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Folate Intake and Ovarian Reserve Among Women Attending a Fertility Center

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B.S.
University of Michigan
2018

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An abstract of
A thesis submitted to the Faculty of the
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Abstract

Folate Intake and Ovarian Reserve Among Women Attending a Fertility Center

By Mumta Kadir

Background: Higher folate intake has been linked to shorter time to pregnancy and greater success of infertility treatments; however, the mechanisms underlying the beneficial effects of folate on female fertility have been less studied. Factors such as diet may affect antral follicle count (AFC), a well-accepted measure of ovarian reserve, although the research is sparse.

Methods: Our analysis included 552 women attending the Massachusetts General Hospital Fertility Center (2007-2019) who participated in the Environment and Reproductive Health Study. We measured folate intake using a validated food frequency questionnaire and AFC using transvaginal ultrasonography. Multivariable Poisson regression models with robust standard errors were used to estimate the association of folate intake with AFC adjusting for calorie intake, age, BMI, physical activity, education, smoking status, year of AFC scan, and intakes of vitamin B12, iron, and vitamin D. Non-linearity was assessed with restricted cubic splines.

Results: Among the 552 women (median age 35.0 years, median folate intake 1,005 μg/day), total and supplemental folate intake had a significant non-linear relationship with AFC (P for non-linearity 0.05 and 0.02, respectively). There was a positive linear association with AFC up to approximately 1200 μg/day for total folate intake and up to 800 μg/day for supplemental folate intake; however, there was no additional benefit of higher folate intakes.

Limitations: Due to the sole inclusion of women undergoing infertility treatment, who tended to be of older reproductive age, White, and of high socioeconomic status, the generalizability of our results to all reproductive aged women is unclear. Given the modest effect estimates, our results may have limited clinical applicability as the reproductive benefits of gaining 1 to 2 additional antral follicles with higher folate intake is unclear.

Conclusions: Our results support current recommendations from the Centers from Disease Control and Prevention that all women planning pregnancy should be consuming at least 400 μg/day of folic acid and suggests that additional benefit may be seen with up to 800 μg/day. Our results provide biological insight into one potential mechanism that could be mediating the positive relationships observed between folate intake and female fertility.

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Introduction

Infertility, defined as the failure to achieve pregnancy after either 6 or 12 months of unprotected sexual intercourse (for women younger or older than 35 years, respectively), affects 15-25% of couples in western countries. [1, 2] Given that the mean age at first birth is rising in most high-income countries and age is the strongest predictor of infertility, the incidence of infertility is only expected to increase.[3] Although treatments for infertility are available, due to the high financial costs, modest success rates, and limited access [4,5] they may not be sufficient to address infertility on a population level, highlighting the need to identify modifiable predictors.

It is well established that daily folic acid supplementation in the periconceptional period can prevent neural tube defects (NTD).[6] There is also suggestive evidence that higher intake of this nutrient leads to great reproductive success in women.[7] One of the earliest studies supporting this link was the Hungarian NTD randomized controlled trial which showed that women consuming a multivitamin containing 800 μg/day of folic acid had higher rates of conception compared to the placebo group.[8] More recent studies also confirm this finding demonstrating that higher folate intake- most notably folic acid from fortified foods and supplements- is associated with lower risk of anovulation[9] and ovulatory infertility,[10] shorter time to pregnancy,[11] and greater success in infertility treatment.[12] Several studies in reproductive aged women have shown that one pathway through which folic acid intake may enhance fertility is through beneficial effects on menstrual cycle function[13-15] including improved hormonal balance and follicular development.[9,16] Studies from women undergoing infertility treatment also suggest that folate could have beneficial effects on fertility though oocyte and embryo quality.[17-20]

Interestingly, no studies to date have assessed whether folate may impact female fertility through effects on ovarian reserve. Antral follicle count (AFC), a standard measure of ovarian reserve,[21] is a well-accepted marker of response to treatments and reproductive aging. While diminished ovarian reserve is major cause of infertility, particularly among older women, the process leading to reproductive senescence is not well understood. Diet, which is a modifiable factor, may affect ovarian reserve, however little research has been done on this topic.[22] Thus, our objective was to evaluate the association between dietary folate intake and AFC among women seeing treatment for infertility.

Methods

The women in our analysis were participants in the Environment and Reproductive Health (EARTH) Study, a prospective cohort of couples seeking infertility treatment and evaluation at the Massachusetts General Hospital (MGH) Fertility Center (2004-2019).[23] In brief, all women 18-46 years were eligible for the study and approximately 60% of eligible women contacted by the research staff participated. A food frequency questionnaire (FFQ) used to assess usual dietary habits was introduced in 2007. Of the initial 1019 antral follicle scans available for analysis, we excluded those done while the woman was on Lupron (n=43), incomplete scans (n=21), those done on women with polycystic ovaries (n=77), and repeated scans (n=107). From these 772 eligible women with a complete AFC, we excluded women who had incomplete or missing diet data (n=200) as well as women who completed the FFQ more than a year after their transvaginal ultrasound (n=20). Our final analytical sample included 552 women. The EARTH study was approved by the Human Studies Institutional Review Boards of MGH and the Harvard T.H. Chan School of Public Health.

We measured dietary intake using a validated FFQ.[24,25] On this questionnaire, women were asked to report how often, on average, they consumed specified amounts of each food and beverage during the previous year. Multivitamin and supplement users were asked to specify brand, dose, and frequency of use. Nutrient intakes were estimated by summing the contribution of all relevant food and supplement items and were expressed as daily intakes. The nutrient content of each food item is obtained from the US Department of Agriculture with supplemental information from manufacturers.[26] To reduce extraneous variation in intake, folate was adjusted for total energy intake using the nutrient residual method.[27] Folate intake assess using this questionnaire has been validated against prospectively collected diet records ($r=0.76$)[25] and red blood cell ($r=0.51$)[28] and plasma folate levels ($r=0.54$).[24]

AFC was measured by one of the reproductive endocrinology and infertility physicians from the MGH Fertility Center using transvaginal ultrasonography performed on the third day of an unstimulated menstrual cycle or on the third day of a progesterone withdrawal bleed. No fertility medications were used in the cycle prior to the ultrasonography assessment. To reduce the influence of very high antral follicle counts, we truncated the measure at 30 (19 women, 3.4%).

At enrollment, height and weight were measured by trained study staff to calculate body mass index (BMI) (kg/m^2) and a brief, staff-administered questionnaire was used to collect data on demographics, medical history, and lifestyle. Participants also completed a detailed take-home questionnaire with additional questions on lifestyle factors, reproductive health, and medical history. Time spent in leisure time physical activities was assessed using a validated questionnaire[29] in which women reported the average time per week they spent during the

preceding year on 11 different activities using 13 response categories ranging from “never” to “40+ hours per week”. Clinical information including infertility diagnosis and protocol type was abstracted from medical records.

We divided women into quartiles of total folate intake and summarized participant characteristics across these categories. We tested for differences across quartiles using Kruskal-Wallis test for continuous variables and Chi-square test for categorical variables. We fit multivariable Poisson regression models with robust standard errors to estimate the mean AFC and 95% confidence interval (CI) by quartile of total, supplemental, and food folate intake. We also estimated the percent difference in AFC by quartile of folate intake using quartile one as the reference. We ran tests for linear trend across quartiles using the median value for each quartile as a continuous variable in the model. Dietary folate was also evaluated as continuous linear variable. Non-linearity was assessed non-parametrically with restricted cubic splines, which used the likelihood ratio test comparing the model with the linear term to the model with the linear and the cubic spline terms.[30]

Confounding was assessed using a priori knowledge in combination with directed acyclic graphs and descriptive statistics. Using these criteria, the final multivariable model included total calorie intake (continuous), age (continuous), BMI (continuous), physical activity (continuous), education (<College degree, College degree, Graduate degree), smoking status (never vs ever), year of AFC scan (continuous), and vitamin B12 (continuous), iron (continuous), and vitamin D intake (continuous). We evaluated whether the association between total folate intake (continuous) and AFC (continuous) was modified by BMI (≥ 25 kg/m^2 and < 25 kg/m^2), age ($\geq$

35 and < 35 years), and smoking status (current/former and never smokers) by introducing cross-product terms to the final multivariable models. We additionally investigated whether intake of other B vitamins (e.g. B1, B2, B3, B5, B6, B12) were related to AFC due to the high collinearity and synergy between these nutrients and folate. All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Two-sided p values ≤0.05 were considered statistically significant.

Results

The 552 women had a mean (standard deviation) age of 35.0 (4.2) years and BMI of 24.2 (4.4) kg/m^2. The majority of women were never smokers (73.4%) and primarily White (83.3%) with a college degree or higher (90.9%). The median (25^{th}, 75^{th} percentile) folate intake was 1005 (699.1, 1292.6), 457 (400.0, 800.0), and 420 (312.0, 542.9) µg/day for total, supplemental, and food folate, respectively. The median antral follicle count was 13 with a range of 1-30. The most common reason for infertility at enrollment was unexplained (46%). There were significant differences in physical activity, total calorie intake, percent of calories from carbohydrates and fat, intake of vitamins D, B1, B2, B3, B5, B6, and B12, and use of a multivitamin across quartiles of total folate intake. There were no significant differences in age, BMI, smoking status, education level, reproductive characteristics, or intake of caffeine, alcohol, or percent of calories from protein across quartiles of total folate intake (**Table 1**). Total folate intake was moderately to highly correlated with intake of most other B vitamins (range: -0.08 for B5 to 0.76 for B1).

When folate was assessed using quartiles, we found no significant associations between total, supplemental, and food folate intake and AFC in the calorie and age-adjusted or multivariable-

adjusted models (**Table 2**). The multivariable adjusted percent difference in AFC (95% CI) for women in the fourth quartile compared to the first quartile was 6.5% (-6.8, 21.7) for total folate intake (p-trend=0.48), 6.6% (-5.6, 20.5) for supplemental folate intake (p-trend=0.32), and 5.2% (-4.8, 16.2) for food folate intake (p-trend=0.33). When total and supplemental folate were assessed as continuous variables, there was evidence of a significant non-linear relationship (P for non-linearity 0.05 and 0.02, respectively). In both instances, there was a positive linear relationship with AFC up to approximately 1200 µg/day for total folate intake and up to 800 µg/day for supplemental folate intake without evidence of additional benefit with higher intakes (**Figure 1**). For food folate intake, no significant non-linear relationship with AFC was detected (P for non-linearity 0.32).

We also investigated whether the intake of the other B-vitamins, which are highly correlated with folate intake, showed similar associations with AFC (**Table 3**). When assessed using quartiles, higher intake of each of these micronutrients was not significantly associated with AFC. The multivariable adjusted percent difference in AFC (95% confidence interval) for women in the fourth quartile compared to the first quartile was 6.2% (-6.8, 21.0) for B1 (p-trend=0.35), 2.0% (-9.9, 15.5) for B2 (p-trend=0.99), -3.0% (-16.0, 12.0) for B3 (p-trend=0.10), -1.6% (-1.9, 9.8) for B5 (p-trend=0.82), 15.5% (1.8, 31.1) for B6 (p-trend=0.27), and 5.1% (-7.8, 19.7) for B12 (p-trend=0.88). After further adjustment for folate intake, these associations remained similar. When assessed continuously, there was also no evidence of a relationship with AFC and B1, B2, B3, B6, or B12 intake, whether modeled linearly or as a restricted cubic spline. For B5 intake, however, a significant non-linear relationship with AFC was detected (P for nonlinearity 0.003) and this association remained after further adjustment for folate intake (P for

nonlinearity <0.001) **(Supplemental Figure 1)**; however, upon further inspection, this association was being entirely driven by outlying B5 intakes above the 95th percentile, and once these intakes were censored, the significant, non-linear association was no longer observed.

As a sensitivity analysis we evaluated whether the association between folate intake and AFC was modified by factors strongly related to AFC (**Table 4**). In all instances, there was no evidence to suggest that the association between folate and AFC was significantly modified by age (p-interaction=0.56), smoking status (p-interaction=0.13), BMI (p-interaction=0.85), or infertility diagnosis (p-interaction=0.35). We also conducted a sensitivity analysis restricted to women who filled out their FFQ prior their antral follicle scan (n=188). Although our power was severely reduced due to the small sample size, we noticed a similar non-linear relationship between total folate intake and AFC (**Supplemental Figure 2**); however, it was no longer statistically significant (P for non-linearity 0.46).

Discussion

Among a prospective cohort of reproductive aged women attending an infertility clinic, we found that higher intake of folate was positively associated with AFC at levels up to 1200 µg/day; however, there was no additional benefit of higher folate intakes. This association was driven by intake of supplemental folate intake as opposed to food folate. While the positive association between folate intake and AFC was robust to adjustment for other lifestyle and dietary characteristics, the magnitude of association was modest – for example, the predicted difference in AFC between a woman consuming 400 versus 800 µg/day of supplemental folate was ~1.5.

Our findings of a beneficial effect of folate intake on ovarian reserve is in concordance with prior studies which found a higher rate of conception,[8,15] lower risk of ovulatory infertility,[10] shorter time to pregnancy, and greater success in infertility treatment[12] among women with higher intake of folate. While, to our knowledge, no other previous studies have directly assessed the relationship between folate intake and markers of ovarian reserve, a handful of studies have evaluated menstrual cycle function, which is closely related to ovarian reserve biomarkers.[31] For example, as follicle numbers gradually decline with age, a sequence of reproductive events begins to occur, starting with reduced fecundity, progressing through menstrual cycle irregularity, towards a complete cessation of menstruation at the menopause. One of the first studies suggesting a possible link between folic acid intake and menstrual cycles came from the Hungarian NTD randomized controlled trial, which found that woman randomized to the preconception multivitamin supplement containing 800 μg/day of folic acid had more regular menstrual cycles during follow-up compared to women taking the placebo-like trace element supplement.[14] Similarly, in a cross-sectional study of Danish pregnancy planners, authors found that compared to non-users, women taking a folic acid supplement had reduced odds of short menstrual cycle length (e.g. <27 days).[13] Interestingly, a meta-analysis of 11 studies found that short cycle length (21–27 days) was the menstrual cycle characteristic most closely related to low ovarian reserve biomarkers.[31] Among a prospective cohort of US reproductive aged women, those with higher intake of synthetic folate had higher progesterone levels during the luteal phase of the menstrual cycle and lower risk of sporadic anovulation, suggesting improved ovarian function.[9] Similarly, a small, double blind, placebo-controlled study found that women assigned to the FertilityBlend for Women supplement (which contained 400 μg/day of folic acid) had a trend toward increased mean mid-luteal progesterone levels compared to women in the placebo

group.[15] Overall, these previous studies are consistent with our findings that folate intake, particularly supplemental folate intake, may be associated with improved ovarian function.

The observed relation between supplemental folate intake and antral follicle count is biologically plausible. Dietary or genetically determined folate deficiency leads to mild hyperhomocysteinemia, which has been associated with increased inflammatory cytokine expression, altered nitric oxide bioavailability, induction of oxidative stress, activation of apoptosis, and defective methylation[32] which could in turn lead to enhanced follicular atresia. Studies among women undergoing in vitro fertilization have shown that women taking a folic acid supplement have a diminished concentration of homocysteine (in both follicular fluid and serum), and subsequently present with better quality oocytes following ovarian stimulation.[18] While current research is limited, there is also a possibility that ovarian response to endogenous follicle stimulating hormone (FSH) pulses is decreased in low folate conditions, which could lead to impaired to ovarian function. In women undergoing controlled hyperstimulation with recombinant FSH, carriers of the T allele in position 677 of the MTHFR gene, had a decreased ovarian responsiveness to this hormone,[17] fewer oocytes retrieved, and granulosa cells that produced less estradiol, basal and stimulated[33].

It is possible that our study found stronger associations between supplemental folate intake as opposed to food folate and AFC because there is a greater absorption rate of supplemental folate.[34] Natural food folate is present primarily in the reduced polyglutamated form while synthetic folic acid is a fully oxidized monoglutamate form of folate. Natural food folate has a lower proportion of folate that is absorbed and available for metabolic reaction and/or storage

compared to folic acid. Also, the poor stability of food folate under cooking conditions can reduce the intake of food folate ingested. Due to the mandatory folic acid fortification of foods (such as ready-to-eat cereals) in the US, there may be a greater bioavailability of synthetic folate which could explain the association between supplemental folate and antral follicle count. These fortified foods also tend to include the other B vitamins, which could possibly explain some of the relationship observed between B5 and antral follicle count.

Strengths of our study include its prospective design which minimizes the possibility of reverse causation and avoids recall bias, the use of a validated FFQ to assess diet, and our standardized assessment of AFC, a well-accepted marker of ovarian reserve. We also benefitted from having a wide range of folate intake in our population which increased our power to discern an effect and investigate non-linear relationships. However, our study was not without limitations. Due to the sole inclusion of women undergoing infertility treatment, who tended to be of older reproductive age, White, and of high socioeconomic status, the generalizability of our results to all reproductive aged women is unclear. However, our study participants are comparable to other fertility patients in the US suggesting that results may be generalizable to other couples seeking infertility treatment and evaluation.[35] Previous work has also shown that infertile women <40 years have similar AFCs compared with women of the same age with no history of infertility,[36] suggesting that our outcome assessment should not be materially impacted by our choice of study population. Self-report of diet by FFQ is subject to measurement error. However, we used a questionnaire known to relate well to biomarker levels.[28,37] Moreover, due to the prospective nature of our study, measurement error would most likely be non-differential with respect to AFC and result in an attenuation of the observed associations. Due to the observational nature of

our study, there remains the possibility of residual confounding by lifestyle factors that were not or poorly measured. As such, our findings need to be confirmed by others and ideally with a randomized trial before causality can be implied. Finally, because the MGH Fertility Center only began routinely measuring anti-Müllerian hormone levels in women starting in 2013 (and the assay changed over time), we did not include this parameter as a marker of ovarian reserve in our analysis.

In conclusion, higher intake of folate, particularly from supplements, in the year prior to infertility exam was associated with modestly improved ovarian reserve as measured by AFC. The positive, linear association between folate and AFC was only present for intakes between 0 to 1200 μg/day, with little to no benefit observed with higher intakes. Our results support current recommendations from the Centers from Disease Control and Prevention that all women planning pregnancy should be consuming at least 400 μg/day of folic acid and suggests that additional benefit may be seen with up to 800 μg/day. Given the modest effect estimates, our results may have limited clinical applicability as the reproductive benefits of gaining 1 to 2 additional antral follicles with higher folate intake is unclear. However, our results do provide interesting biological insight into one potential mechanism that could be mediating the positive relationships observed between folate and female fertility. Given the lack of data on this topic, it is important that future studies, particularly in more diverse populations, are conducted to better understand the clinical relevance and biological implications of our findings.

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Table 1. Baseline characteristics of 552 women by quartile of total folate intake in the EARTH Study.

	Total Folate Intake (N=552)				
Quartile (Range), µg/day	**Q1 (215.5-742.6)**	**Q2 (746.7-1003.4)**	**Q3 (1006.6-1325.6)**	**Q4 (3140.1-1515.7)**	**p-value[1]**
N	138	138	138	138	
Personal Characteristics					
Age, years	34.4 (4.8)	35.2 (4.0)	35.5 (4.1)	34.8(4.1)	0.24
BMI, kg/m	24.5 (5.3)	24.0 (3.8)	23.8 (4.3)	24.5 (4.3)	0.48
Total Physical Activity (hrs/wk)	7.05 (8.9)	6.99 (8.5)	8.75 (11.0)	7.72 (7.8)	0.05
Ever smoker, N (%)	32 (23.2)	39 (28.3)	39 (28.3)	37 (26.8)	0.75
White, N (%)	114 (82.6)	114 (82.6)	117 (84.8)	115 (83.3)	0.99
Education, N (%)					0.38
High school or less	14 (10.1)	10 (7.3)	9 (6.5)	6 (4.4)	
College	50 (36.2)	52 (37.7)	45 (32.6)	42 (30.4)	
Graduate school	74 (53.7)	76 (55.1)	84 (60.9)	90 (65.2)	
Dietary Characteristics					
Total Calories, kcal/day	1805.0 (608.8)	1641.6 (556.7)	1890.5 (551.2)	1656.7 (632.9)	<0.001
Carbohydrates, % of kcal/day	46.9 (9.3)	48.1 (7.1)	48.9 (7.3)	50.1 (7.8)	0.001
Protein, % of kcal/day	17.1 (3.3)	17.1(2.6)	16.3 (2.6)	16.7 (3.2)	0.13
Fat, % of kcal/day	34.6 (7.2)	33.2(5.9)	33.9 (6.9)	32.6 (6.1)	0.05
Alcohol, g/day	7.8 (8.1)	8.3 (10.1)	8.6 (9.2)	6.7 (6.8)	0.19
Caffeine, mg/day	146.4 (132.5)	129.2 (111.1)	127.2 (112.6)	111.3 (100.5)	0.19
Vitamin B1, µg/day	2.06 (3.30)	3.50 (5.68)	4.35 (7.58)	8.26 (14.9)	<0.001
Vitamin B2, µg/day	2.79 (3.40)	4.36 (5.71)	5.24 (7.57)	9.75 (14.8)	<0.001
Niacin, µg/day	26.9 (8.98)	39.2 (107.0)	42.8 (26.3)	59.3 (76.0)	<0.001
Vitamin B5, µg/day	8.27 (5.17)	12.6 (7.65)	10.4 (9.71)	12.1 (16.0)	<0.001
Vitamin B6, µg/day	3.83 (8.87)	7.67 (16.3)	8.53 (11.9)	24.6 (30.7)	<0.001
Vitamin B12, µg/day	17.2 (66.5)	32.7 (107.0)	47.5 (128.1)	78.0 (191.4)	<0.001
Vitamin D, IU/day	455.2 (464.0)	726.5 (521.0)	812.6 (525.4)	925.6 (571.6)	<0.001
Total Folate Intake, µg/day	548.4 (187.5)	812.9 (145.9)	1215.5 (189.3)	1555.0 (398.1)	<0.001
Supplemental Folate, µg/day	137.0 (146.0)	401.7 (87.6)	733.4 (151.0)	1073.7 (360.7)	<0.001
Food Folate, µg/day	404.5 (95.0)	437.3 (88.2)	446.2 (118.2)	503.2 (153.6)	<0.001
Multivitamin Intake, N (%)	79 (57.3)	119 (86.2)	133 (96.4)	135 (97.8)	<0.001
Reproductive Characteristics					
Infertility diagnosis, N (%)					0.39
Female factor	51 (37.0)	35 (25.4)	35 (25.4)	36 (26.1)	
Diminished ovarian reserve	10 (7.3)	10 (7.25)	15 (10.9)	11 (8.0)	
Ovulatory	17 (12.3)	14 (10.1)	11 (8.0)	12 (8.7)	
Endometriosis	10 (7.3)	5 (3.6)	3 (2.2)	4 (2.9)	
Uterine	5 (3.6)	1 (0.7)	1 (0.7)	1 (0.7)	
Tubal	9 (6.5)	5 (3.6)	5 (3.6)	8 (5.8)	
Male factor	29 (21.0)	31 (22.5)	36 (26.1)	41 (29.7)	
Unexplained	58 (42.0)	70 (50.7)	66 (47.8)	60 (43.5)	
Gravid, N (%)	69 (50.0)	54 (39.1)	52 (37.8)	65 (47.1)	0.18
Previous Infertility Exam, N (%)	108 (78.3)	108 (78.3)	113 (81.9)	113 (81.9)	0.68
Previous Infertility Treatment, N (%)	63 (45.7)	61 (44.2)	70 (50.7)	67 (48.6)	0.63
Regular menstrual cycles, N (%)					0.24
Yes	115 (83.3)	122 (88.4)	122 (88.4)	123 (89.1)	
No	21 (15.2)	13 (9.4)	15 (10.9)	10 (7.2)	

Missing	2 (1.4)	3 (2.2)	1 (0.7)	5 (3.6)	
Menstrual cycle length, N (%)					0.79
24-38 days	122 (88.4)	126 (91.3)	124 (90.0)	127 (92.0)	
<24 or >38 days	9 (6.5)	7 (5.1)	9 (6.5)	9 (6.5)	
Missing	7 (5.1)	5 (3.6)	5 (3.6)	2 (1.4)	

Data are presented as mean (standard deviation) or N (%) unless otherwise noted.

[1]P-values were calculated using a Kruskal-Wallis test for continuous variables and a chi-square test for categorical variables.

Table 2. Association between folate intake and antral follicle count among 552 women in the EARTH Study.

		Adjusted mean (95% CI)		% Difference in AFC (95% CI)	
Folate Intake (range)	n	Age and total energy	Multivariable[1]	Age and total energy	Multivariable[1]
Total Folate					
Q1 (215.5-742.6)	138	13.7 (12.7, 14.7)	13.6 (12.4, 14.9)	REF	REF
Q2 (746.7-1003.4)	138	14.1 (13.1, 15.2)	14.1 (13.0, 15.4)	3.3 (-7.0, 14.7)	4.0 (-6.7, 15.8)
Q3 (1006.6-1325.6)	138	13.5 (12.5, 14.5)	13.9 (12.7, 15.2)	-1.6 (-11.4, 9.3)	2.3 (-9.0, 15.0)
Q4 (3140.1-1515.7)	138	13.6 (12.6, 14.6)	14.5 (13.1, 16.0)	-0.8 (-10.7, 10.2)	6.5 (-6.8, 21.7)
P-trend		0.64	0.48		
Supplemental Folate					
Q1 (0-399)	152	13.5 (12.6, 14.5)	13.3 (12.2, 14.5)	REF	REF
Q2 (400-500)	138	13.9 (13.0, 15.0)	14.0 (13.0, 15.2)	2.9 (-6.9, 13.7)	5.2 (-5.0, 16.4)
Q3 (501-800)	142	14.0 (13.0, 15.1)	14.6 (13.4, 16.0)	3.8 (-6.3, 15.0)	9.5 (-2.2, 22.6)
Q4 (801-2400)	120	13.3 (12.3, 14. 4)	14.2 (12.9, 15.6)	-1.7 (-11.8, 9.6)	6.6 (-5.6, 20.5)
P-trend		0.81	0.32		
Food Folate					
Q1 (206.3-361.8)	138	13.2 (12.3, 14.2)	13.5 (12.5, 14.6)	REF	REF
Q2 (362.1-420.3)	138	13.8 (12.8, 14.9)	14.0 (12.9, 15.3)	4.4 (-6.0, 15.8)	3.9 (-6.4, 15.3)
Q3 (420.4-513.6)	138	13.8 (12.8, 15.0)	14.3 (13.1, 15.7)	4.7 (-5.8, 16.4)	6.0 (-4.8, 18.0)
Q4 (513.6-1135.8)	138	14.0 (13.0, 15.0)	14.2 (13.1, 15.4)	5.7 (-4.3, 16.7)	5.2 (-4.8, 16.2)
P-trend		0.34	0.33		

[1]Adjusted for total calorie intake, age, BMI, physical activity, education level, smoking status, year of AFC (antral follicle count), Vitamin B12, Iron, and Vitamin D intake.

Table 3. Association between B vitamin intakes and antral follicle count among 552 women in the EARTH Study.

		Adjusted mean (95% CI)		% Difference in AFC (95% CI)	
Nutrient Intake (range)	n	Multivariable[1]	Further Adjusted for Folate	Multivariable[1]	Further Adjusted for Folate
B1 (Thiamin), mg/day					
Q1 (0.74-2.24)	138	13.5 (12.3, 14.7)	13.7 (12.4, 15.2)	REF	REF
Q2 (2.24-2.92)	138	13.8 (12.7, 15.0)	13.9 (12.8, 15.2)	2.5 (-8.3, 14.6)	1.2 (-9.8, 13.6)
Q3 (2.93-3.28)	138	14.4 (13.3, 15.6)	14.3 (13.2, 15.5)	6.8 (-4.4, 19.3)	4.3 (-7.9, 18.1)
Q4 (3.28-65.2)	138	14.3 (13.0, 15.8)	14.1 (12.7, 15.6)	6.2 (-6.8, 21.0)	2.6 (-12.0, 19.5)
P-trend		0.35	0.70		
B2 (Riboflavin), mg/day					
Q1 (0.92-2.93)	138	13.7 (12.6, 14.9)	14.0 (12.6, 15.5)	REF	REF
Q2 (2.93-3.58)	138	14.7 (13.5, 16.0)	14.7 (13.6, 16.0)	7.3 (-3.7, 19.5)	4.8 (-6.4, 17.5)
Q3 (3.58-4.43)	138	13.8 (12.6, 15.0)	13.8 (12.6, 15.0)	0.5 (-10.4, 12.7)	-2.2 (-13.2, 10.3)
Q4 (4.44-64.5)	138	14.0 (12.7, 15.3)	13.6 (12.3, 15.1)	2.0 (-9.9, 15.5)	-3.0 (-16.0, 12.0)
P-trend		0.99	0.50		
B3 (Niacin), mg/day					
Q1 (10.5-29.8)	138	13.2 (12.1, 14.5)	13.3 (12.1, 14.7)	REF	REF
Q2 (29.8-38.4)	138	13.9 (12.7, 15.1)	13.9 (12.8, 15.1)	4.8 (-6.4, 17.5)	4.2 (-7.0, 16.7)
Q3 (38.4-42.8)	138	13.8 (12.7, 15.1)	13.8 (12.7, 15.1)	-2.2 (-13.2, 10.3)	3.7 (-8.1, 17.0)
Q4 (42.8-694.6)	138	15.0 (13.9, 16.3)	15.0 (13.8, 16.2)	-3.0 (-16.0, 12.0)	12.1 (-1.0, 27.0)
P-trend		0.10	0.06		
B5 (Pantothenic acid), mg/day					
Q1 (1.29-4.85)	138	14.3 (13.1, 15.6)	14.3 (13.1, 15.6)	REF	REF
Q2 (4.86-7.71)	138	13.8 (12.7, 15.0)	13.8 (12.7, 15.0)	-3.5 (-13.4, 7.5)	-3.2 (-13.1, 7.8)
Q3 (7.72-13.8)	138	13.8 (12.6, 15.0)	13.8 (12.6, 15.1)	-3.8 (-13.5, 6.9)	-3.4 (-13.2, 7.5)
Q4 (13.8-66.8)	138	14.1 (12.9, 15.4)	14.1 (12.9, 15.4)	-1.6 (-1.9, 9.8)	-1.7 (-11.9, 9.8)
P-trend		0.82	0.83		
B6 (Pyridoxine), mg/day					
Q1 (0.97-3.13)	138	12.8 (11.7, 14.1)	12.9 (11.6, 14.3)	REF	REF
Q2 (3.13-4.17)	138	13.4 (12.3, 14.6)	13.4 (12.3, 14.6)	4.4 (-6.7, 16.8)	4.2 (-7.2, 17.1)
Q3 (4.18-5.48)	138	15.1 (13.9, 16.3)	15.1 (13.9, 16.3)	17.6 (4.4, 32.5)	17.3 (3.0, 33.6)
Q4 (5.56-153.2)	138	14.8 (13.5, 16.2)	14.8 (13.4, 16.3)	15.5 (1.8, 31.1)	15.1 (-0.8, 33.5)
P-trend		0.27	0.48		
B12 (Cyanocobalamin), µg/day					
Q1 (1.12-8.73)	138	13.7 (12.5, 15.0)	13.9 (12.6, 15.4)	REF	REF
Q2 (8.74-11.5)	138	13.8 (12.7, 15.1)	13.9 (12.7, 15.2)	1.3 (-9.3, 13.2)	-0.1 (-11.0, 12.1)
Q3 (11.6-15.8)	138	14.1 (13.0, 15.3)	14.1 (13.0, 15.3)	3.5 (-8.0, 16.4)	1.4 (-10.5, 14.8)
Q4 (15.8-978.9)	138	14.3 (13.1, 15.8)	14.1 (12.8, 15.6)	5.1 (-7.8, 19.7)	1.5 (-12.6, 17.7)
P-trend		0.88	0.56		

[1]Adjusted for total calorie intake, age, BMI, physical activity, education level, smoking status, year of AFC (antral follicle count), Iron, and Vitamin D intake.

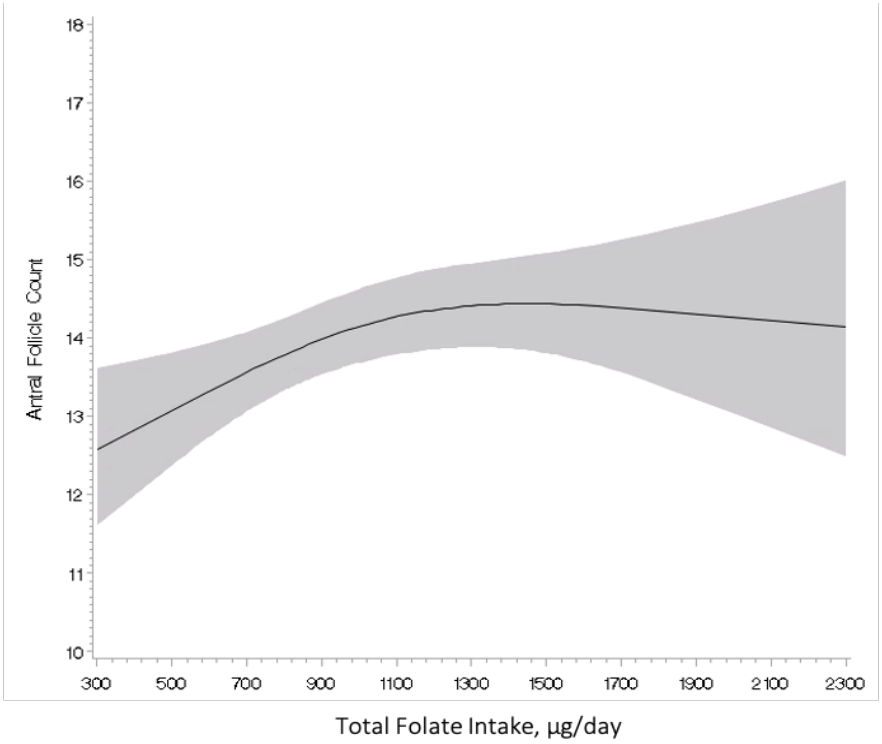
Table 3. Effect modification of the association between folate intake and antral follicle count among 552 women in the EARTH Study.

		% Difference in AFC (95% CI) Quartiles of Total Folate Intake (Range, µg/day)				
	N	Q1 (215.5-742.6)	Q2 (746.7-1003.4)	Q3 (1006.6-1325.6)	Q4 (3140.1-1515.7)	
Age						
<35 yrs	258	REF	1.8 (-11.6, 17.3)	-5.3 (18.9, 10.7)	-5.4 (-21.4, 13.8)	
≥ 35 yrs	294	REF	2.4 (-12.4, 19.7)	8.1 (-9.2, 28.7)	18.6 (-3.4, 45.4)	
P-interaction						0.56
BMI						
<25 kg/m^2	373	REF	0.4 (-12.1, 14.7)	0.0 (-12.5, 14.4)	6.1 (-10.6, 25.8)	
≥ 25 kg/m^2	179	REF	11.2 (-7.4, 33.6)	5.1 (-15.8, 31.1)	7.8 (-14.6, 35.9)	
P-interaction						0.85
Smoking Status						
Never smoker	405	REF	8.5 (-4.1, 22.6)	-3.8 (-15.8, 9.9)	5.3 (-9.0, 21.9)	
Ever smoker	147	REF	-7.2 (-26.9, 17.8)	18.2 (-9.4, 54.3)	11.5 (-20.9, 56.9)	
P-interaction						0.13
Infertility Diagnosis						
Female	157	REF	-2.3 (-21.5, 21.8)	13.2 (-14.3, 49.5)	16.6 (-12.6, 55.5)	
Male & Unexplained	391	REF	2.5 (-9.4, 15.9)	-3.8 (-15.0, 8.9)	-2.2 (-15.5, 13.1)	
P-interaction						0.35

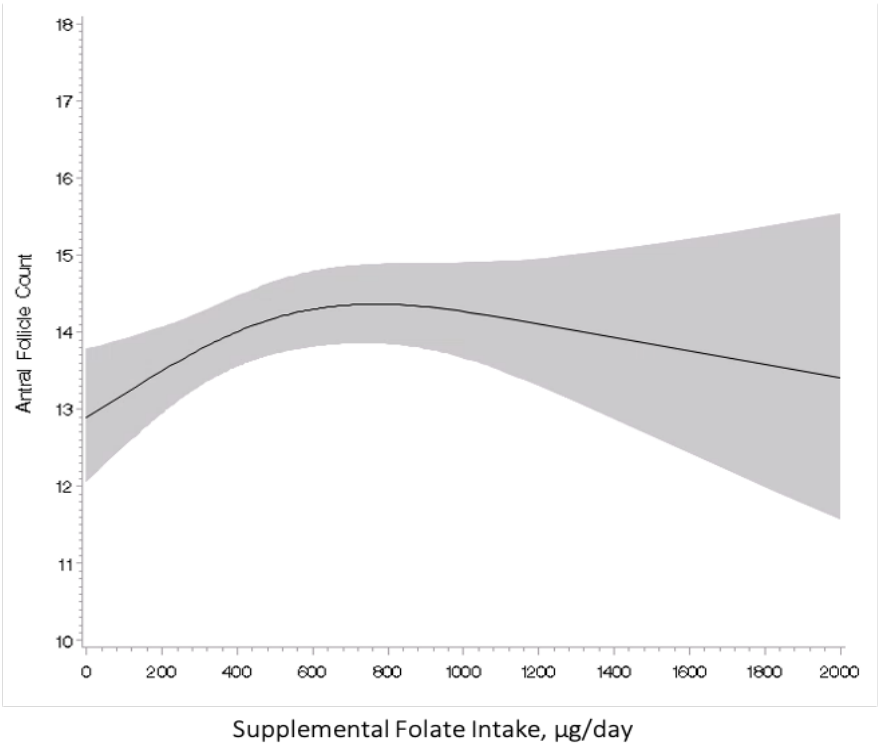
[1]Adjusted for total calorie intake, age, BMI, physical activity, education level, smoking status, year of AFC (antral follicle count), vitamin B12, iron, and vitamin D intake.

Figure 1. Associations between total folate intake (panel A), supplemental folate intake (panel B), and food folate (panel C) and antral follicle count fit using restricted cubic splines among 552 women in the EARTH Study.

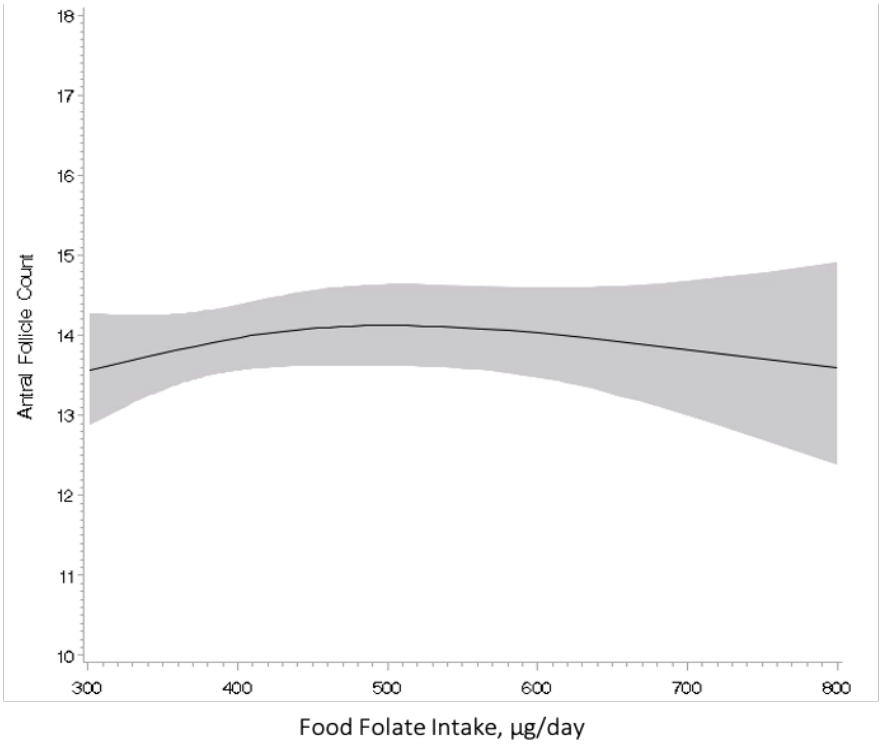
A.



B.



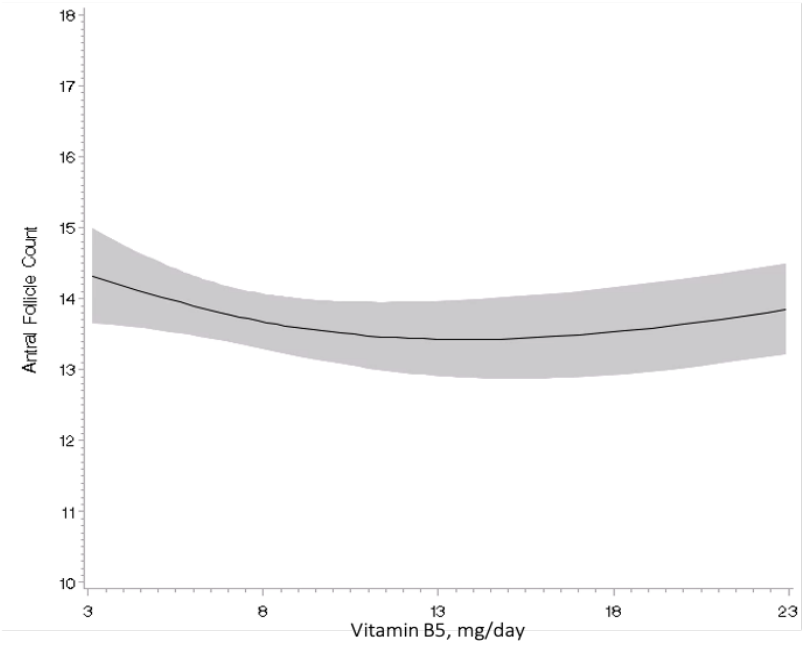
C.



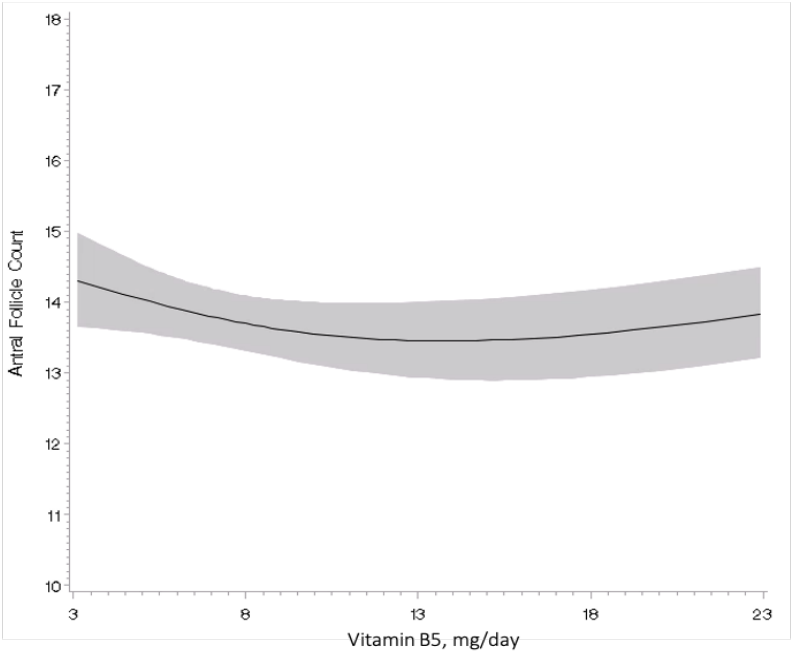
The solid line represents the adjusted mean AFC and the shaded grey area represents the 95% confidence interval. The models are adjusted for total calorie intake, age, BMI, physical activity, education level, smoking status, year of AFC (antral follicle count), vitamin B12, iron, and vitamin D intake. The p-value for non-linearity was 0.05 for total folate, 0.02 for supplemental folate, and 0.23 for food folate. For all figures, the x-axis extends from the 5th to 95th percentile of the intake distribution for that nutrient.

Supplemental Figure 1. Associations between B5 intake and antral follicle count fit using restricted cubic splines among 552 women in the EARTH Study after multivariable adjustment (Panel A) and with further adjustment for total folate intake (Panel B).

A.



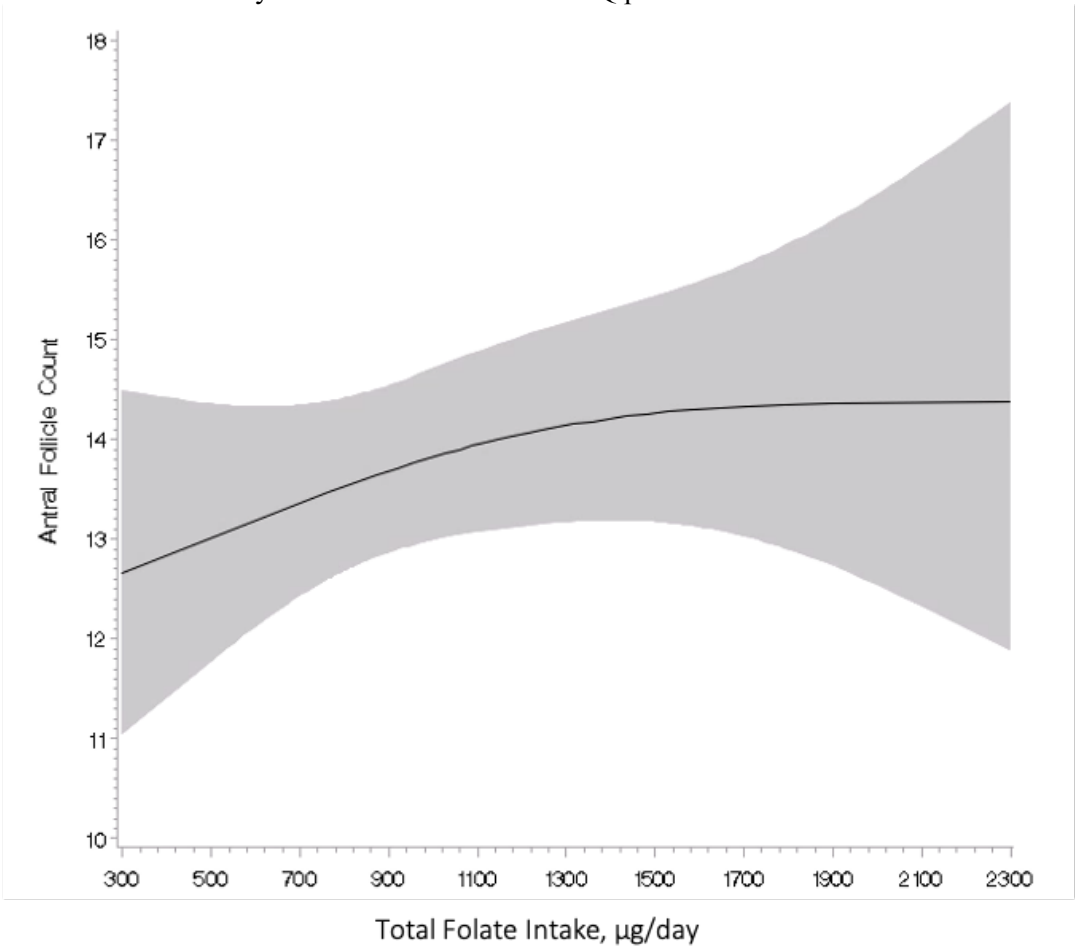
B.



The solid line represents the adjusted mean AFC and shaded grey area represents the 95% confidence interval. Model A is adjusted for total calorie intake, age, BMI, physical activity, education level, smoking status, year of AFC (antral follicle count), vitamin B12, iron, and vitamin D intake. Model B adjusts for the same variables in Model A and folate intake. The p-value for non-linearity was 0.003 for Model A and 0.0006 for Model B; however, after excluding outlying B5 intakes above the 95th percentile,

this non-linear relationship was no longer statistically significant. The x-axis extends from the 5th to 95th percentile of vitamin B5 intake.

Supplemental Figure 2. Association between total folate intake and antral follicle count among the among the 188 EARTH Study women who filled out a FFQ prior to their antral follicle scan.



The solid line represents the adjusted mean AFC and the shaded grey area represents the 95% confidence interval. The models are adjusted for total calorie intake, age, BMI, physical activity, education level, smoking status, year of AFC (antral follicle count), vitamin B12, iron, and vitamin D intake. The p-value for non-linearity was 0.46. The x-axis extends from the 5th to 95th percentile of the intake distribution for that nutrient.