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Dietary intakes of vitamins B2, B6, and B12 and ovarian cycle function among premenopausal women

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Abstract

Background: Vitamins B2, B6, and B12 are key players in one-carbon metabolism as enzymatic cofactors, and deficiency of these nutrients may influence reproductive outcomes possibly through affecting reproductive hormones.

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Author Contributions: Keewan Kim and Sunni L. Mumford designed the research; Keewan Kim conducted statistical analyses; Jean Wactawski-Wende collected the data; Keewan Kim wrote the manuscript with contributions from Ellen N. Chaljub; James L. Mills, Kara A. Michels, Jean Wactawski-Wende, Torie C. Plowden, and Sunni L. Mumford critically reviewed the manuscript; and Keewan Kim and Sunni L. Mumford had primary responsibility for final content. All authors reviewed and commented on subsequent drafts of the manuscript.

Objective: The goal was to investigate associations between dietary intakes of vitamin B2, B6, and B12, and menstrual function among premenopausal women.

Design: This was a secondary analysis of a prospective cohort study conducted at the University at Buffalo in 2005-2007.

Participants/Setting: Participants were 259 healthy, regularly-menstruating women (aged 18-44 years) with self-reported menstrual cycles between 21 and 35 days, who were not trying to conceive, and who had not used hormonal contraception in the past three months.

Main Outcome Measure(s): Intakes of B-vitamins were assessed via 24-hour dietary recalls four times per menstrual cycle for two cycles. Serum reproductive hormones and plasma homocysteine were measured eight and three times, respectively, per cycle for two cycles. Anovulatory cycles were determined by progesterone concentrations ≤5 ng/mL (15.9 nmol/L) and no observed serum luteinizing hormone peak during the mid or late luteal phase visit.

Statistical Analysis: Weighted linear mixed regressions were used to evaluate associations between cycle-averaged B-vitamin intakes and hormones and homocysteine, and generalized linear regressions for associations with anovulation. Models were adjusted for age, race, BMI, physical activity, alternate Mediterranean diet score, intakes of total energy, protein, fiber, and folate, and percentage of energy intake from fat.

Results: Higher intakes of vitamin B2 (per 0.1 mg increase in intake) were inversely correlated with estradiol (−0.87%, 95% confidence interval [CI] −1.67, −0.06) and homocysteine levels (−0.61%, 95% CI −1.10, −0.12). Higher vitamin B6 intakes were suggestive of higher follicle-stimulating hormone though the results were not statistically significant (0.63% difference, 95% CI −0.03, 1.29, per 0.1 mg increase in intake; P=0.06). Small increases in testosterone and decreases in homocysteine were found with vitamin B12 intake. No associations were observed between intake of B-vitamins and a risk of sporadic anovulation.

Conclusion: Higher intakes of vitamin B2 were associated with a small decrease in serum estradiol among healthy, regularly-menstruating women. Higher intakes of B2 and B12 were associated with lower plasma homocysteine concentrations. Overall, vitamins B2, B6, and B12 that are one-carbon nutrients do not appear to influence the ovarian cycle among premenopausal women.

Keywords

B-vitamin; riboflavin; reproductive hormones; homocysteine; anovulation

Introduction

Folate-mediated one-carbon metabolism is a complex and cyclical metabolic process that is important in human reproduction.[1] Studies have indicated that key players in one-carbon metabolism, including vitamins B2 (riboflavin), B6 (pyridoxine), B9 (folate), and B12 (cobalamin), homocysteine, and genetic variation in the gene encoding 5, 10-methylene tetrahydrofolate reductase (MTHFR), are associated with ovarian responsiveness [2, 3] and pregnancy outcomes [4, 5] among women using assisted reproductive technology (ART). However, much remains to be understood with regard to specific mechanisms by which

factors involved in one-carbon metabolism may influence reproductive outcomes, particularly among healthy women.

To date, most studies on folate-mediated one-carbon metabolism and reproduction focus on folate.[1, 6] Our previous study found that higher levels of serum folate concentrations were associated with higher luteal phase progesterone.[7] In addition, increased dietary intake of synthetic folate (i.e., folic acid) reduced risk for sporadic anovulation,[8] possibly through reduced levels of homocysteine, an amino acid which is elevated during B-vitamin insufficiency. Indeed, higher levels of plasma homocysteine measured around ovulation increased risk for sporadic anovulation by 33% in the same cohort of healthy women.[7] Though B-vitamins other than folate, including B2, B6, and B12, act as enzymatic cofactors in one-carbon metabolism, the influence of these nutrients among reproductively healthy women is not well understood as most studies were done in women using ART [5] or only evaluated outcomes such as ovulatory infertility.[9] To our knowledge, no studies have examined dietary B-vitamin intakes other than folate in the context of ovarian cycle function, such as evaluating reproductive hormone concentrations or ovulation in healthy women. Thus, the objective of this study was to investigate the associations between intakes of dietary vitamins B2, B6, and B12, and both serum reproductive hormone and plasma homocysteine concentrations and sporadic anovulation among healthy, regularly menstruating women who were not taking dietary supplements.

Methods

Study design and sample collection

This was a secondary analysis of the BioCycle Study, a prospective cohort study, that included 259 women in western New York (NY), ages 18-44, with self-reported cycle length between 21-35 days for each menstrual cycle, who were followed for two menstrual cycles from 2005 to 2007. Specific details of the study design and sample collection are published elsewhere.[10] Briefly, regularly menstruating premenopausal women were recruited using a variety of recruitment methods (e.g., advertisements in local newspapers, other print media, radio, television interview, and posting flyers) from the western New York region and the University at Buffalo. Regardless of recruitment method, women were instructed to directly contact the clinical research center for information and to schedule a screening visit appointment. At the screening visit, women were excluded based on specific criteria that included current or recent use of hormonal or oral contraceptives; vitamin or mineral supplement use; gynecologic problems, including a history of abnormal Pap smears, polycystic ovarian syndrome (PCOS), uterine fibroids, or endometriosis; pregnancy or breast feeding in the past 6 months; and diagnosis of chronic conditions such as metabolic or gastrointestinal conditions associated with malabsorption. Women were excluded if they had a self-reported body mass index (BMI) of <18 or >35 kg/m^2 at screening or if they were planning to restrict their diet.

Participants were asked to complete questionnaires about demographic factors (e.g., age, race, education, marital status, etc.), lifestyle factors (e.g., physical activity, cigarette smoking, alcohol consumption, etc.), and reproductive history (e.g., parity, past oral contraceptive use, etc.) The International Physical Activity Questionnaire (IPAQ) long form

was used to estimate physical activity, which was categorized into high, moderate, and low.[11] Height and weight were measured at baseline by trained study staff to calculate BMI. Participants were requested to remove street clothing and shoes prior to anthropometric measurement, and put on the provided paper gown. Height was measured twice using a fixed stadiometer and if the two measurements differed by more than 0.5 cm, then a third measurement was taken. Measures of height were averaged to calculate BMI. Weight was measured using a certified scale. The University at Buffalo Health Sciences Institutional Review Board (IRB) approved the study and served as the IRB for the National Institutes of Health for this study under a reliance agreement. All participants provided written informed consent.

Fasting blood samples for hormonal assessment were collected at eight timepoints across the menstrual cycle (Figure 1 online only). Overall, samples were collected across two cycles in 250 women and one cycle in nine women. Clinic visits for the sample collection were timed to occur on the second day of menstruation (one visit), the mid follicular phase (one visit), the periovulatory phase (three visits), and during the early, mid-, and late luteal phase (three visits). Self-reported information on menstrual cycle length was used along with daily morning urine fertility monitors to help schedule clinic visits (Clearblue Easy Fertility Monitor, Inverness Medical, Waltham, MA, US) and 94% of the women completed at least seven clinic visits per cycle.[10]

Dietary assessment

Dietary intake was assessed four times per cycle by using 24-hour dietary recalls by trained dietary interviewers in person on the same days as blood sample collections during menstruation, the mid follicular phase, predicted ovulation, and the mid luteal phase (Supplemental Figure 1). In the women who completed two cycles, eight dietary recalls were available in total and 87% of women completed all of the four dietary recalls in each cycle. The dietary data were analyzed using the Nutrition Data System for Research software version 2005 developed by the Nutrition Coordinating Center of the University of Minnesota, Minneapolis, MN.[12] Vitamin B2, B6, and B12 intakes, as well as total energy, protein, dietary fiber, and folate (dietary folate equivalent) intakes, percentage of energy from fat intake, and the alternate Mediterranean diet score to assess healthy eating patterns [13] were calculated from the recall data. Intakes of dietary B-vitamins did not vary substantially throughout the menstrual cycle,[14] and thus averages per cycle were used for the analysis. Women who were taking dietary supplements and multivitamins regularly at screening and unwilling to stop taking them during the study period were excluded from the study.

Analysis of reproductive hormones and homocysteine

Estradiol, progesterone, LH, FSH, sex hormone-binding globulin (SHBG), and testosterone were measured in the fasting serum samples which were collected at each cycle visit. Estradiol, progesterone, LH, FSH, and SHBG were measured using solid-phase competitive chemiluminescent enzymatic immunoassays (Specialty Laboratories Inc., Valencia, CA) on the DPC Immulite 2000 analyzer (Siemens Medical Solutions Diagnostics, Deerfield, IL) at the Kaleida Health Center for Laboratory Medicine in Buffalo, NY. Total testosterone

concentrations were determined by liquid chromatography/tandem mass spectrometry (Shimadzu Prominence Liquid Chromatograph, Shimadzu Scientific Instruments, Columbia, MD and ABSceix 5500 tandem mass spectrometer, Sciex, Framingham, MA) at the University of Minnesota, Minneapolis, MN. Increased sensitivity was obtained by the use of mobile phase B (100% acetonitrile) with a low standard of 4 ng/dL (0.14 nmol/L) added to the standard curve. Calculations of free testosterone and free androgen index (the ratio of total testosterone to SHBG, multiplied by 100) were performed via standardized methods.[15, 16] Homocysteine was measured in plasma samples collected at the menstrual, expected ovulation, and mid luteal phase visits using an IMMULITE 2000 Homocysteine Competitive Immunoassay at the Kaleida Center for Laboratory Medicine at Buffalo, NY. Over the study period, the coefficients of variation for these tests were <10% for estradiol, <5% for FSH and LH, <14% for progesterone, <10% for SHBG, 6% for total testosterone, and <10% for homocysteine. Cycles with progesterone concentrations ≤5 ng/mL (15.9 nmol/L) and no observed serum LH peak during the mid or late luteal phase visit were considered anovulatory cycles.[17]

Statistical Analysis

Demographic and dietary characteristics were compared between women with intakes of vitamin B2, B6, and B12 at or above versus below the recommended dietary allowance (RDA) (1.1 mg/day for vitamin B2, 1.3 mg/day for vitamin B6, and 2.4 μg/day for vitamin B12), using chi-square tests and analysis of variance.[18] Average intakes of vitamin B2, B6, and B12 were compared to the levels reported for women 20-29 years of age in the US population based on data from NHANES, which are 1.8 mg/day for B2, 1.8 mg/day for B6, and 4.7 μg/day for B12.[19]

Cycle-averaged vitamin B2, B6, and B12 intakes were evaluated as continuous variables in weighted linear mixed regression models to identify associations between B-vitamin intakes and reproductive hormones, SHBG, and the free androgen index. Reproductive hormones and homocysteine concentrations for these models were log transformed for normality and allowed to vary across the menstrual cycle; effect estimates were back-transformed and interpreted as percent difference in hormone concentrations associated with a one-unit increase in dietary intake. Models for progesterone concentrations were limited to measurements during the luteal phase visits. Models were adjusted for age, race, BMI, physical activity, alternate Mediterranean diet score, cycle-averaged intakes of energy, protein, dietary fiber, and folate (dietary folate equivalents), and percentage of energy intake from fat. The models were weighted to account for hormonal feedback mechanisms and potential confounders using inverse probability weights for each hormone at each visit that accounted for other hormones and homocysteine concentrations measured in the previous phase and visit.[20] Associations between cycle-averaged vitamin B2, B6, and B12 intakes and plasma homocysteine were evaluated using weighted linear mixed models. These models were adjusted for prior hormone concentrations and the potential confounders, as listed above. Separately, analyses were repeated after excluding cycles with extremely low (<1,000 kcal/day) or high (>3,000 kcal/day) mean energy intake.

Then generalized linear models were used to estimate the risk of sporadic anovulation associated with the B-vitamin intakes. The models were adjusted for age, race, BMI, physical activity, alternate Mediterranean diet score, cycle-averaged intakes of energy, protein, fiber, and folate, and percentage of energy intake from fat. These results are presented as relative risk (RR) and 95% confidence interval (CI). A P <0.05 was considered statistically significant. SAS version 9.4 (SAS Institute, Cary, NC) was used for all statistical analyses.[21]

Results

BioCycle Study participants had a mean ± standard deviation (SD) age of 27 ± 8 years, mean BMI of 24 ± 4 kg/m^2, and were mostly nonsmokers (96%), nulliparous (72%), and past users of oral contraceptives (54%; Table 1). Overall, 86%, 61%, and 71% of women consumed vitamin B2, B6, and B12 at or above the RDA, respectively. The mean ± SD dietary intakes for B2, B6, and B12 were 1.7 ± 0.5 mg/day, 1.5 ± 0.5 mg/day, and 4.2 ± 4.8 μg/day, respectively. These means were higher than the RDAs [18] and comparable to the levels reported for women 20-29 years of age in the US population.[19] Women who consumed vitamins B2 and B12 at or above the RDA were more likely to be married and to self-identify as white than women who consumed less than the RDA (Table 1). There were no significant differences in demographic characteristics by vitamin B6 status at or above and below the RDA. In general, intakes of total energy, protein, and folate were significantly higher among women who consumed B-vitamins at or greater than the RDA compared with women who consumed below the RDA.

Higher intakes of vitamin B2 (per 0.1 mg increase in intake) were associated with decreases in estradiol levels (−0.87% difference, 95% CI −1.67, −0.06) (Table 2). Higher vitamin B6 intakes (per 0.1 mg increase in intake) were suggestive of a small increase in FSH levels, though the results were statistically not significant (0.63% difference, 95% CI −0.03, 1.29; P=0.06). Higher cycle-averaged vitamin B12 intakes (per 1 μg increase in intake) were also associated with small increases in testosterone (0.17% difference, 95% CI 0.03, 0.31) and free testosterone (0.18% difference, 95% CI 0.03, 0.33).

Homocysteine concentrations were lower among women who consumed vitamin B12 at or above the RDA compared to below the RDA, whereas no substantial differences were found by vitamin B2 and B6 intakes (Table 1). Decreasing homocysteine concentrations were observed with higher B2 (−0.61% difference per 0.1 mg increase in B2 intake, 95% CI −1.10, −0.12) and B12 intakes (−0.30% difference per 0.1 mg increase in B12 intake, 95% CI −0.46, −0.13; Table 2). No associations were detected between a unit increase in B6 intakes and homocysteine. There were 23 cycles (4.5%) with mean energy intake <1,000 kcal/day and 1 cycle (0.2%) >3,000 kcal/day. Excluding these cycles from the analyses did not change the results (data not shown).

In general, this data does not support associations between intake of B-vitamins and a risk of sporadic anovulation (Table 3). Specifically, intakes of vitamin B2 (RR 0.99, 95% CI 0.89, 1.10, per 0.1 mg increase in intake), B6 (RR 0.94, 95% CI 0.84, 1.06, 0.1 mg increase in

intake), and B12 (RR 0.97, 95% CI 0.86, 1.09, 1 μg increase in intake) were not associated with risk for sporadic anovulation, adjusted for potential confounders.

Discussion

Among healthy regularly menstruating women, higher intakes of vitamin B2 were associated with lower serum estradiol, whereas vitamin B12 intakes were correlated with small increases in testosterone levels. Higher intakes of vitamin B2 and B12 were also associated with lower plasma homocysteine concentrations. Intakes of vitamin B6 were largely not associated with reproductive hormones or homocysteine. No associations were observed between intake of these B-vitamins and sporadic anovulation. Overall, these findings suggest that at intakes typical of the US population, B-vitamins do not greatly influence the ovarian cycle among healthy regularly menstruating women. To our knowledge, this is the first study to assess measures of ovarian cycle function among healthy women in association with dietary intake of non-folate B-vitamins involved in one-carbon metabolism.

In this cohort of healthy premenopausal women, vitamin B2 intake was associated with lower serum estradiol levels. Due to the difficulty in measuring hormones and their variability across the menstrual cycle in reproductive aged women, it is unsurprising that few studies investigated reproductive hormone concentrations and B-vitamin intake. However, the universal importance of vitamins B2, B6, and B12 as enzymatic cofactors makes connections with sex steroid hormone metabolism biologically plausible. For example, vitamin B2 serves as a precursor to flavin mononucleotide and flavin adenine dinucleotide, which are incorporated into proteins; these flavoproteins interact with enzymes crucial to cholesterol synthesis and sex-steroid hormone metabolism, including cytochrome P450s.[22, 23] In early vitamin B research among a small number of reproductive aged women (n = 14), no differences in estradiol levels by vitamin B deficiency status were reported.[24] A more recent analysis from the Nurses' Health Study II data among premenopausal women who took a multivitamin or nutrition supplements found an inverse association between intakes of vitamin B2, B6, and B12 and risk of ovulatory infertility.[9] Studies of ovulatory disorders, such as PCOS, reported lower serum vitamin B12 and higher homocysteine concentrations among women with PCOS relative to those without the disease.[25, 26] Recently, a study among women seeking ART treatment reported that higher serum vitamin B12 levels were associated with improved implantation, clinical pregnancy, and live birth.[5] Interestingly, no associations between vitamin B12 intake and reproductive hormones and sporadic anovulation were observed in this current analysis among healthy premenopausal women, except for small changes in testosterone levels.

Many factors may influence homocysteine, but vitamins B6 and B12 serve as cofactors for enzymes that have the ability to decrease intracellular homocysteine, while vitamin B2 is a cofactor for MTHFR, an enzyme that produces 5-methyltetrahydrofolate, which is necessary for converting homocysteine into methionine.[1] While vitamins B6 intakes were not correlated with homocysteine, an inverse association was detected for B2 and B12. This is in line with other studies in which small decreases in homocysteine levels were detected with increasing dietary [27] and plasma vitamin B2 levels.[28] Our earlier work showed a 33% increased risk of sporadic anovulation per unit increase in plasma homocysteine levels.[7]

However, observed changes in homocysteine levels did not translate into subsequent associations with risk of sporadic anovulation, presumably due to a small decrease in homocysteine concentrations.

It should be noted that there were several limitations. Compared to dietary data, blood concentrations of B-vitamins may provide a more sensitive assessment of how B-vitamin status relates to ovarian function as correlations between dietary intake and biomarkers of B-vitamins were weak, though the correlations for vitamin B2 tend to be stronger than for B6 and B12.[29] However, blood measures of B-vitamin were not available to confirm deficiencies or more accurately estimate bioavailability of these vitamins for each participant. However, multiple 24-hour dietary recalls were used, which minimizes potential misclassification of B-vitamin intake as well as measurement errors.[30, 31] Only women who were not planning to be on a special diet and agreed to discontinue taking supplements during the study period were included in the study, which may limit the generalizability of these results. However, this allowed examination of the impact of vitamins B2, B6, and B12 from dietary sources—as opposed to supplements, among the usual diets of healthy women. The intakes in this study were similar to those of reproductive age women in the US general population.[19] Of note, data regarding the time between the last intake of vitamin supplementation prior to study enrollment and study start when participants stopped taking supplementation are unavailable. Daily progesterone measures or transvaginal ultrasounds were not available to confirm ovulation. However, timely serum hormone measurements and fertility monitor usage, which measured LH in urine daily, helped to classify ovulatory cycles.[17, 32] Women enrolled in the study had self-reported regular cycles and were followed up for only two menstrual cycles. Therefore, there were a small number of sporadic anovulatory cycles (42 among 509 cycles), which may have limited the power of this study. Nevertheless, this study is uniquely rigorous, as dietary intake and reproductive hormones were measured at multiple times throughout the menstrual cycle in a large number of premenopausal women.

Conclusion

Overall, higher intakes of vitamin B2 were associated with differences in estradiol and both B2 and B12 intakes were correlated with a small decrease in homocysteine concentrations among healthy premenopausal women, though careful interpretation is needed given a lack of blood measurements. Intakes of B-vitamins were largely not associated with risk of sporadic anovulation. In conclusion, these findings suggest that at dietary intakes typical of the US population, B-vitamins other than folate, specifically vitamins B2, B6, and B12, that play a key role in one-carbon metabolism, do not appear to greatly influence ovulatory functioning among healthy premenopausal women.

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Research Snapshot

Research Question: Are dietary intakes of vitamin B2, B6, and B12, that play a key role in one-carbon metabolism, associated with menstrual function among premenopausal women?

Key Findings: In a secondary analysis of a prospective cohort of 259 premenopausal women in the US, higher intakes of vitamin B2 were associated with lower estradiol levels and intakes of B2 and B12 were correlated with a decrease in homocysteine concentrations. Vitamin B6 was largely not associated with reproductive hormones and homocysteine. These findings suggest a potential role of vitamin B2 in regulation of hormones, though these B-vitamins do not appear to influence ovulatory functioning among premenopausal women.

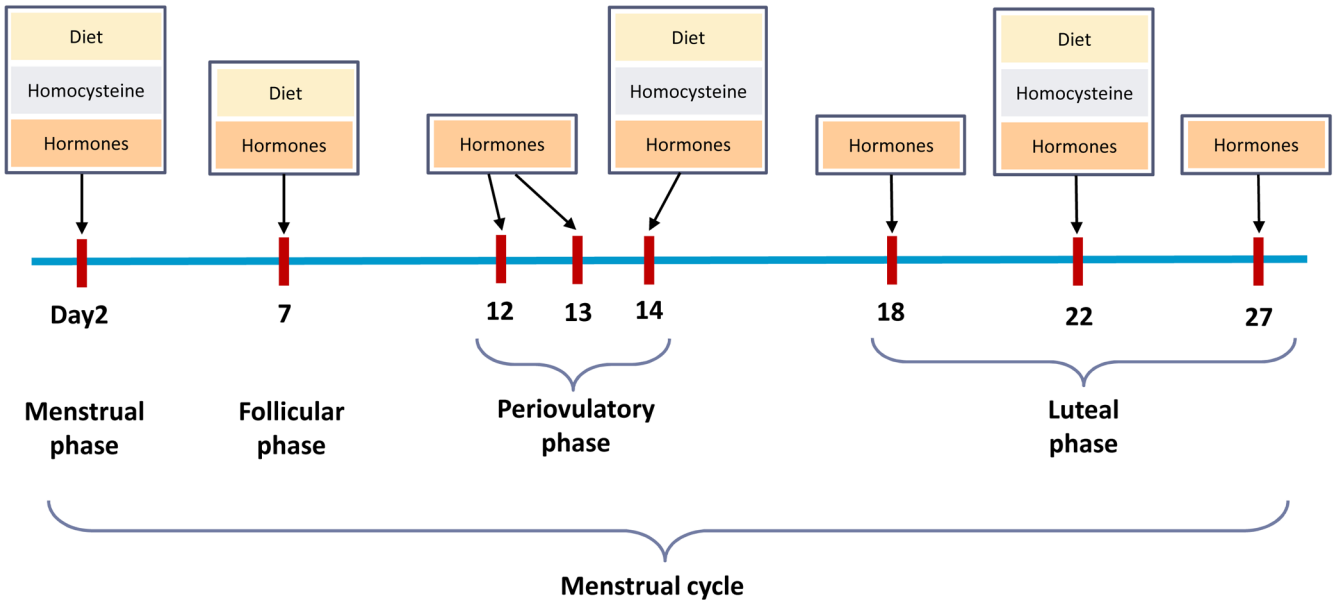

Figure 1.

Collection of blood specimens (8 times) and dietary data (4 times) per menstrual cycle: BioCycle Study, New York, 2005-2007.

Table 1.

Demographic, lifestyle, and dietary factors, and plasma homocysteine concentrations of the study cohort described by reported consumption of the recommended dietary allowance (RDA) of B-vitamins[18] across the study period: BioCycle Study, New York, 2005-2007.[a]

		Vitamin B2		Vitamin B6		Vitamin B12	
	Overall	**< 1.1 mg /day**	**≥ 1.1 mg /day**	**< 1.3 mg /day**	**≥ 1.3 mg /day**	**< 2.4 µg/day**	**≥ 2.4 µg/day**
Number of women (N)	259	36	223	101	158	74	185
Demographics factors							
Age, years: mean ± SD[b]	27.3 ± 8.2	25.8 ± 7.6	27.5 ± 8.3	27.0 ± 8.3	27.4 ± 8.2	25.8± 7.6	27.9 ± 8.3
Body mass index, kg/m^2: mean ± SD[b]	24.1 ± 3.9	24.2 ± 4.5	24.1 ± 3.8	23.9 ± 3.6	24.2 ± 4.0	24.0 ± 4.0	24.1 ± 3.8
Race, n (%)[c]							
White	154 (59)	7 (19)	147 (66) ***	57 (55)	97 (62)	35 (47)	119 (64) *
Black	51 (20)	18 (50)	33 (15) ***	23 (22)	28 (18)	22 (30)	20 (16) *
Other[d]	54 (21)	11 (31)	43 (19) ***	23 (22)	31 (20)	17 (23)	37 (20) *
≤ High school education, n (%)	33 (13)	5 (14)	28 (13)	13 (13)	20 (13)	7 (9)	26 (14)
Lifestyle factors: n (%)							
Physical Activity[e]							
Low	25 (10)	4 (11)	21 (9)	11 (11)	14 (9)	9 (12)	16 (9)
Moderate	92 (36)	14 (39)	78 (35)	39 (38)	53 (34)	29 (39)	63 (34)
High	142 (55)	18 (50)	124 (56)	53 (51)	89 (57)	36 (49)	106 (57)
Current smoker	10 (4)	0 (0)	10 (4)	2 (2)	8 (5)	3 (4)	7 (4)
Married	66 (25)	4 (11)	62 (28) *	29 (28)	37 (24)	12 (16)	54 (29) *
Reproductive history: n(%)							
Nulliparous	187 (72)	28 (78)	159 (71)	76 (74)	111 (71)	61 (82)	126 (68) *
Past oral contraceptive use	140 (54)	11 (31)	129 (58) **	54 (52)	86 (55)	35 (47)	105 (57)
Dietary Intake: mean ± SD[b]							
% calories from fat	34 ±5	34 ± 6	34 ± 5	34 ± 5	34 ± 5	33 ± 6	34 ± 5 *
Total energy intake, kcal/day	1607 ±354	1262 ± 277	1663 ± 334 ***	1412 ± 317	1733 ± 319 ***	1425 ± 311	1680 ± 345 ***

		Vitamin B2		Vitamin B6		Vitamin B12	
	Overall	**< 1.1 mg /day**	**≥ 1.1 mg /day**	**< 1.3 mg /day**	**≥ 1.3 mg /day**	**< 2.4 µg/day**	**≥ 2.4 µg/day**
Total protein, g/day	62 ± 16	47 ± 11	65 ± 15***	53 ± 12	68 ± 15***	51 ± 12	67 ± 15***
Total dietary fiber, g/day	14 ± 6	10 ± 6	14 ± 5***	11 ± 4	15 ± 6***	13 ± 7	14 ± 5
Total folate, dietary folate equivalent/day	501 ± 187	344 ± 118	527 ± 183***	393 ± 120	571 ± 189***	419 ± 139	535 ± 193***
Vitamin B2, mg/day	1.7 ± 0.5	0.9 ± 0.1	1.8 ± 0.5***	1.4 ± 0.4	1.9 ± 0.5***	1.3 ± 0.3	1.8 ± 0.5***
Vitamin B6, mg/day	1.5 ± 0.5	1.0 ± 0.3	1.5 ± 0.4***	1.1 ± 0.2	1.7 ± 0.4***	1.2 ± 0.3	1.6 ± 0.4***
Vitamin B12, µg/day	4.2 ± 4.8	2.0 ± 1.2	4.5 ± 5.0**	3.0 ± 5.1	4.9 ± 4.4**	1.8 ± 0.5	5.1 ± 5.3***
Plasma homocysteine concentration							
Homocysteine, µg/mL[f]	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.2**

[a] P-values were derived by Chi-square test or ANOVA comparing B-vitamin intakes below versus at or above the recommended dietary allowance.

[b] SD = standard deviation.

[c] 11 women self-identified as Spanish/Hispanic/Latino.

[d] Comprised of 1 American Indian/Alaska Native, 13 Asian Indian, 10 Chinese, 2 Filipino, 2 Japanese, 3 Korean, 3 Vietnamese, 6 other Asian, 1 Native Hawaiian, and 13 other race.

[e] The International Physical Activity Questionnaire (IPAQ) long form [11] to estimate participants' physical activity.

[f] To convert µg/mL homocysteine to µmol/L, multiply µg/mL by 7.3975.

* P<0.05;

** P<0.01;

*** P<0.001.

Table 2.

Associations between vitamin B2, B6, and B12 intakes and log transformed reproductive hormone concentrations using weighted linear mixed models: BioCycle Study, New York, 2005-2007.

	Unadjusted	Adjusted[a]
	% difference (95% CI[b])	% difference (95% CI[b])
Vitamin B2 (per 0.1 mg)		
Estradiol, pg/mL[c]	−0.19 (−0.69, 0.30)	−0.87 (−1.67, −0.06) *
Progesterone, ng/mL[d]	0.96 (−0.23, 2.16)	−1.62 (−3.54, 0.35)
LH, ng/mL[e]	0.27 (−0.25, 0.80)	0.40 (−0.49, 1.30)
FSH, mIU/mL[f]	0.18 (−0.24, 0.60)	0.11 (−0.57, 0.80)
SHBG, μg/mL[g]	−0.35 (−0.65, −0.05) *	−0.15 (−0.57, 0.26)
Testosterone, ng/dL[h]	−0.03 (−0.29, 0.24)	0.04 (−0.39, 0.47)
Free Testosterone, ng/dL[h]	−0.13 (−0.47, 0.21)	0.03 (−0.42, 0.48)
Free Androgen Index	0.27 (−0.15, 0.68)	0.19 (−0.48, 0.86)
Homocysteine, μg/mL[j]	−0.66 (−0.95, −0.36) ***	−0.61 (−1.10, −0.12) *
Vitamin B6 (per 0.1 mg)		
Estradiol, pg/mL[c]	0.25 (−0.23, 0.74)	0.12 (−0.66, 0.91)
Progesterone, ng/mL[d]	1.61 (0.37, 2.86) *	0.32 (−1.67, 2.34)
LH, ng/mL[e]	0.32 (−0.21, 0.85)	0.48 (−0.38, 1.36)
FSH, mIU/mL[f]	0.25 (−0.16, 0.67)	0.63 (−0.03, 1.29)
SHBG, μg/mL[g]	−0.24 (−0.53, 0.05)	−0.06 (−0.51, 0.40)
Testosterone, ng/dL[h]	0.11 (−0.14, 0.35)	0.09 (−0.29, 0.47)
Free Testosterone, ng/dL[h]	0.19 (−0.07, 0.45)	0.08 (−0.32, 0.49)
Free Androgen Index	0.35 (−0.04, 0.74)	0.05 (−0.55, 0.66)
Homocysteine, μg/mL[i]	−0.47 (−0.77, −0.16) **	−0.12 (−0.60, 0.36)
Vitamin B12 (per 1 μg)		
Estradiol, pg/mL[c]	−0.07 (−0.36, 0.21)	−0.14 (−0.43, 0.14)
Progesterone, ng/mL[d]	0.30 (−0.39, 1.00)	−0.08 (−0.74, 0.58)
LH, ng/mL[e]	0.19 (−0.09, 0.48)	0.21 (−0.09, 0.50)
FSH, mIU/mL[f]	−0.15 (−0.38, 0.09)	−0.18 (−0.41, 0.05)
SHBG, μg/mL[g]	−0.04 (−0.20, 0.12)	−0.02 (−0.18, 0.14)
Testosterone, ng/dL[h]	0.14 (−0.01, 0.28)	0.17 (0.03, 0.31) *
Free Testosterone, ng/dL[h]	0.15 (0.00, 0.30)	0.18 (0.03, 0.33) *
Free Androgen Index	0.17 (−0.06, 0.40)	0.03 (−0.22, 0.27)

	Unadjusted	Adjusted[a]
	% difference (95% CI[b])	% difference (95% CI[b])
Homocysteine, μg/mL[i]	−0.34 (−0.50, −0.18) ***	−0.30 (−0.46, −0.13) **

[a] Models were adjusted for age, race, body mass index, physical activity, alternate Mediterranean diet score, mean energy intake, mean protein intake, mean dietary fiber intake, mean percent of calories from fat, mean folate (dietary folate equivalent) intake, and concurrent hormone levels with the use of inverse probability weights.

[b] CI = confidence interval.

[c] To convert pg/mL estradiol to pmol/L, multiply pg/mL by 3.6713.

[d] Models were limited to measurements during luteal phase visit. To convert ng/mL progesterone to nmol/L, multiply ng/mL by 3.1801.

[e] LH = luteinizing hormone. To convert ng/mL LH to nmol/L, multiply ng/mL by 0.03846.

[f] FSH = follicle-stimulating hormone. To convert mIU/mL FSH to IU/L, multiply by 1.00.

[g] SHBG = sex hormone-binding globulin. To convert μg/mL SHBG to nmol/L, multiply μg/mL by 10.5263.

[h] To convert ng/dL testosterone to nmol/L, multiply ng/dL by 0.0347.

[i] To convert μg/mL homocysteine to μmol/L, multiply μg/mL by 7.3975.

* P<0.05;

** P<0.01;

*** P<0.001.

Table 3.

Associations between vitamin B2, B6, and B12 intakes and anovulation using generalized linear models: BioCycle Study, New York, 2005-2007.

	Unadjusted	Adjusted[a]
	RR[b] (95% CI)[c]	RR[b] (95% CI)[c]
Vitamin B2 (per 0.1 mg/day)	0.96 (0.90, 1.02)	0.99 (0.89, 1.10)
Vitamin B6 (per 0.1 mg/day)	0.97 (0.92, 1.02)	0.94 (0.84, 1.06)
Vitamin B12 (per 1 μg/day)	0.92 (0.77, 1.10)	0.97 (0.86 1.09)

[a]Models were adjusted for age, race, body mass index, mean energy intake, physical activity, alternate Mediterranean diet score, mean protein intake, mean dietary fiber intake, mean percent of calories from fat, and mean folate (dietary folate equivalent) intake.

[b]RR = relative risk.

[c]CI = confidence interval.