidity, education, wealth, time from pre-pregnancy blood test to conception, BMI and living lake-side
[d]Adjusted for G2 gestational age, gravidity, education, wealth and living lake-side
[e]Adjusted for G2 gestational age, gravidity, education, wealth and BMI
[f]Adjusted for G2 gestational age, gravidity, education, wealth, BMI and living lake-side

4. Discussion

These results show that serum ferritin concentrations prior to conception have an influence on blood concentrations of iron, Mn, Cd, Pb and Se during pregnancy. Pre-pregnancy serum ferritin concentrations were inversely associated with higher whole blood concentrations of Mn, Cd and Pb and positively associated with higher Se concentrations in the first trimester. With iron deficiency pre-pregnancy strongly related to iron deficiency in the first trimester, similar associations are seen between ANC2 serum ferritin concentrations and Mn, Cd, Pb and Se, as would be expected.

4.1 Iron

The number of women found to be anemic prior to pregnancy was high (57.9%) with that due to iron deficiency at 17%. Although iron deficiency is commonly assumed to cause half of all cases of anemias (WHO, 2015), this figure is in line with a recent meta-analysis of national survey data which found where anemia prevalence was greater than 40% in rural areas the percentage associated with iron deficiency was 16% (Petry et al., 2016).

All women received iron supplements from their first ANC visit and overall serum ferritin concentrations increased, and the numbers of anemic and/or iron deficient women decreased between the pre-pregnancy and ANC2 visit blood tests implying that supplementation was effective. It was not however possible to know from the data collected the compliance of individual women in regularly taking iron supplements. Women were asked at each ANC visit if they had taken iron supplements the previous day but no relationship was found between the number of positive responses and iron status. Iron status prior to pregnancy had an important effect on iron status in early pregnancy. The odds of being iron deficient at the ANC2 visit were 41.2 (95%CI 6.2;275.0) times more likely if iron deficient pre-pregnancy. The wide confidence interval of this result reflects the small number of women in the sample who remained iron deficient at ANC2. For this reason, a different cutoff was also tested based on an estimate of iron stores that are needed to be present at the start of pregnancy to fully meet requirements and was consistent with the previous result with the odds of a woman remaining without sufficient iron stores for pregnancy at ANC2 being 12.5 (95%CI 5.11;30.6) times higher if low in iron stores prior to pregnancy. This finding suggests that for women who are not classified as iron deficient but have lower iron stores, the iron supplementation that they receive is not sufficient to carry them through all the iron requirements of pregnancy.

The current recommendations for iron supplementation in pregnancy in Benin is daily oral iron and folic acid supplementation usually provided through ANC visits and coverage is relatively high with 85% of women who had a live birth in the previous 5 years reporting having taken oral iron supplement in the most recent national demographic and health survey (INSAE & ICF, 2019). A comparison of rates of adherence to iron supplementation during pregnancy between 22 sub-Saharan found Benin had the 4th highest reported rate of these countries and varies as may be expected with the number of antenatal visits, and levels of education and family wealth (Ba et al., 2019). It appears that iron supplementation is well accepted in Benin but may start later than ideal as seen in some of the quite late first ANC visits in this cohort, despite regular follow-up. It is also possible that the same socio-economic factors that contribute to lower adherence to iron supplementation may also contribute to higher environmental exposures to metals.

4.2 Manganese, Cadmium and Lead

Blood Mn concentrations in the range 4–15 µg/L are considered normal (ATSDR, 2012b). Although the majority of blood samples taken at ANC2 in this study fell within this range, 15% of women recorded concentrations above 15 µg/L. As blood manganese concentrations are known to increase during pregnancy and be significantly higher in cord than in maternal blood samples at delivery (Gunier et al., 2014; Takser et al., 2004; Tholin et al., 1995; Yamamoto et al., 2019), it is likely that an even greater percentage of women would have been exposed to higher concentrations of Mn by the end of pregnancy than that measured at ANC2. Several studies link adverse effects on birth size parameters with both low and high concentrations of Manganese in maternal and/or cord blood (Guan et al., 2014; Yamamoto et al., 2019; Zota et al., 2009) and between higher concentrations of manganese and impaired child neurodevelopment (Chung et al., 2015; Claus Henn et al., 2017; Lin et al., 2013; Takser et al., 2003). Evidence from other studies also supports a close interaction between manganese and iron, particularly in an iron deficient state, in terms of their transport and absorption mechanisms into the body (Bjørklund et al., 2017; Margrete Meltzer et al., 2010). Blood Mn concentrations have been found to be higher among pregnant women (Tholin et al., 1995). Low iron status may also increase manganese concentration in certain brain areas as demonstrated in animal studies (Erikson et al., 2004, 2005).

The results for Mn, Cd and Pb appear to be consistent with reported transport affinities of DMT1 whereby Mn and Cd are transported in preference to iron and Pb (Garrick et al., 2006). The slightly lower increase in Pb concentrations compared to Mn and Cd for the same change in serum ferritin concentrations for both pre-pregnancy and ANC2 results may indicate the competition between these metals for absorption through this transport mechanism. A similar pattern was found in a Norwegian cohort monitored for metals mid-pregnancy where blood concentrations of Mn and Cd were found to be significantly higher in women who were iron deficient, with a greater difference in Mn concentrations compared to Cd concentrations. Pb concentrations in this cohort however were not found to be significantly different between iron deficient and non-iron deficient women (Caspersen et al., 2019).

4.3 Selenium

For optimal activity of Se dependent proteins, concentrations in whole blood would be expected to be in the range 125-163 µg/L (Muecke et al., 2018). This is similar to the concentrations measured in the first trimester for women in this study where the median value was 149.1 and the inter-quartile range 132.1-167.3 µg/L. Although a significant positive association was found between serum ferritin concentrations and Se concentrations, the effect was quite small. For example all other parameters being equal, the difference between Se concentration if increasing from the minimum pre-pregnancy serum ferritin concentration of 4.3 to the average of 42 µg/l would be an increase of 7 µg/l which is a relatively small difference on the scale of the Se results measured. Similar results were found in a mid-pregnancy cohort where a small but significant increase in blood Se concentrations was found in non iron deficient women compared to iron deficient women (Caspersen et al., 2019). The adjusted R squared values for the Se models in this study were the lowest of those that found a significant association explaining only 7% to 8% of the variation in the dataset indicating that other important factors are certainly influencing Se values. Although there is some evidence that low selenium concentrations can be associated with anaemia and iron deficiency anaemia, increasing iron stores is not thought to have a direct effect on selenium uptake (Gürgöze et al., 2004; Semba et al., 2009; Viita et al., 1989; Yetgin et al., 1992).

4.4 Limitations and Strengths

There are several factors that add to measurement uncertainty in the dataset. The time between the blood draw before pregnancy and pregnancy confirmation varied widely from just over 2 weeks to almost 2 years and iron status may have changed over time. There were also differences in the timing of ANC visits meaning that iron supplementation would have started at different points during the pregnancy and that what was intended as the first trimester blood draw did not take place until early in the second trimester for 16% of women in the sample and both of these could have affected the concentrations of metals measured. In particular Mn has a relatively low half-life in blood of around 30 days (Finley et al., 2003) so changes in iron status between the pre-pregnancy and ANC2 blood draw may not fully reflect Mn exposure over that time period. Nevertheless, there was a strong correlation between serum ferritin concentrations at the pre-pregnancy and ANC2 blood draws (r_s=0.65) which implies that the differing time periods did not change relationships too much. If some women experienced longer periods of iron supplementation than others, this may have resulted in some low pre-pregnancy serum ferritin concentrations not corresponding to higher concentrations of Mn at ANC2. This would have reduced the strength of the associations found in the regression analysis as well as reducing the value of the coefficients. Conversely, for the women who were not tested for metals until the second trimester of pregnancy, metal concentrations may look higher than they actually were for a normal serum ferritin concentration, given that the uptake of some metals increase during pregnancy. This would have had the opposite effect on the regression coefficients, potentially increasing above their true value while also reducing the strength of the association.

The major strength of this study is that detailed information was collected prior to conception which is not often available in comparable pregnancy cohorts. The number of metals assessed and the ability to correct for inflammation in this context adds to understanding of environmental exposures

in Benin for which there are few existing studies. It highlights that women with lower iron stores prior to pregnancy, even if not classified as iron deficient, are at risk of absorbing higher amounts of metals from their environments that are detrimental to the health of both mother and child. It is suggested that testing the effects of iron and folic acid supplementation beginning prior to conception on concentrations of Mn, Cd and Pb during pregnancy would be worthwhile to determine if fetal exposures can be reduced without any other negative effects.

5. Conclusion

This study shows that serum ferritin concentrations are negatively associated with increased uptake of the toxic metals Mn, Cd and Pb in pregnancy. Importantly, lower serum ferritin concentrations prior to conception were found to be associated with higher concentrations of Mn, Cd and Pb suggesting that increasing iron both before and during pregnancy may limit uptake of these metals. These results argue in favour of testing the effects of iron supplementation prior to pregnancy to better safeguard foetal development.

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Supplementary Materials

Table S1: Maternal blood metal concentrations at ANC2 visit (µg/l)

Metal	n	LOQ (µg/L)	%<LOQ	Geometric Mean (95%CI)	Median	Min	Max	IQR
Mn	262	5	0	11.0 (10.6, 11.4)	11.2	5.12	41.7	8.76-13.2
Cu	262	200	0	1318 (1289, 1348)	1333	743.4	2378	1165-1490
Zn	262	200	0	5157 (5019, 5298)	5074	2596	13167	4541-5830
Se	202	20	0	148.1 (144.4, 151.9)	149.1	89.6	235.7	132.1-167.3
Pb	262	2	0	31.6 (30.3, 32.9)	31.3	14.7	366.5	25.7-38.9
Cd[a]	262	0.25	43	0.254 (0.242, 0.266)	0.253	0.177	1.01	0.177-0.331
Hg[a]	202	0.4	1.5	1.20 (1.13, 1.27)	1.20	0.283	3.98	0.907-1.56
As	202	1.0	0	5.96 (5.67, 6.26)	5.84	2.04	16.6	4.65-7.22

[*]Results below the limit of quantification (LOQ) were replaced by the LOQ divided by the square root of 2. For Cd the number of results below LOQ was 113 and for Hg 3.

Table S2: Correlations between metals at ANC2 – Spearman rank correlation test

Metal	Mn	Cu	Zn	Se	Pb	Cd[a]	Hg[a]	As
Mn	1							
Cu	0.03	1						
Zn	0.29	-0.03	1					
Se	-0.01	0.01	0.10	1				
Pb	0.31	0.03	0.14	0.08	1			
Cd[a]	0.37	0.03	0.21	0.008	0.29	1		
Hg[a]	0.11	0.08	0.14	0.65	0.14	0.07	1	
As	-0.003	0.17	-0.04	0.12	0.10	0.04	0.20	1

[a]Results below the limit of quantification (LOQ) were replaced by the LOQ divided by the square root of 2. For Cd the number of results below LOQ was 113 and for Hg 3