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Maternal Iron and Vitamin D Status during the Second Trimester Is Associated with Third Trimester Depression Symptoms among Pregnant Participants in the APrON Cohort

Jenna L. Evanchuk [1], Anita Kozyrskyj [2], Elnaz Vaghef-Mehrabani [3], Yvonne Lamers [4,5], Gerald F. Giesbrecht [3,6], Nicole Letourneau [3,6,7], Fariba Aghajafari [6,8], Deborah Dewey [3,6], Brenda Leung [9], Rhonda C. Bell [1], Catherine J. Field [1,*]

[1] *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada;* [2] *Department of Pediatrics, University of Alberta, Edmonton, AB, Canada;* [3] *Department of Pediatrics, University of Calgary, Calgary, AB, Canada;* [4] *Food, Nutrition and Health Program, University of British Columbia, Vancouver, BC, Canada;* [5] *British Columbia's Children's Hospital Research Institute, Vancouver, BC, Canada;* [6] *Department of Community Health Sciences, University of Calgary, Calgary, AB, Canada;* [7] *Department of Psychiatry, University of Calgary, Calgary, AB, Canada;* [8] *Department of Family Medicine, University of Calgary, Calgary, AB, Canada;* [9] *Faculty of Health Sciences, University of Lethbridge, Lethbridge, AB, Canada*

A B S T R A C T

Background: The maternal status of multiple micronutrients during pregnancy and postpartum and their potential associations with maternal health outcomes are largely undescribed.

Objectives: This study aimed to examine associations between maternal iron and vitamin D status, individually and in combination, on depression symptoms in pregnant individuals.

Methods: The Alberta Pregnancy Outcomes and Nutrition cohort study included pregnant participants and their children from Calgary and Edmonton, Canada. Iron biomarkers (serum ferritin [SF], soluble transferrin receptor, and hepcidin) were measured via immunoassays and vitamin D [25-hydroxyvitamin D3 (25(OH)D3) and 3-epi-25-hydoxyvitamin D3 (3-epi-25(OH)D3)] metabolites were quantifed using liquid chromatography with tandem mass spectroscopy. Four categories of maternal iron and vitamin D status during the second trimester were conceptualized using concentrations of SF and total 25-hydoxyvitamin D [25(OH)D], respectively. Maternal Edinburgh Postnatal Depression Scale (EPDS) scores during the third trimester (n = 1920) and 3 mo postpartum (n = 1822) were obtained.

Results: Concentrations of maternal 25(OH)D3, 3-epi-25(OH)D3, and the ratio of both metabolites were significantly higher during the second trimester compared with their status at 3 mo postpartum. Higher second trimester maternal concentrations of SF (β: −0.8; 95% confidence interval [CI]: −1.5, −0.01), hepcidin (β: −0.5; 95% CI: −0.9, −0.2), and 25(OH)D3 (β: −0.01; 95% CI: −0.02, −0.004) predicted lower maternal EPDS scores during the third trimester. Pregnant individuals with a low iron (SF <15 μg/L) and replete vitamin D (25(OH)D ≥75 nmol/L) (β: 1.1; 95% CI: 0.03, 2.1) or low iron (SF <15 μg/L) and vitamin D (25(OH)D <75 nmol/L) (β: 2.2; 95% CI: 0.3, 4.2) status during midpregnancy had higher third trimester EPDS scores compared with those that were replete in both micronutrients.

Conclusions: A higher midpregnancy maternal iron and vitamin D status, independently or in combination, predicted fewer maternal depression symptoms in the third trimester. Concentrations of maternal 25(OH)D3 and 3-epi-25(OH)D3 may be lower in the postpartum period compared with midpregnancy.

Keywords: pregnancy, iron, vitamin D, nutrient status, multiple micronutrients, maternal depression

Abbreviations: APrON, Alberta Pregnancy Outcomes and Nutrition; CFG, Canada's Food Guide; CV, coefficient of variation; DAG, directed acyclic graphs; EPDS, Edinburgh Postnatal Depression Scale; IQR, interquartile range; LLOQ, lowest limit of quantitation; MS/MS, tandem mass spectrometry; NIST, National Institute for Standards and Technologies; SF, serum ferritin; SIQ, Supplemental Intake Questionnaire; sTfR, soluble transferrin receptor; sTfR:SF, soluble transferrin receptor-serum ferritin index; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 3-epi-25(OH)D3, 3-epi-25-hydroxyvitamin D3; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D2, 25-hydroxyvitamin D2; 25(OH)D3, 25-hydroxyvitamin D3.

* Corresponding author. *E-mail address:* cjfield@ualberta.ca (C.J. Field).

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Introduction

An adequate status of iron and vitamin D during pregnancy can be difficult to achieve for many reasons, including limited access to food sources, low bioavailability of nutrient forms in consumed foods, the observance of specific dietary patterns, location or seasonality of residence, certain medical conditions, and demographic factors such as ethnicity and age [1–3]. Pregnant people have a higher risk of low status or deficiency in one or both of these micronutrients due to the increased requirements necessitated for optimal fetal neurodevelopment and maternal health [4–8]. Iron deficiency anemia is estimated to affect 20% of pregnancies globally [9]. There are limited data, however, on the global prevalence of maternal vitamin D sufficiency, most often defined as 25-hydroxyvitamin D [25(OH)D] concentrations >50 nmol/L [10] throughout pregnancy [11,12]. Biomarker cutoffs for vitamin D deficiency vary between studies and remain controversial. Despite a few investigations that attempted to estimate risk of iron or vitamin D deficiency during gestation [13,14], very few have assessed the maternal status of both nutrients in healthy pregnant cohorts.

Even though iron and vitamin D are thought to be essential for gestational health and early childhood development [4–8], they are often not monitored among pregnant people from high-income countries [15–17]. Health Canada recommends iron supplementation for pregnant individuals to meet their increased iron needs [18,19]. Nevertheless, recent investigations have questioned whether the current recommended daily allowance of 27 mg of iron each day during pregnancy is sufficient to sustain increased maternal, placental, and fetal iron demands [13,20]. The recommendations for vitamin D do not increase during the reproductive years, pregnancy, or lactation relative to the dietary vitamin D requirements for children and nonpregnant adults aged <50 y in Canada [21]. Moreover, pregnant individuals living at higher latitudes are less likely to be replete in vitamin D when consuming the current Canadian recommended daily allowance of 600 International Units/d [22]. The use of iron and vitamin D biomarkers could aid in a more objective assessment of their adequacy among pregnant people, compared with dietary assessments [23], if they are measured in a variety of populations. Status refers to the biological adequacy of a given nutrient within the body that accounts for differences in dietary intake, bioavailability, and absorption. Fortunately, biomarkers that estimate the status of these nutrients, including hepcidin, serum ferritin (SF), and soluble transferrin receptor (sTfR) for iron and 25(OH)D for vitamin D, are becoming more clinically available [20,24,25].

Evidence related to the potential combined impact of maternal iron and vitamin D status on pregnancy-related health outcomes is limited, despite an emergence of studies reporting mechanistic interactions between their biological pathways [26–31]. For example, Braithwaite et al. [29] reported associations between erythropoietin concentrations, indicative of hypoxia and iron deficiency [32], and an increased expression of fibroblast growth factor 23, a negative regulator of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] [33]. Relationships between 1, 25$(OH)_2$D and erythropoietin receptors have also been reported [26]. Further, elevated 1,25$(OH)_2$D has been shown to reduce the expression of hepcidin [27,31], a negative regulator of nonheme iron absorption [34]. Positive associations between biomarkers of iron and vitamin D have been described in pregnant individuals [35], adolescents [36], athletes [37], and older adults [38]. The findings from these studies suggest that a history of anemia [35] or other measures of iron depletion, such as SF and total iron binding capacity [36–38], are related to a lower vitamin D status in humans. However, there were other studies that did not detect associations between iron and vitamin D status [39,40], reinforcing the need for future investigation in diverse populations. To our knowledge, whether there is a combined effect of concurrent maternal vitamin D and iron deficiency on maternal health outcomes is unknown.

Neurobiological evidence links iron status to energy and neurotransmitter balance in the brain [41–43] and vitamin D to neuronal messaging and immunomodulation [44,45], suggesting their importance for mental health. Previous observational studies have reported associations between higher iron and vitamin D status, independently, with a reduced risk of maternal depression [46–49], but others did not [50,51]. Nonetheless, most of the available evidence suggests that a higher maternal iron or vitamin D status predicts less prenatal or postpartum depression symptoms [44,52,53]. Few studies have assessed nutrient risk factors for maternal depression at both timepoints in the same investigation [47,54]. A recent study by Lin et al. [49] may be one of the only investigations that reported on the maternal status of iron and vitamin D (as well as folic acid), and their associations with gestational depression symptoms, but more studies are required espeically in the postpartum period.

There are notable differences in study designs, particularly in the type and timing of outcome assessments and biochemical quantification of biomarkers that complicate the synthesis of the body of evidence linking maternal iron and vitamin D status to mental health among pregnant people. In addition, very few studies to date have recruited large numbers of generally healthy pregnant individuals to explore how this critical outcome may be impacted by maternal iron or vitamin D status. Considering previous evidence independently linking both micronutrients to maternal depression [46–49], an investigation that examines potential differences in these outcomes depending on the combined adequacy of maternal iron and vitamin D is warranted.

One of the primary aims of the Alberta Pregnancy Outcomes and Nutrition (APrON) cohort study is to examine if nutrient status in pregnancy and postpartum is related to maternal mental health [55]. The goal of this substudy was to examine the individual and combined associations of maternal iron and vitamin D status during and after pregnancy on depression symptoms among participants.

Methods

The APrON study recruited 2189 pregnant people from Calgary and Edmonton, Alberta, Canada between 2009 and 2012 [55]. Detailed methods have been published elsewhere [55,56]. Briefly, pregnant people were eligible for this prospective cohort if they were over 16 y of age, could read and write in English, and intended to reside in Calgary or Edmonton throughout gestation and to 3 mo postpartum. These participants visited the clinic up to three times during pregnancy (average weeks of gestation [range]: 10.8 [3.1–13.9] in the first, 19.0 [14.0–26.9] in the second, and 32.5 [27.0–39.0] in the third trimester) and at multiple timepoints during the postpartum period [55,56]. We

employed data for this secondary analysis from the pregnancy and 3 months (mo) postpartum visits. The Calgary Health Research Ethics Board (REB14-1702) and the University of Alberta Health Research Biomedical Panel (Pro00002954) provided ethical approval, and informed consent was obtained from all participants [55,56].

Maternal demographic information

An extensive list of self-reported demographic characteristics, including maternal ethnicity, age, highest level of education, and average family income, were collected at the first study visit [55,56]. The occurrence of gestational medical conditions was determined through self-report questionnaires at several pregnancy visits. Heights of the pregnant participants were measured at the first visit, and weight was measured at all prenatal visits to estimate prepregnancy BMI and weight gain in pregnancy, as previously reported [57].

Predictors

Maternal nutrition information.

Maternal intakes of iron and vitamin D from supplements was estimated via Supplemental Intake Questionnaires (SIQs) by self-report and participants confirmed the type (often brand name) and dose of individual nutrients or multivitamins [55,56]. This data was then converted into an estimated daily supplement intake for different micronutrients including iron and vitamin D, which were utilized for the current analysis. Maternal 24-h dietary recalls and SIQs were collected at each prenatal study visit and at ~3 mo postpartum [56]. A maternal healthy eating score was calculated based on Canada's Food Guide (CFG) to estimate overall diet quality [58,59].

Iron and vitamin D biomarker quantification.

Maternal venous blood samples were drawn at each study visit by a trained phlebotomist [55]. Whole blood was immediately fractioned into red blood cell, serum, and plasma portions, which were stored separately at −80 °C. Maternal SF was quantified at all 3 trimesters and at ~3 mo postpartum using a i2000sr Architect Plus blood analyzer machine (Abbott), which uses chemiluminescent microparticle immunoassay techniques. The variation in SF concentrations of random samples that were run daily was <5%. Iron storage depletion was defined as SF <15 μg/L [17]. Enzyme-linked immunosorbent assays (R&D Systems) were used to measure hepcidin (detection range: 3.13–800 pg/mL) and soluble transferrin receptor (sTfR) (5–80 mg/L) concentrations in serum samples in duplicate. Maternal hepcidin was measured in second and third trimester samples, and maternal sTfR was quantified in first and third trimester samples. A coefficient of variation (CV) of <10% between the absorbances of duplicate samples was considered reproducible. If a particular sample had a CV ≥10% or had a concentration that fell outside of the detection range, the sample was requantified after dilution or concentration. If maternal SF and sTfR concentrations were quantified during the first ($n = 267$) or third trimester ($n = 280$), the ratio of sTfR to the logarithm of SF [sTfR/$\log_{10}$(SF)] was used to calculate the sTfR–SF ratio (sTfR:SF) [60].

Second trimester plasma concentrations of 25-hydroxyvitamin D (25(OH)D) and the prevalence of maternal vitamin D deficiency (25(OH)D <50 nmol/L) [61,62] and insufficiency (25(OH)D <75 nmol/L) [63] among 537 pregnant participants were published elsewhere [22]. For the present investigation, vitamin D metabolites were quantified in an additional 2500 maternal samples during the second trimester ($n = 1249$) and at 3 mo postpartum ($n = 1251$). In brief, vitamin D metabolites were extracted from human plasma by liquid-liquid extraction using hexane and ethyl acetate [64–66]. The analytes, including 25-hydroxyvitamin D2 (25(OH)D2), 1,25-dihydroxyvitamin D2 (1,25$(OH)_2$D2), 25-hydroxyvitamin D3 (25(OH)D3), 3-epi-25hydroxyvitamin D3 (3-epi-25(OH)D3), and 1,25-hydroxyvitamin D3 (1,25$(OH)_2$D3), were separated by liquid chromatography (Agilent 1290) using reverse-phase conditions and detected by tandem mass spectrometry (MS/MS) in positive-ion mode (Agilent 6495B MS/MS). Quantitation was achieved by isotope dilution method using stable-isotope-labeled standards (Cayman Chemicals, Sigma Supelco, Inc). The lowest limit of quantitation (LLOQ) for each of the vitamin D metabolites was 0.78 ng/mL. The assay was validated using pooled plasma samples and the Standard Reference Material 2970 (National Institute for Standards and Technologies, NIST). The assay showed high accuracy (compared with NIST values) and precision reflected in the <6% interassay CV for mean ± standard deviation (SD) of 9.65 ± 0.58 ng/mL 25(OH)D3 (certified value: 9.63 ± 0.31 ng/mL), 23.19 ± 1.45 ng/mL 25(OH)D2 (certified value: 23.5 ± 0.3 ng/mL) and 1.98 ± 0.11 ng/mL 3-epi-25(OH)D3. Concentrations of the forms 25(OH)D2, 1,25$(OH)_2$D2, and 1,25$(OH)_2$D3 were below the LLOQ in all maternal samples in the most recent liquid chromatography-MS/MS analysis. Maternal 25(OH)D concentrations are the sum of maternal 25(OH)D3 and 25(OH)D2 concentrations during the second trimester in the case both forms were quantified over the LLOQ ($n = 644$) [22].

To examine relationships between different levels of maternal iron and vitamin D status adequacy on the outcome of interest, a categorical variable was created. Four categories were defined: *1*) replete iron (SF ≥15 μg/L) and replete vitamin D (25(OH)D ≥75 nmol/L); *2*) replete iron (SF ≥15 μg/L) and low vitamin D (25(OH)D <75 nmol/L); *3*) low iron (SF <15 μg/L) and replete vitamin D (25(OH)D ≥75 nmol/L); and *4*) low iron (SF <15 μg/L) and low vitamin D (25(OH)D <75 nmol/L). Participants with SF and 25(OH)D quantified in the second trimester ($n = 627$) were categorized.

Outcome

Maternal depression.

The 10-item Edinburgh Postnatal Depression Scale (EPDS) was used to assess maternal depression symptoms at the third trimester and 3 mo postpartum [67]. This questionnaire is a widely researched tool to measure depressive symptoms in pregnant individuals, and it asks participants about varied aspects of their feelings in the past week. Scores could range from 0, indicating the absence of depressive symptoms, to 30, suggesting a high frequency or severity of depressive symptoms [67,68]. An EPDS score of ≥13, estimated to have a sensitivity of 66% and specificity of 95% in a recent meta-analysis [69], was used as a threshold to indicate probable depression.

Statistical analyses

Variables that are normally distributed are presented as mean ± SD; skewed data are presented as medians (interquartile range (IQR); iron and vitamin D daily supplement intakes, 3-epi-25(OH)D3, 3-epi-25(OH)D3–25(OH)D3 ratio) and were log

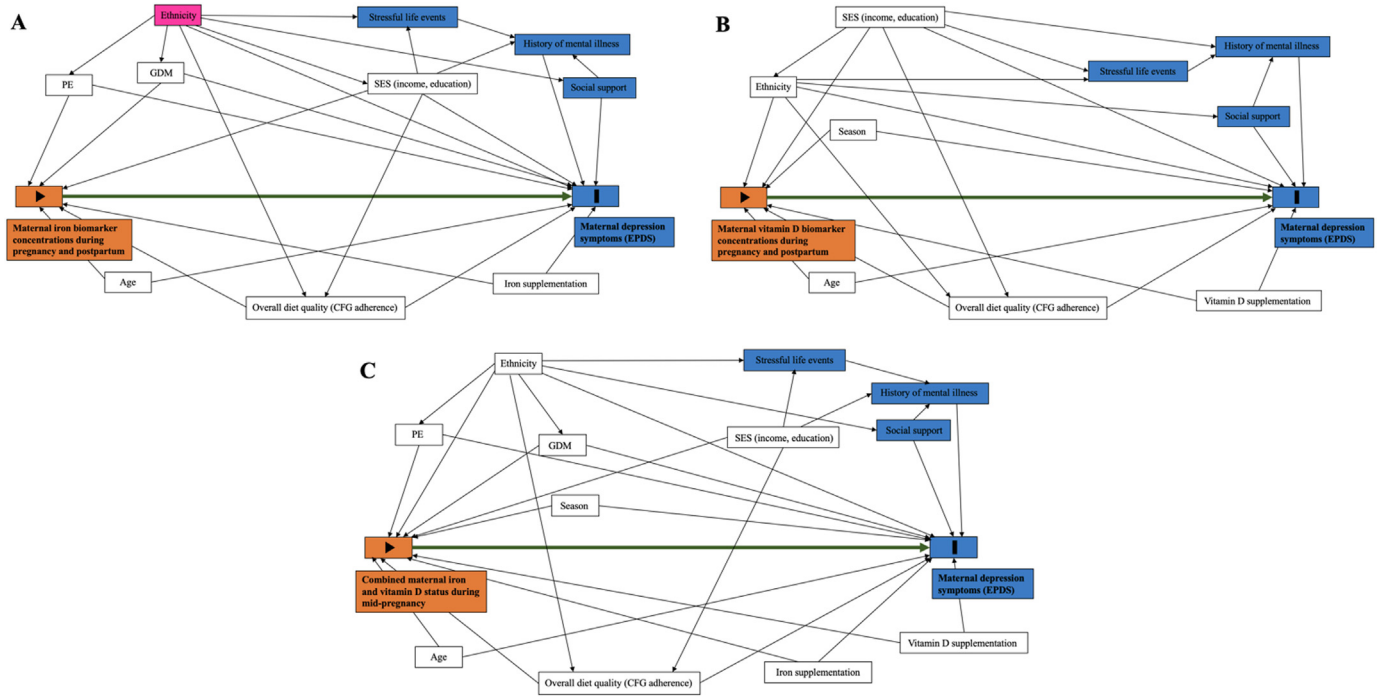

FIGURE 1. Directed acyclic graphs (DAGs) constructed for relationships between (A) maternal iron, (B) vitamin D biomarker concentrations, and (C) the combined status of iron and vitamin D status on maternal depression symptoms. Green lines depict the path between the exposure (orange box) and outcome (blue box) of interest, whereas the black lines highlight paths with potential confounders of the relationship [80]. White boxes with black borders show the minimum set of covariates that must be adjusted to isolate the exposure and outcome association. Ancestors of the exposures and outcomes (pink boxes) and outcomes (blue boxes) are also shown. The DAGs were constructed using DAGitty.net. Abbreviations: CFG, Canadian Food Guide; EPDS, Edinburgh Postnatal Depression Scale; GDM, gestational diabetes mellitus; PE, preeclampsia; SES, socioeconomic status.

transformed as needed for statistical tests (hepcidin, SF, sTfR, sTfR:SF, 3-epi-25(OH)D3). Maternal vitamin D biomarker concentrations during the second trimester and 3 mo postpartum were compared using paired *t* tests.

Multivariate regression models were utilized to explore relationships between maternal iron or vitamin D biomarkers at different study timepoints and EPDS scores during the third trimester and 3 mo postpartum. The availability of EPDS data dictated the number of pregnant participants that were eligible for inclusion into the statistical models (see Supplemental Figure 1). Directed acyclic graphs (DAGs) were constructed based on evidence in the literature to identify variables that could be associated with both the exposures and outcomes of interest (Figure 1) [70]. This information was used to guide multivariate adjustment. Change-in-estimate rules were applied to each model as a means of adjusting for the minimum number of covariates for each relationship to reduce risk of over-adjustment [71]. A threshold of 10% was used because of the large sample sizes in the APrON cohort (refer to Supplemental Table 1 for a detailed description of variables that were included in the models). Generalized linear models, adjusted for maternal age, ethnicity, income, educational attainment, CFG scores, gestational diabetes, preeclampsia, iron and vitamin D supplementation during pregnancy, and season were used to assess relationships between the iron and vitamin D status adequacy categorical variable and maternal depressive symptoms. If data for a variable was missing, then data from that participant was excluded. SPSS (V28.0, IBM Corporation) was used to conduct all statistical analyses, and 2-tailed P values < 0.05 were considered statistically significant.

Results

Characteristics of the cohort are shown in Table 1 [72]. Generally, participants were married or cohabiting with a partner, highly educated, and had a household income >$70,000. The majority self-identified as White and were born in Canada, and 53% were pregnant with their first child. The prevalence of pregnancy-related medical conditions was low. Most of the pregnant individuals consumed iron and vitamin D supplements at each trimester of pregnancy (Table 2).

Mean maternal concentrations of 25(OH)D3 were significantly lower at 3 mo postpartum (83.8 ± 24.8 nmol/L) compared with the second trimester of pregnancy (90.7 ± 25.8 nmol/L) (Figure 2A). The same relationship was detected between the second trimester (median [IQR]: 6.7 [3.2]) and postpartum timepoint (5.4 [2.1]) for 3-epi-25(OH)D3 (Figure 2B) and the ratio of 3-epi-25(OH)D3 to 25(OH)D3 (median [IQR]: 7.1 [0.04] during the second trimester and 6.7 [0.03] at 3 mo postpartum) (Figure 2C). When participants were categorized based on their iron and vitamin D status during the second trimester, about 65% were replete for both micronutrients; however, one-third had a low status in either iron or vitamin D, and less than 5% had a low

TABLE 1
Maternal characteristics in the full (n=2189) APrON cohort

Variables	Participants with data (n)	Point estimate	Participants with missing data (n)
Age (y)[1]	2134	31.5 ± 4.5	55
Prepregnancy BMI (kg/m^2)[2]	1947	23.0 (5.4)	242
Marital status[3]			97
Married	1772	86.7	
Common-law	236	11.3	
Separated or divorced	15	0.7	
Single	69	3.3	
Education[3]			117
Completed post-grad	470	22.7	
Completed university	943	45.5	
Completed trade/tech	401	19.4	
Completed high school	200	9.6	
Less than high school	58	2.8	
Household income[3]			120
$100 000 or more	1143	55.2	
$70 000–$99 999	463	22.4	
$40 000–$69 999	276	13.3	
$20 000–$39 999	122	5.9	
Less than $20 000	65	3.1	
Primiparous[3]			99
Yes	1119	53.5	
No	971	46.5	
Immigrated to Canada[3]			98
No	1612	77.1	
Yes	479	22.9	
Ethnicity[3]			103
White	1674	80.2	
Non-white	412	19.7	
Maternal pregnancy conditions[3,4]			
Gestational diabetes mellitus	1695	3.0	494
Preeclampsia	1695	0.3	494
Edinburgh Depression Scale (EPDS)[5]			
Third trimester	1920	4 (0–23)	269
Probable depression (EPDS ≥13), %	107	5.6	
3 mo postpartum	1822	4 (0–22)	367
Probable depression (EPDS ≥13), %	80	4.4	

Abbreviations: APrON, Alberta Pregnancy Outcomes and Nutrition; IQR, interquartile range.
[1] Mean ± SD
[2] Median (IQR)
[3] Percentage
[4] Information collected during the last gestational visit (third trimester). The proportion of participants with the condition is given in the third column for these variables
[5] Median scores (range of scores). Table adapted from [72].

status for both (Table 2). The changes in maternal iron biomarker concentrations in APrON study participants are extensively detailed elsewhere [73].

Associations between maternal iron and vitamin D biomarker concentrations and third trimester maternal EPDS scores were observed after adjustment (Table 3). Higher maternal hepcidin (P = 0.005), SF (P = 0.048) and 25(OH)D3 (P = 0.001) concentrations during the second trimester were significantly associated with lower EPDS scores during the third trimester. There were no relationships between maternal sTfR, sTfR:SF or 3-epi-25(OH)D3 and third trimester EPDS scores or between maternal biomarkers and EPDS scores at 3 mo postpartum (Supplemental Table 2).

There were differences in EPDS scores during the third trimester depending on the combined status of maternal iron and vitamin D during midpregnancy (Table 4). Compared with pregnant participants who were iron replete (SF ≥15 μg/L) and vitamin D replete [25(OH)D ≥75 nmol/L], EPDS scores were significantly higher when iron status was low (SF <15 μg/L) and vitamin D was replete [25(OH)D ≥75 nmol/L] (P = 0.044) or when iron (SF <15 μg/L) and vitamin D [25(OH)D <75 nmol/L] were both low (P = 0.024). There were no differences in EPDS scores collected at 3 mo postpartum by the combined midpregnancy maternal status of iron and vitamin D.

Discussion

A higher maternal iron status in the second trimester was associated with lower maternal EPDS scores during the third trimester. Maternal depression scores differed depending on the combined adequacy of maternal iron and vitamin D during midpregnancy. A summary of these relationships is presented in Table 5. This study provides evidence that maternal iron and vitamin D status are important predictors of maternal mental health during pregnancy.

TABLE 2
Maternal iron and vitamin D supplementation and status adequacy during pregnancy

	First Trimester[1]	Second Trimester	Third Trimester
Supplement intake			
Iron			
Proportion taking, % (*n*)	93.0 (1769)	95.4 (2008)	93.7 (1299)
Median intake (mg/d)[2]	22.9 (13.5)	27.0 (7.4)	27.0 (19.3)
Vitamin D			
Proportion taking, % (*n*)	65.2 (1241)	70.2 (1478)	68.2 (1059)
Median intake (IU/d)[2]	250.0 (461.5)	342.9 (857.1)	400.0 (1000.0)
Iron and vitamin D status adequacy, % (*n*)[3]			
(1) Iron and vitamin D replete	—	63.3 (397)	—
(2) Iron replete, low vitamin D	—	14.8 (93)	—
(3) Low iron, vitamin D replete	—	18.4 (115)	—
(4) Low iron and vitamin D	—	3.5 (22)	—

Abbreviations: IU, international unit; SF, serum ferritin; 25(OH)D, 25-hydroxyvitamin D.
[1] Timepoints: First (mean [range] 10.8 [3.1–13.9] wk], second (19.0 [14.0–26.9] wk), and third (32.5 [27.0–39.0] wk).
[2] Supplement intakes given as median (IQR).
[3] Determined in pregnant participants with SF and 25(OH)D quantified during the second trimester ($n = 627$): *1*) iron (SF ≥15 μg/L) and vitamin D (25(OH)D ≥75 nmol/L) replete; *2*) iron replete (SF ≥15 μg/L), low vitamin D (25(OH)D <75 nmol/L); *3*) low iron (SF <15 μg/L), vitamin D replete (25(OH)D ≥75 nmol/L); and *4*) low iron (SF <15 μg/L) and vitamin D (25(OH)D <75 nmol/L).

Results of other work examining the effects of maternal iron or vitamin D status in pregnancy on maternal depression outcomes are difficult to compare, and some have found contradictory results. Similar to the present study, Lin et al. [49] did not find a relationship between first trimester maternal SF concentrations and third trimester EPDS scores. Gowtham et al. [47] observed that concentrations of SF in pregnant people during early gestation were not related to maternal EPDS scores in the second trimester of pregnancy. Other studies reported that a low maternal iron or vitamin D status was simultaneously related to higher maternal EPDS scores [48,53]; for example, in Jani et al. [53], pregnant participants with first trimester EPDS scores ≥13 were more likely to have a lower vitamin D status during the first trimester. In contrast, we did not observe a significant association between any of the third trimester maternal iron biomarker concentrations and third trimester EPDS scores. However, in the present study, maternal vitamin D metabolites were not quantified during the third trimester, preventing us from comparing third trimester maternal depression symptoms to concurrent maternal 25(OH)D3 and 3-epi-25(OH)D3 concentrations. Considering this body of evidence, reported associations between iron or vitamin D biomarkers and symptoms of maternal depression appear to be temporally nuanced among different study populations, including ours. While other vitamin D metabolites, including 25(OH)D2, 25(OH)D3, and 1, $25(OH)_2D$, were measured in a few investigations [74, 75], maternal concentrations of total 25(OH)D were measured in the majority of cases [46,48,53]. The analysis of a more diverse set of nutrient biomarkers across an array of timepoints has the potential to inform the association between maternal nutrient status and maternal depression before, during and after pregnancy, which should be a focus of future research.

A particularly novel finding in this investigation was that there appeared to be differences in maternal EPDS scores during the third trimester depending on the combined adequacy of maternal iron and vitamin D status during the second trimester. For example, the mean difference in EPDS scores when pregnant individuals had a low status of both micronutrients [SF <15 μg/L and 25(OH)D <75 nmol/L] was more than 2 points above that of the reference group, suggesting that individuals with a concurrent depletion of iron and vitamin D during midpregnancy may experience more symptoms associated with depression during the last trimester [67]. At this point, it is difficult to discuss these findings in relation to other comparable evidence as it is either extremely limited or nonexistent.

Others have postulated bidirectional influences of inadequate nutrient status and poor mental health [76]. Micronutrient availability may impact the development of mental health issues, but to the contrary, people with low moods or depression may consume food or supplements in an inconsistent or disordered manner, which could have consequences on nutrient status [77]. Nevertheless, based on previous data suggesting a mechanistic role for poor nutrition in the development of maternal mental illness [78, 79], we deemed there was enough evidence to justify DAGs and statistical analyses that assumed one direction, with maternal micronutrient status as exposures and EPDS scores as

TABLE 3
Relationships between second trimester maternal hepcidin, SF, and 25(OH)D3 concentrations and third trimester maternal EPDS scores

Biomarker model	Univariate β (95% CI)	Multivariate β (95% CI)
Hepcidin[1]	−0.5 (−0.9, −0.2)	−0.5 (−0.9, −0.2)
SF[2]	−0.5 (−1.1, 0.1)	−0.8 (−1.5, −0.01)
25(OH)D3[3]	−0.01 (−0.02, −0.004)	−0.01 (−0.02, −0.004)

Abbreviations: CI, confidence interval; EPDS, Edinburgh Postnatal Depression Scale; PE, preeclampsia; SF, serum ferritin; 25(OH)D3, 25-hydroxyvitamin D3.
This table only includes variables that were significant predictors of maternal EPDS scores during the third trimester ($P < 0.05$). Hepcidin and SF concentrations were log transformed before regression analysis. The use of directed acyclic graphs identified potential confounding variables which were maternal age, ethnicity, income, educational history, diet quality, gestational diabetes mellitus, PE, season, and current iron or vitamin D supplementation (Figure 1A, B). Minimum adjustment covariates in each multivariate model:
[1] Maternal age.
[2] Third trimester maternal PE and third trimester maternal iron supplementation (mg/d).
[3] No covariates (Supplementary Table 1).

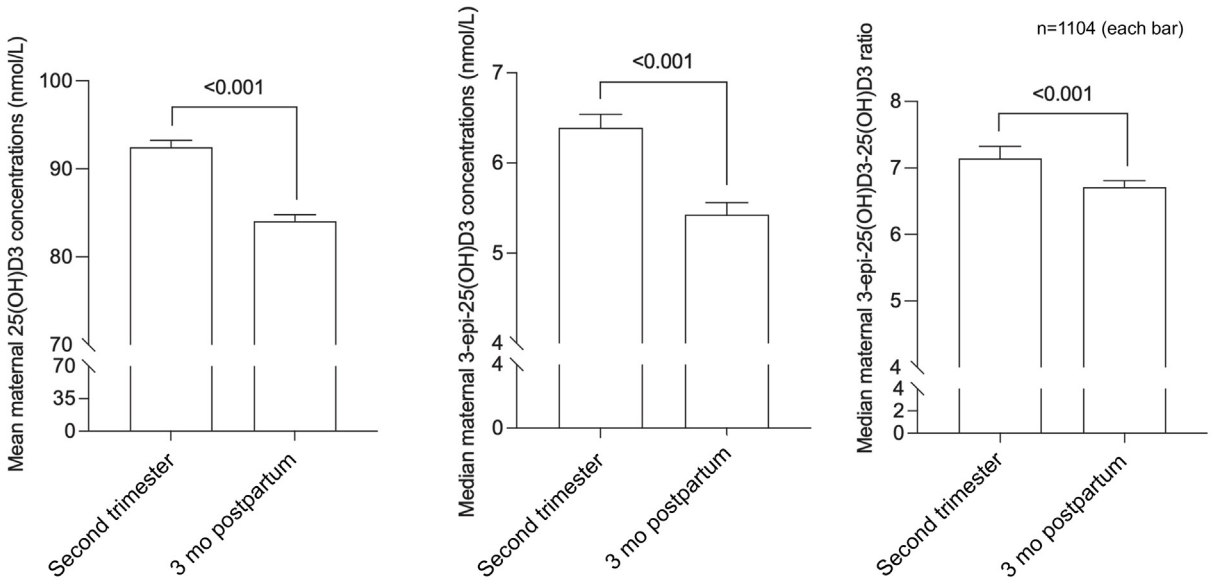

FIGURE 2. Changes in maternal (A) 25(OH)D3, (B) 3-epi-25(OH)D3, and (C) the 3-epi-25(OH)D3-25(OH)D3 ratio between the second trimester and 3 mo postpartum. Concentrations of 3-epi-25(OH)D3 and the 3-epi-25(OH)D3-25(OH)D3 ratio were skewed and log transformed before conducting paired *t* tests. Bars and error bars respectively represent the mean and SD in panel (A) and the median and 95% confidence intervals in panels (B) and (C). Abbreviations: 3-epi-25(OH)D3, 3-epi-25-hydroxyvitamin D3; 25(OH)D3, 25-hydroxyvitamin D3.

the outcome [71]. Although our results suggest that a low maternal status of iron or vitamin D may be related to more symptoms of maternal depression at a later pregnancy timepoint, bidirectional associations are plausible and should be considered [76, 77]. Specifically, it may be prudent to analyze relationships between multiple nutrients, including those without a clear link to brain health, and maternal depression to monitor the impact of overall nutritional adequacy before, during, and after the onset of these symptoms.

Recent evidence also suggests that the unique trajectories of maternal stress or mental illness may be influenced by different risk factors depending on whether they exist during pregnancy or postpartum [80–82]. Indeed, in our investigation, concentrations of all the iron or vitamin D biomarkers, even those that were significantly related to depressive symptoms during the third trimester, were not associated with maternal EPDS scores at 3 mo postpartum, consistent with the findings of Armony-Sivan et al. [50]. Nevertheless, others have reported that maternal iron or vitamin D biomarkers measured during pregnancy were related to postpartum depression symptoms among people who recently gave birth [47,75]. Considering this mixed evidence, the temporal trajectory of maternal depression is an important variable to consider in the future [82].

Finally, in addition to previous estimates of vitamin D status during the second trimester among a subcohort of pregnant participants [22], this investigation reported significant changes in maternal 25(OH)D3, 3-epi-25(OH)D3, and 3-epi-25(OH)D3:25(OH)D3 ratios between midpregnancy to approximately 3 mo postpartum for the first time in the APrON study. Other studies that describe maternal vitamin D status

TABLE 4
Differences in maternal EPDS scores depending on the adequacy of maternal iron and vitamin D status during midpregnancy

	Maternal iron and vitamin D status adequacy (second trimester)[1]			
	Both replete **β[2] (95% CI)**	Replete iron, low vitamin D **β (95% CI)**	Low iron, replete vitamin D **β (95% CI)**	Both low **β (95% CI)**
Maternal EPDS scores[3]				
Third trimester	Ref.	0.5 (-0.6, 1.7)	**1.1 (0.03, 2.1)**	**2.2 (0.3, 4.2)**
3 mo postpartum	Ref.	0.1 (-1.0, 1.3)	0.2 (-0.9, 1.2)	1.0 (-1.1, 0.9)

Abbreviations: CFG, Canada's Food Guide; CI, confidence interval; EPDS, Edinburgh Postnatal Depression Scale; GDM, gestational diabetes mellitus; GLM, generalized linear model; PE, preeclampsia; SF, serum ferritin; 25(OH)D, 25-hydroxyvitamin D.

[1] Groups (left to right) of maternal iron and vitamin D adequacy: *1*) iron (SF ≥15 μg/L) and vitamin D (25(OH)D ≥75 nmol/L) replete; *2*) iron replete (SF ≥15 μg/L), low vitamin D (25(OH)D <75 nmol/L); *3*) low iron (SF <15 μg/L), vitamin D replete (25(OH)D ≥75 nmol/L); and *4*) low iron (SF <15 μg/L) and vitamin D (25(OH)D <75 nmol/L).

[2] Reference group for GLM assessment. The β and 95% CI for the remainder of the categories represent the mean difference in the outcome variable between that category and the reference category.

[3] Maternal GLM models were adjusted for maternal age, ethnicity, income, education, CFG scores, GDM, PE, iron and vitamin D supplementation (pregnancy) and season (Figure 1C).

TABLE 5
A summary of important findings from this investigation

1. Associations between individual maternal iron and vitamin D biomarkers and maternal depression
- Inverse association: maternal SF concentrations (second trimester) and EPDS scores (third trimester)
- Inverse association: maternal hepcidin concentrations (second trimester) and EPDS scores (third trimester)
- Inverse association: maternal 25(OH)D3 concentrations (second trimester) and EPDS scores (third trimester)

2. Differences in depression outcomes depending on the combined midpregnancy adequacy of maternal iron and vitamin D
- Compared with those that were iron and vitamin D replete (SF ≥ 15 μg/L and 25(OH)D ≥75 nmol/L):
  - Maternal EPDS scores (third trimester) were higher in those with either low iron (SF <15 μg/L) but replete vitamin D (25(OH)D ≥ 75 nmol/L) or low iron and vitamin D (SF <15 μg/L and 25(OH)D <75 nmol/L) during the second trimester

Only the significant relationships associated with the study objectives (after appropriate covariate adjustment) are provided in this table. Abbreviations: EPDS, Edinburgh Postnatal Depression Scale; SF, serum ferritin; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D3, 25-hydroxyvitamin D3.

after birth are sparse, but Narchi et al. [83] found that the proportion of pregnant individuals at risk for deficiency increased across gestation to 6 mo postpartum. As maternal vitamin D biomarker concentrations were lower at 3 mo after birth in this investigation, the postnatal status of this micronutrient should be explored in different pregnant populations.

This study has several strengths and limitations. Multiple biomarkers for each micronutrient were quantified during pregnancy and at 3 mo postpartum. The inclusion of prenatal and postpartum maternal depression scores provided evidence of its potential risk factors during both lifestages [82]. However, although 25(OH)D3 concentrations are known to have a relatively stable (weeks long) half-life [84], it is possible that the half-lives of 3 carbon vitamin D epimers, including 3-epi-25(OH)D3, and iron biomarkers may impact their ability to accurately estimate the status of either micronutrient. Another limitation is that gestational concentrations of maternal vitamin D metabolites have only been measured during midpregnancy, but we are hopeful that they will be quantified during the first and third trimesters in the future. Maternal 25(OH)D3 and 3-epi-25(OH)D3 are precursors to their activated derivatives, $1,25(OH)_2D3$ and 3-epi-1α,25-dihroxyvitamin D3, respectively, which retain many of the functional capabilities of vitamin D [25,85]. Nevertheless, maternal 25(OH)D metabolites, including 25(OH)D3, cross the placenta [86] and are known to significantly contribute to the fetal status of vitamin D [87,88]. We used a 25(OH)D cutoff of <75 nmol/L to define maternal vitamin D insufficiency despite other reports that recommend <50 nmol/L [10–12]. However, the use of a more conservative threshold may be helpful to detect individuals that could be at risk of developing a poor vitamin D status during pregnancy [22,63] when the demand of this micronutrient increases [6–8], but more research is needed. Moreover, pregnant participants in the APrON cohort are generally well-nourished, which likely limited the group of individuals that had a low status of both iron and vitamin D (SF <15 μg/L and 25(OH)D <75 nmol/L) in this investigation. In line with this, we also recognize that the pregnant participants in our study are of a generally high socioeconomic status and would caution generalizing to populations at a higher risk of poor mental health, obstetric complications, or malnutrition.

To conclude, maternal iron and vitamin D biomarkers, measured during midpregnancy, were independently associated with third trimester maternal depression symptoms. That is, an adequate maternal status of iron and vitamin D during the second trimester was related to less maternal depression symptoms during the third trimester. This investigation is one of the first to report on the combined adequacy of maternal iron and vitamin D status during pregnancy and its impact on maternal depression. The novelty of this work reinforces the need to ask similar questions in other pregnant populations. Future investigations should report on the status of multiple micronutrients and explore their independent and combined impact on the health outcomes of pregnant individuals and their children.

Author contributions

The authors' responsibilities were as follows—CJF, GFG, NLL, DD, BL, RCB: designed the research; JLE, YL: conducted the research; JLE, AK, CJF, EVM, RCB: analyzed or reviewed the data; JLE, CJF, EVM, YL, GFG, NL, FA, DD, BL, RCB wrote the paper; JLE: had primary responsibility for final content; and all authors: read and approved the final manuscript.

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Data availability

Please contact the APrON Project Director (email: apron@ucalgary.ca or visit https://apronstudy.ca/contact-us/) to request access to the data utilized in this investigation.

Conflict of interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2023.10.029.

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