

## Vitamin B12 content in breast milk of vegan, vegetarian, and non-vegetarian lactating women in the United States

By: Roman Pawlak, Paul Vos, Setareh Shahab-Ferdows, Daniela Hampel, Lindsay H. Allen, and [Maryanne Tigchelaar Perrin](#)

**This is a pre-copyedited, author-produced version of an article accepted for publication in *American Journal of Clinical Nutrition* following peer review. The version of record,**

Pawlak R, Shahab-Ferdows S, Hampel D, Allen LH, Perrin MT. Vitamin B12 content in breast milk of vegan, vegetarian, and non-vegetarian lactating women in the United States. *American Journal of Clinical Nutrition*, Volume 108, Issue 3, September 2018, Pages 525–531, <https://doi.org/10.1093/ajcn/nqy104>

is available online at: <https://doi.org/10.1093/ajcn/nqy104>

\*\*\*© 2018 American Society for Nutrition. Reprinted with permission. No further reproduction is authorized without written permission from Oxford University Press. This version of the document is not the version of record. \*\*\*

### Abstract:

**Background:** The nutritional profile of human milk varies significantly between women, and the impact of maternal diet on these variations is not well understood. **Objective:** We analyzed breast-milk vitamin B-12 concentration and vitamin B-12 supplement use pattern among women who adhered to different dietary patterns: vegan, vegetarian, and nonvegetarian. **Design:** A total of 74 milk samples, 26 from vegan, 22 from vegetarian, and 26 from nonvegetarian breastfeeding mothers, were analyzed. **Results:** The prevalences of low vitamin B-12 (<310 pmol/L) were 19.2% for vegans, 18.2% for vegetarians, and 15.4% for nonvegetarians, which was not significant by diet group ( $P = 1.00$ ). The median (quartile 1, quartile 3) vitamin B-12 values were 558 pmol/L (331, 759 pmol/L) for vegans, 509 pmol/L (368, 765 pmol/L) for vegetarians, and 444 pmol/L (355, 777 pmol/L) for nonvegetarians ( $P = 0.890$ ). The use of individual vitamin B-12 supplements was higher in vegans (46.2%) than in vegetarians (27.3%) and nonvegetarians (3.9%) ( $P = 0.001$ ). In linear regression analysis, the use of individual vitamin B-12 supplements was a significant positive predictor of milk vitamin B-12 concentration ( $\beta \pm \text{SE}$ :  $172.9 \pm 75.2$ ; standardized  $\beta = 0.263$ ;  $P = 0.024$ ;  $R^2 = 0.069$ ), the use of a multivitamin had a significant negative relation with milk vitamin B-12 concentrations ( $\beta \pm \text{SE}$   $-222.0 \pm 98.7$ ; standardized  $\beta = -0.258$ ;  $P = 0.028$ ,  $R^2 = 0.067$ ), whereas the use of a B-complex vitamin and prenatal vitamin were not predictive of vitamin B-12 milk concentration ( $P > 0.05$ ). **Conclusions:** Almost 20% of our study participants were classified as having low breast-milk vitamin B-12 concentrations (<310 pmol/L), independent of maternal diet pattern. Approximately 85% of participants categorized as having low vitamin B-12 were taking vitamin B-12 supplements at doses in excess of the Recommended Dietary Allowance, which suggests that more research is needed to determine breast-milk adequacy values.

**Keywords:** vitamin B-12 | vegan | vegetarian | breast milk | supplements

## Article:<sup>1</sup>

### INTRODUCTION

Breastfeeding is the recommended feeding method for infants, exclusively for the first 6 mo of life and with complementary feeding until 12–24 mo of age (1–3). The nutritional profile of human milk varies significantly between women, and the impact of maternal diet on these variations is not well understood (4–7). Allen (8) concluded that some B-vitamins, including vitamin B-12, were significantly affected by maternal intake.

Vegetarian diets are associated with several health advantages, including lower risk of ischemic heart disease, type 2 diabetes, hypertension, and obesity (9–12). In 2012, ~5% of American adults were vegetarians (13). It has also been estimated that 2% of children aged 6–17 y in the United States are vegetarians (14).

Despite numerous health benefits, vegetarians are at a high risk of inadequate intake and status of several nutrients, with vitamin B-12 deficiency being a major concern (15, 16). Research in German lacto-ovo-vegetarian pregnant women showed that 40% of participants ingested less than the Institute of Medicine's Estimated Average Requirement for vitamin B-12 (<2.2 µg/d) (17, 18). Cases of vitamin B-12–deficient infants breastfed by vegan mothers have been reported, with deficiencies manifesting in profound developmental and neurological symptoms (19). Low serum and breast-milk vitamin B-12 concentrations have been reported in pregnant and lactating vegetarian mothers (20–26). Recently, in part due to concern about poor vitamin B-12 status among vegans, the German Nutrition Society recommended against a vegan diet for pregnant and lactating women, infants, children, and adolescents (15).

Vegan and vegetarian mothers are more likely to breastfeed their children, compared with nonvegetarian mothers, and they do so for a longer duration (27). Thus, if these mothers' vitamin B-12 milk concentration is inadequate, their offspring are at risk of receiving inadequate nutrition until appropriate complementary feeding begins. Two studies on vitamin B-12 breast-milk composition in vegetarians in the United States were conducted >20 y ago (22, 23). In these studies, vitamin B-12 supplement use among vegetarians was low or nonexistent. It is thus prudent to assess vitamin B-12 in breast milk of vegetarians, especially vegans.

Our goal was to determine vitamin B12 concentrations in breast milk and the pattern of vitamin B-12–containing supplement use among American women who adhered to vegan, vegetarian, and nonvegetarian diets. The objectives included the following: 1) to assess whether there is a difference between vitamin B-12 milk concentration from vegan, vegetarian, and nonvegetarian breastfeeding women; 2) to calculate whether there is a difference in the percentage of women in each diet group with vitamin B-12 milk concentration <310 pmol/L, a cutoff used by others and derived from data to compute Adequate Intakes (21, 25); 3) to determine the prevalence of

---

<sup>1</sup> Supported by a grant from the Academy of Nutrition and Dietetics Foundation, Vegetarian Nutrition Dietary Practice Group.

Abbreviations used: BSQ, basic screening questionnaire; Hcy, homocysteine; MMA, methylmalonic acid.

vitamin B-12–containing supplement use; and 4) to assess the association between the use of vitamin B-12–containing supplements and vitamin B-12 milk concentration.

## METHODS

### Recruitment

The study was approved by the institutional review board offices at the University of North Carolina Greensboro and East Carolina University.

### Sample

Participants ( $n = 74$ ) of this cross-sectional study were recruited through vegetarian and vegan organizations, faith-based institutions, and online parenting and breastfeeding support groups. Participants were asked to complete a basic online screening questionnaire (BSQ) to determine eligibility for participation. Inclusion criteria included living in the United States, maternal age 18–46 y, giving birth to a healthy term infant currently older than 2 wk, and willingness to collect 1 milk sample per the study collection protocol and to complete a diet survey. Exclusion criteria included being diagnosed with methylene tetrahydrofolate reductase (*MTHFR*) gene mutation; health conditions that affect vitamin B-12 status (e.g., patients with known intrinsic factor deficiency, history of bariatric surgery, inflammatory bowel disease, celiac disease); hypo- or hyperthyroidism; advanced liver disease (cirrhosis, hepatitis); myeloproliferative disorders; or being pregnant.

With the use of means  $\pm$  SDs published by Patel and Lovelady (23) for vitamin B-12 milk composition in a small study in omnivorous women in the United States, the sample size needed to detect a 15–20% difference in vitamin B-12 concentration, with a power of 80% and an  $\alpha$  of 0.05, was 15–26. Once eligibility was determined via the BSQ, individuals were selectively invited to participate in the study on the basis of their self-reported diet type, with a goal of recruiting a similar number of participants ( $n = 25$ ) per diet group. These individuals were also sent the institutional review board–approved consent form. Recruitment and milk sample collection took place between November 2016 and April 2017. Study participants received a \$25 gift card.

### Milk sample and survey collection

Each participant was asked to provide a breast-milk sample, which was either collected in person or received via a shipment on dry ice to our laboratory. Participants were instructed to collect the sample in the morning, during the first or second feeding of the day and  $\geq 2$  h since the previous feeding. Milk was to be collected in a dimly lit room to help protect light-sensitive nutrients. Women were instructed to completely express the content of 1 breast using the expression method of their choice. Expressed milk was to be transferred to a storage bag appropriate for freezing breast milk, labeled with the collection date, wrapped in aluminum foil for further protection, and stored in a home freezer until samples were collected or shipped to the laboratory at the University of North Carolina, Greensboro. Participants also completed a digital survey with demographic, anthropometric, dietary, and supplement use information.

## Diet and supplement classification

Vegans were defined as participants who did not ingest any meat but may have ingested other animal products <1 time/mo. Vegetarians consisted of participants who did not eat meat but regularly ingested other animal products, such as eggs or dairy. Any B-vitamin supplementation was defined as using any of the following: individual vitamin B-12 supplements, B-complex supplements, multivitamin supplements, or prenatal vitamin supplements. A low milk vitamin B-12 concentration was defined as <310 pmol/L (21, 25). A high milk vitamin B-12 concentration was defined as above the assay detection threshold of 1122 pmol/L.

## Sample analyses

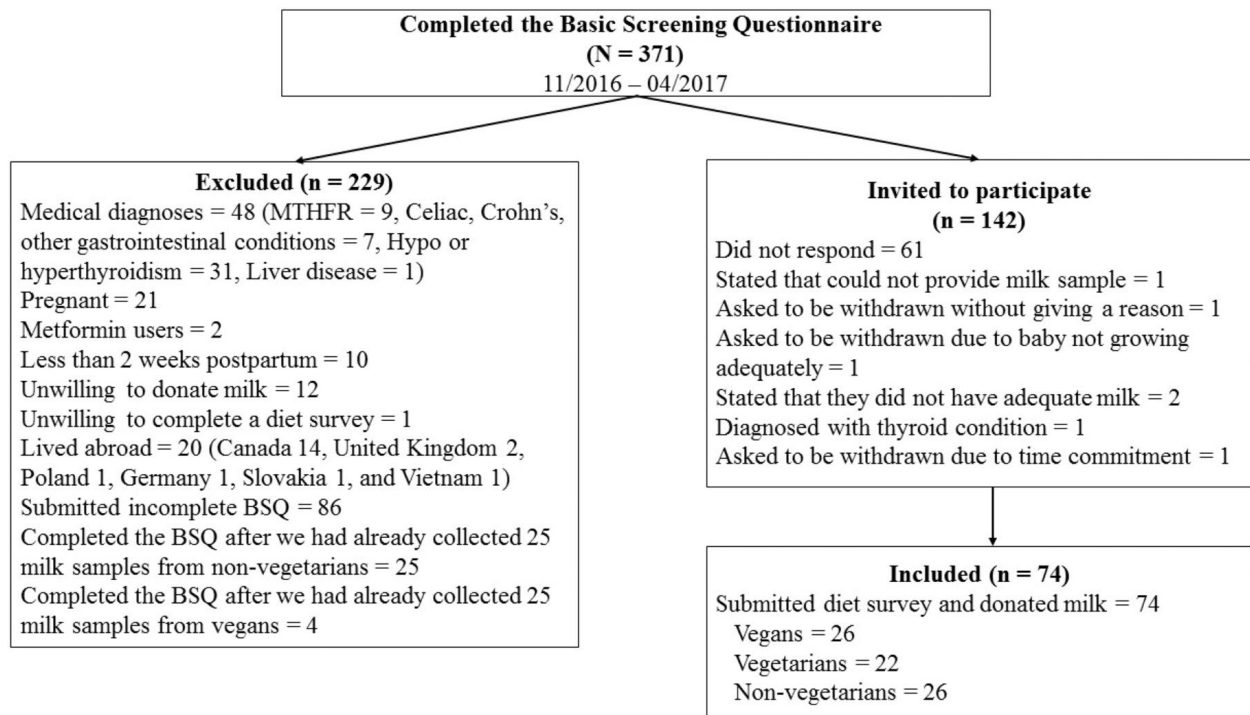
The outcome of the study was to evaluate the vitamin B-12 content of breast milk. Milk samples were thawed, placed into aliquots, and stored at  $-20^{\circ}\text{C}$  at the laboratory at the University of North Carolina, Greensboro. De-identified samples were shipped on dry ice to the USDA/Agricultural Research Service–Western Human Nutrition Research Center (University of California, Davis, California) for vitamin B-12 analysis. The analysis was performed by using a competitive chemiluminescent enzyme immunoassay that accounts for interference effects caused by high concentrations of haptocorrin in breast milk, as previously described (28). This assay has the ability to detect vitamin B-12 in the range of 24–1122 pmol/L. Milk samples with vitamin B-12 concentrations above the upper detection limit were assigned a maximum vitamin B-12 value of 1122 pmol/L and were flagged as being above assay detection range.

## Statistical analyses

The distributions of vitamin B-12 among the 3 diet groups were compared by using side-by-side boxplots and summarized with the use of means and medians. The null hypothesis that all 3 distributions were the same was tested by using the F-statistic from a 1-factor ANOVA and by using the Kruskal-Wallis test. The groups were compared on dichotomous variables with the use of Fisher's exact test. Linear regression was performed to probe for significant predictors of vitamin B-12 milk concentration. The significance level of  $P < 0.05$  was used in all analyses. Data analysis was performed with the statistical software R, version 3.4.2, and SAS 9.4 (SAS Institute) (28).

## RESULTS

The BSQ was completed by 371 women, 142 of whom were contacted and asked to participate in the study. Seventy-four of the contacted women completed the diet survey and donated their milk. Figure 1 depicts participant selection.



**FIGURE 1.** PRISMA flow chart of participant selection process. BSQ, basic screening questionnaire; MTHFR, methylene tetrahydrofolate reductase; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

**TABLE 1.** Characteristics of study participants<sup>1</sup>

	Vegans (n = 26)	Vegetarians (n = 22)	Nonvegetarians (n = 26)	P
Age, y	32.7 ± 5.2	32.2 ± 4.6	31.0 ± 4.7	0.438
BMI, kg/m <sup>2</sup>	22.8 <sup>a</sup> ± 3.1	23.9 <sup>a,b</sup> ± 3.8	25.8 <sup>b</sup> ± 4.5	0.021
Gravida, n	2.3 ± 1.3	2.0 ± 1.1	2.8 ± 2.0	0.170
Parity, n	1.8 ± 0.8	1.6 ± 0.8	2.3 ± 1.8	0.158
Stage of lactation, mo	36.6 <sup>a,b</sup> ± 27.7	54.6 <sup>b</sup> ± 46.0	27.5 <sup>a</sup> ± 19.8	0.017
Diet duration, y	6.2 <sup>a</sup> ± 5.5	7.5 <sup>a</sup> ± 5.5	25.8 <sup>b</sup> ± 11.5	<0.001
Ethnicity, n (%)				0.900
Black	0 (0.0)	0 (0.0)	0 (0.0)	
Asian	1 (3.9)	0 (0.0)	0 (0.0)	
Hispanic	1 (3.9)	1 (4.6)	1 (3.9)	
White	22 (84.6)	20 (90.9)	21 (80.8)	
Mixed/other	2 (7.7)	1 (4.6)	4 (15.4)	
Education, n (%)				0.279
HS/GED/other	1 (3.9)	0 (0.0)	1 (3.9)	
Some college/technical	2 (7.7)	3 (13.6)	7 (26.9)	
4-y college degree	8 (30.8)	11 (50.0)	9 (34.6)	
Graduate degree	15 (57.7)	8 (36.4)	9 (34.6)	

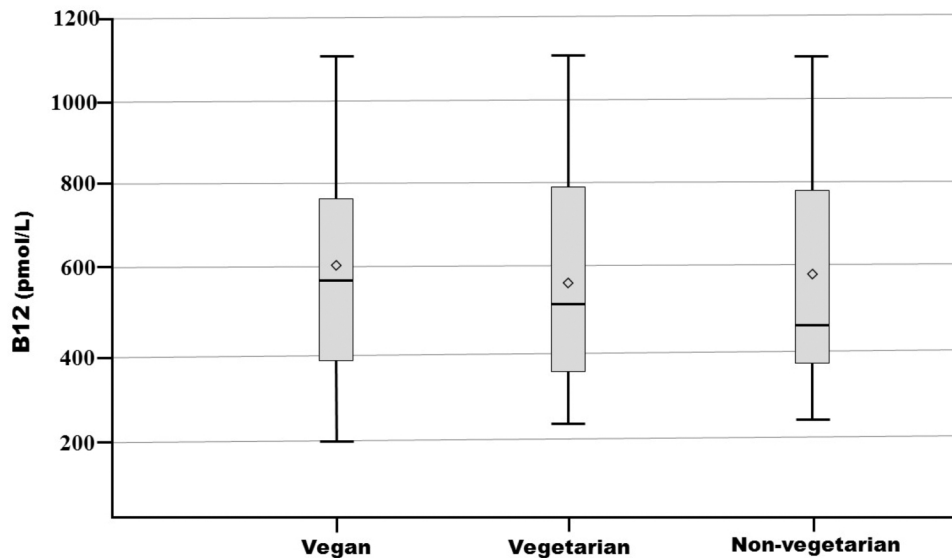
<sup>1</sup>Values are means ± SDs unless otherwise indicated. Means ± SDs were evaluated for differences between groups by using 1-factor ANOVA and Tukey's test was used for multiple comparisons. Groups with a common superscript letter did not differ; values for n (%) were evaluated for differences between groups by using Fisher's exact test. GED, General Education Diploma; HS, high school.

Of the 74 total collected milk samples, 26 were from vegan, 22 from vegetarian, and 26 from nonvegetarian breastfeeding mothers. Vegan participants adhered to their diet for a mean ± SD of 6.2 ± 5.5 y (range: 0.5–23 y; median: 4.3 y), whereas vegetarians practiced their diet for a

mean of  $7.5 \pm 5.5$  y (range: 1 mo to 20 y; median: 6.0 y). Details with regard to demographic and socioeconomic characteristics of the study participants are included in Table 1.

There were no significant differences between the mean age of participants ( $P = 0.438$ ), gravida ( $P = 0.170$ ), parity ( $P = 0.158$ ), ethnicity ( $P = 0.900$ ), or education ( $P = 0.279$ ) by diet group. There was a significant difference in stage of lactation ( $P = 0.017$ ) and maternal BMI between diet groups ( $P = 0.021$ ), with vegans having the lowest and nonvegetarians having the highest BMI (Table 1).

#### Comparison of milk vitamin B-12 concentrations between diet groups



**FIGURE 2.** Vitamin B-12 concentrations (expressed as pmol/L) by diet type. Quartile 1 to quartile 3 values are represented within the gray rectangle; mean values are represented by horizontal lines; median values are represented by diamonds. The median (quartile 1, quartile 3) vitamin B-12 values were 558 pmol/L (331, 759 pmol/L) for vegans, 509 pmol/L (368, 765 pmol/L) for vegetarians, and 444 pmol/L (355, 777 pmol/L) for nonvegetarians. No significant differences by diet group with the use of both ANOVA ( $P = 0.890$ ) and the Kruskal-Wallis test ( $P = 0.936$ ) were found. B12, vitamin B-12.

Boxplots of vitamin B-12 distribution by diet group are provided in Figure 2 and showed no significant difference by group with the use of both ANOVA ( $P = 0.890$ ) and the Kruskal-Wallis tests ( $P = 0.936$ ). Median (quartile 1, quartile 3) vitamin B-12 values were 558 pmol/L (331, 759 pmol/L) for vegans, 509 pmol/L (368, 765 pmol/L) for vegetarians, and 444 pmol/L (355, 777 pmol/L) for nonvegetarians. When the ANOVA analysis for BMI and lactation stage, which were different between groups, was controlled for, there was still no significant difference in the vitamin B-12 values observed between groups ( $P = 0.888$ ). Eight of the 74 participants (10.8%), including 4 (15.4%) vegans, 2 (9.1%) vegetarians, and 2 (7.7%) nonvegetarians, had vitamin B-12 values above the assay threshold of 1122 pmol/L. The prevalence of high vitamin B-12 was not significant by diet group ( $P = 0.724$ ). The fact that there were capped vitamin B-12 values violates the assumptions required for both statistical tests. The side-by-side boxplots and large  $P$  value suggest that, if the noncapped versions been available, the differences would still

fail to be significant. Only gravida ( $\beta \pm \text{SE}$ :  $56.6 \pm 21.0$ ; standardized  $\beta = 0.305$ ;  $P = 0.009$ ,  $R^2 = 0.093$ ) and parity ( $\beta \pm \text{SE}$ :  $79.6 \pm 25.2$ ; standardized  $\beta = 0.351$ ;  $P = 0.002$ ,  $R^2 = 0.123$ ), but not maternal age, BMI, or stage of lactation, were predictive of milk vitamin B-12 concentrations across the study population.

#### Assessment of low milk vitamin B-12 concentration

Thirteen of the 74 participants (17.6%) had milk vitamin B-12 concentrations  $<310$  pmol/L. The prevalences of low vitamin B-12 were 19.2% for vegans, 18.2% for vegetarians, and 15.4% for nonvegetarians, which were not significant by diet group ( $P = 1.00$ ) (Table 2).

**TABLE 2.** Vitamin B-12 supplement use and prevalence of participants with low ( $<310$  pmol/L) and high (1122 pmol/L) vitamin B-12 milk concentration by diet group<sup>1</sup>

Attribute	Vegan ( <i>n</i> = 26)	Vegetarian ( <i>n</i> = 22)	Nonvegetarian ( <i>n</i> = 26)	<i>P</i>
Supplement use				
Vitamin B-12 supplement	12 (46.2)	6 (27.3)	1 (3.9)	0.001
B-complex	2 (7.7)	0 (0.0)	1 (3.9)	0.771
Multivitamin	4 (15.4)	0 (0.0)	5 (19.2)	0.083
Prenatal vitamin	14 (53.9)	13 (59.1)	14 (53.8)	0.915
Any B-vitamin	24 (92.3)	16 (72.7)	18 (69.2)	0.093
Vitamin B-12				
$<310$ pmol/L	5 (19.2)	4 (18.2)	4 (15.4)	1.0
$>1122$ pmol/L	4 (15.4)	2 (9.1)	2 (7.7)	0.724

<sup>1</sup>Values are *n* (%) by treatment group. Differences between groups were evaluated by using Fisher's exact test.

#### Patterns of use of vitamin B-12-containing supplements

Fifty-eight of the 74 participants (78.4%) used supplements containing vitamin B-12. The doses of vitamin B-12 in the various supplements ranged from 4 to 5000  $\mu\text{g}$  and were all higher than the Recommended Dietary Allowance for lactating women of 2.8  $\mu\text{g}/\text{d}$ . Of those who used an individual vitamin B-12 supplement, 72% used one with a dose of  $\geq 100$   $\mu\text{g}$ . Prenatal vitamins were used by 55.4% (41 of 74) of participants, individual vitamin B-12 supplements were used by 25.7% (19 of 74), multivitamin supplements were used by 12.2% (9 of 74), and B-complex vitamins were used by 4.1% of participants (3 of 74). There was a significant difference in the percentage of participants who used individual vitamin B-12 supplements by diet group (vegan = 44.8%, vegetarian = 26.3%, nonvegetarian = 3.9%;  $P = 0.001$ ). There was no difference in the prevalence of use of B-complex vitamins, multivitamins, or prenatal vitamins by diet group. Data on participant supplement use are presented in Table 2.

#### Impact of vitamin B-12 supplements on vitamin B-12 milk concentration

In a linear regression analysis, the use of individual vitamin B-12 supplements was a significant positive predictor of milk vitamin B-12 concentration ( $\beta \pm \text{SE}$ :  $172.9 \pm 75.2$ ; standardized  $\beta = 0.263$ ;  $P = 0.024$ ,  $R^2 = 0.069$ ) and the use of a multivitamin had a significant negative relation with milk vitamin B-12 concentrations ( $\beta \pm \text{SE}$ :  $-222.0 \pm 98.7$ ; standardized  $\beta = -0.258$ ;  $P = 0.028$ ,  $R^2 = 0.067$ ). The use of a B-complex vitamin and prenatal vitamin were not predictive of vitamin B-12 milk concentration ( $P > 0.05$ ). The percentage of participants classified as having low vitamin B-12 ( $<310$  pmol/L) was not significantly different on the basis

of whether participants used any B-vitamin-containing supplements (users = 19.0%, nonusers = 12.5%;  $P = 0.721$ ) or whether they used individual vitamin B-12 supplements (users = 10.5%, nonusers = 20.0%;  $P = 0.494$ ). The percentage of participants classified as having high vitamin B-12 ( $>1122$  pmol/L) was significantly different on the basis of use of an individual vitamin B-12 supplement (users = 26.3%, nonusers = 5.5%;  $P = 0.023$ ).

## DISCUSSION

We analyzed the vitamin B-12 concentration of milk samples from women in a resource-rich setting who adhered to vegan, vegetarian, and nonvegetarian diets and found no significant difference by diet, with median vitamin B-12 concentrations ranging from 444 pmol/L to 558 pmol/L within the diet groups. Our findings differ from those in 2 other studies conducted in the United States (22, 23). In these studies, healthy women who consumed omnivorous diets had significantly higher concentrations of breast-milk vitamin B-12 than did women who consumed either a macrobiotic vegan or a vegetarian diet, with both studies reporting concentrations  $<310$  pmol/L in the plant-based diet groups (22, 23). Neither of these studies used analytical methods that accounted for matrix effects from the high concentration of haptocorrin in breast milk, which can interfere with vitamin B-12 measurements in a random fashion; therefore, results should be interpreted with caution (29). Moreover, the prevalence of vitamin B-12 supplement use was nonexistent or lower among vegetarian subjects in these studies compared with their omnivorous counterparts.

### Impact of vitamin B-12 supplementation

There is consistent evidence that maternal vitamin B-12 supplementation increases the vitamin B-12 concentration in breast milk. In a randomized controlled trial in HIV-infected women in Malawi, breast-milk vitamin B-12 concentrations were higher in lactating women who received a 2.6  $\mu\text{g}$  vitamin B-12 supplement/d than in the control group, although the use of antiretroviral therapy drugs abolished this effect (25). Chebaya et al. (30) reported higher breast-milk vitamin B-12 concentrations in Canadian women who received 12  $\mu\text{g}/\text{d}$  of a vitamin B-12 supplement than in a population of unsupplemented women in Cambodia (geometric mean: 452 compared with 310 pmol/L;  $P < 0.0001$ ), although maternal dietary intake was not explored. In a study in which poorly nourished Bangladeshi women were given pharmacologic doses of 250  $\mu\text{g}$  vitamin B12 supplements/d from 20 wk of pregnancy through 3 mo postpartum, supplemented women had higher breast-milk vitamin B-12 concentrations than the control group (31).

There are conflicting findings in the literature with regard to the relation between human-milk vitamin B-12 concentration and infant serum vitamin B-12 (24, 26). Chebaya et al. (30) proposed that maternal vitamin B-12 intake during pregnancy may be a better predictor of infant deficiency risk than breast-milk vitamin B-12 concentrations, suggesting an important potential role of infant stores at birth. In our study, supplementation use was the norm among vegan participants and we did not see a difference in breast-milk vitamin B-12 concentrations between participants who consumed only plant-based diets and those who also consumed animal-source foods. In addition, individual vitamin B-12 supplement use was a significant predictor of vitamin B-12 milk concentration, and 12 of 19 (63.2%) of the individual vitamin B-12 supplement users were vegan, which likely contributed to breast-milk vitamin B-12 status in this vegan population.



## Defining vitamin B-12 adequacy

There are limited data available to establish adequate vitamin B-12 concentrations in human milk. Specker et al. (22) found a correlation between low breast-milk vitamin B-12 concentrations and infant urinary methylmalonic acid (MMA) concentrations, a biomarker of vitamin B-12 deficiency, and proposed 362 pmol/L as a cutoff for deficiency. However, there are major methodologic flaws in how vitamin B-12 was assessed in the Specker et al. study; thus, results may not have reflected actual vitamin B-12 concentrations. Chebaya et al. (30) recently found that 95% of Canadian infants at 2 mo postpartum had serum vitamin B-12 >221 pmol/L and 50% of their mothers had breast-milk vitamin B-12 concentrations <362 pmol/L. Although this seem to provide evidence that the deficiency thresholds proposed by Specker et al. may be too high, it is not clear what constitutes adequate serum vitamin B-12 in infants. Recently, Williams et al. (21) suggested a vitamin B-12 deficiency threshold of 310 pmol/L for breast milk on the basis of estimated values in the milk of healthy mothers. With the use of this threshold for adequacy, almost 20% of our study participants were classified as having low breast-milk vitamin B-12 concentrations, independent of maternal diet pattern.

Greibe et al. (32) studied longitudinal changes in vitamin B-12 breast-milk composition in 25 healthy Danish women who consumed an omnivorous diet and described a decline between 2 wk postpartum (median: 760 pmol/L) and 4 mo postpartum (median: 290 pmol/L), with corresponding changes in infant vitamin B-12 and MMA markers. The majority of Danish women studied were supplemented with 1.0–4.5 µg vitamin B-12/d via a multivitamin. The authors concluded that it remained to be determined whether the lower breast-milk vitamin B-12 concentration observed at 4 mo postpartum could adequately meet an infant's needs.

Inadequate vitamin B-12 status at any time is associated with increased serum homocysteine (Hcy) concentration and increased urinary MMA. In adults, serum Hcy concentration is most strongly reflective of poor folate and vitamin B-12 intake and status. However, in infants ≤6 mo of age, Hcy concentration depends more on vitamin B-12 than on folate (33).

Hyperhomocysteinemia in infants is associated with such adverse health outcomes as developmental delays, neurological problems, and hematologic abnormalities. A longitudinal study in >200 healthy Norwegian infants found that serum MMA and Hcy were significantly higher in breastfed infants than in nonbreastfed infants, although there were no differences in hematologic values, suggesting that different infant feeding patterns may generate unique metabolomic profiles not necessarily indicative of deficiency (32). Future research is warranted to develop vitamin B-12 deficiency thresholds on the basis of infant outcomes with the use of vitamin B-12 analytical methods that account for breast-milk matrix interference effects.

## Limitations

Participants were recruited online and represent primarily white, well-educated women. Findings may not be generalizable to other populations who consume low-animal-source foods. This was a cross-sectional study and there were differences in stage of lactation between groups, which may have contributed to our findings. Only 2 of the vegan participants in our study did not use any vitamin B-12-containing supplements. Thus, we were unable to compare vitamin B-12 milk

concentrations between the unsupplemented vegan participants and those who utilized supplements.

## Conclusions

Findings from this study showed a high level of vitamin B-12 supplement use among vegan lactating women in the United States, with no differences in breast-milk vitamin B-12 concentration by maternal diet pattern. It can thus be concluded that vegan lactating mothers who utilize individual vitamin B-12 supplements can attain vitamin B-12 milk concentrations comparable to those in nonvegetarians. Almost 20% of study participants had a milk vitamin B-12 concentration below suggested cutoffs for inadequate intake.

## Areas of further research

There is an urgent need to establish normative breast-milk vitamin B-12 concentrations associated with favorable infant health outcomes, the impact of maternal diet and supplementation on breast-milk vitamin B-12 concentration, and appropriate biomarkers for assessing vitamin B-12 deficiency in breastfed infants. More research is warranted into how fortified foods, and other nonanimal foods, contribute to the vitamin B-12 intake and status of vegans and vegetarians. Future research should also assess whether vegan women who do not utilize vitamin B-12-containing supplements can attain vitamin B-12 milk concentrations above the suggested deficiency cutoffs using commonly fortified foods.

## Acknowledgements

We thank Larisse Melo from the University of British Columbia for laboratory support. The authors' responsibilities were as follows—RP: created the study design, performed data collection, and aided with manuscript preparation; PV: performed the statistical analysis; SS-F, DH, and LHA: performed the vitamin B-12 assessment and manuscript review; MTP: created the study design and performed data collection, and aided with manuscript preparation; and all authors: read and approved the final manuscript. None of the authors had a conflict of interest of any type.

## REFERENCES

1. American Academy of Pediatrics. Breastfeeding and the use of human milk. *Pediatrics* 2012;129:e827–41.
2. Lessen R, Kavanagh K. Position of the Academy of Nutrition and Dietetics: promoting and supporting breastfeeding. *J Acad Nutr Diet* 2015;115:444–49.
3. Kramer MS, Kakuma R; WHO. The optimal duration of exclusive breastfeeding. [cited 2017 Oct 26]. Available from: [http://www.who.int/nutrition/publications/optimal\\_duration\\_of\\_exc\\_bfeeding\\_review\\_eng.pdf](http://www.who.int/nutrition/publications/optimal_duration_of_exc_bfeeding_review_eng.pdf).

4. Michaelsen KF, Skaftø L, Badsberg JH, Jørgensen M. Variation in macronutrients in human bank milk: influencing factors and implications for human milk banking. *J Pediatr Gastroenterol Nutr* 1990;11:229–39.
5. Wojcik KY, Rechtman DJ, Lee ML, Montoya A, Medo ET. Macronutrient analysis of a nationwide sample of donor breast milk. *J Am Diet Assoc* 2009;109:137–40.
6. Perrin MT, Fogleman AD, Newburg DS, Allen JC. A longitudinal study of human milk composition in the second year postpartum: implications for human milk banking. *Matern Child Nutr* 2017;13. doi: 10.1111/mcn.12239
7. Bravi F, Wiens F, Decarli A, Dal Pont A, Agostoni C, Ferraroni M. Impact of maternal nutrition on breast-milk composition: a systematic review. *Am J Clin Nutr* 2016;104:646–62.
8. Allen L. B vitamins in breast milk: relative importance of maternal status and intake, and effects on infant status and function. *Adv Nutr* 2012;3:362–9.
9. Orlich MJ, Fraser GE. Vegetarian diets in the Adventist Health Study 2: a review of initial published findings. *Am J Clin Nutr* 2014;100:353S–8S.
10. Pawlak R. Vegetarian diets in the prevention and management of diabetes and its complications. *Diabetes Spectrum* 2017;30:82–8.
11. Pawlak R, Ding Q, Sovyanhadi M. Vegetarian children and adolescents' anthropometric characteristics do not significantly differ from their non-vegetarian counterparts. *Integr Food Nutr Metab* 2017;4:1–4.
12. Sabaté J, Wien M. Vegetarian diets and childhood obesity prevention. *Am J Clin Nutr* 2010;91:1525S–9S.
13. Stahler C. How often do Americans eat vegetarian meals? And how many adults in the U.S. are vegetarian? [cited 2017 Oct 10]. Available from: <http://www.vrg.org/journal/vj2011issue4/vj2011issue4poll.php>.
14. The Vegetarian Resource Group. How many teens are vegetarian? How many kids don't eat meat? *Vegetarian Journal*. 2001. [cited 2017 Oct 11]. Available from: [www.vrg.org/journal/vj2001jan/2001janteen.htm](http://www.vrg.org/journal/vj2001jan/2001janteen.htm).
15. Richter M, Boeing H, Grünewald-Funk D, Heseker H, Kroke A, Leschik-Bonnet E, Oberritter H, Strohm D, Watzl B; German Nutrition Society. Vegan diet. *Ernährungs Umschau* 2016;63:92–102.
16. Pawlak R. Is vitamin B12 deficiency a risk factor for cardiovascular disease in vegetarians? *Am J Prev Med* 2015;48:e11–26.
17. Koebnick C, Hoffmann I, Dagnelie PC, Heins UA, Wickramasinghe SN, Ratnayaka ID, Gruendel S, Lindemans J, Leitzmann C. Long-term ovo-lacto vegetarian diet impairs vitamin B-12 status in pregnant women. *J Nutr* 2004;134:3319–26.
18. Institute of Medicine. Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. [cited 2017 Sep 20]. Available from: [https://www.ncbi.nlm.nih.gov/books/NBK114310/pdf/Bookshelf\\_NBK114310.pdf](https://www.ncbi.nlm.nih.gov/books/NBK114310/pdf/Bookshelf_NBK114310.pdf).

19. Dror DK, Allen LH. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. *Nutr Rev* 2010;66:250–5.
20. Pawlak R. To vegan or not to vegan when pregnant, lactating or feeding young children. *Eur J Clin Nutr* 2017;71:1259–62.
21. Williams AM, Chantry CJ, Young SL, Achando BS, Allen LH, Arnold BF, Colford JM Jr., Dentz HN, Hampel D, Kiprotich MC et al. . Vitamin B-12 concentrations in breast milk are low and are not associated with reported household hunger, recent animal-source food, or vitamin B-12 intake in women in rural Kenya. *J Nutr* 2016;146:1125–31.
22. Specker BL, Black A, Allen L, Morrow F. Vitamin B-12: low milk concentrations are related to low serum concentrations in vegetarian women and to methylmalonic aciduria in their infants. *Am J Clin Nutr* 1990;52:1073–6.
23. Patel K, Lovelady C. Vitamin B12 status in East Indian lactating women living in the United States. *Nutr Res* 1998;18:1839–46.
24. Deegan KL, Jones KM, Zuleta C, Ramirez-Zea M, Lildballe DL, Nexo E, Allen LH. Breast milk vitamin B-12 concentrations in Guatemalan women are correlated with maternal but not infant vitamin B-12 status at 12 months postpartum. *J Nutr* 2012;142:112–6.
25. Allen LH, Hampel D, Shahab-Ferdows S, York ER, Adair LS, Flaz VL, Tegha G, Chasela CS, Kamwendo D, Jamieson DJ et al. . Antiretroviral therapy provided to HIV-infected Malawian women in a randomized trial diminishes the positive effects of lipid-based nutrient supplements on breast-milk B vitamins. *Am J Clin Nutr* 2015;102:1468–74.
26. Shabad-Ferdows S, Engle-Stone R, Hampel D, Ndjebayi AO, Nankap M, Brown KH, Allen LH. Regional, socioeconomic, and dietary risk factors for vitamin B-12 deficiency differ from those for folate deficiency in Cameroonian women and children. *J Nutr* 2015;145:2587–95.
27. Pawlak R, Ding C, Sovyanhadi M. Pregnancy outcome and breastfeeding pattern among vegans, vegetarians and non-vegetarians. *J Diet Res Nutr* 2014;1:004.
28. R Core Team R: a language and environment for statistical computing. Vienna (Austria):R Foundation for Statistical Computing; 2017. Available from: <https://www.R-project.org/>. Accessed on January, 2018.
29. Hampel D, Shahab-Ferdows S, Domek JM, Siddiqua T, Raqib R, Allen LH. Competitive chemiluminescent enzyme immunoassay for vitamin B12 analysis in human milk. *Food Chem* 2014;153:60–65.
30. Chebaya P, Karakochuk CD, March KM, Chen NN, Stamm RA, Kroeun H, Sophonneary P, Borath M, Shahab-Ferdows S, Hampel D et al. . Correlations between maternal, breast milk, and infant vitamin B12 concentration among mother-infant dyads in Vancouver, Canada and Prey Veng, Cambodia: an exploratory analysis. *Nutrients* 2017;9. DOI: 10.3390/nu9030270.
31. Siddiqua TJ, Ahmad SM, Ahsan KB, Rashid M, Roy A, Rahman SM, Shahab-Ferdows S, Hampel D, Ahmed T, Allen LH et al. . Vitamin B12 supplementation during pregnancy and postpartum improves B12 status of both mothers and infants but vaccine response in mothers only: a randomized clinical trial in Bangladesh. *Eur J Nutr* 2016;55:281–93.

32. Greibe E, Lildballe DL, Streym S, Vestergaard P, Rejnmark L, Mosekilde L, Nexø E. Cobalamin and haptocorrin in human milk and cobalamin-related variables in mother and child: a 9-mo longitudinal study. *Am J Clin Nutr* 2013;98:389–95.
33. Minet JC, Bisse E, Aebischer CP, Beil A, Wieland H, Lutschg J. Assessment of vitamin B-12, folate, and vitamin B-6 status and relation to sulfur amino acid metabolism in neonates. *Am J Clin Nutr* 2000;72:751–7.