Use of multivitamins, intake of B vitamins, and risk of ovulatory infertility

Jorge E. Chavarro, M.D., Sc.D.,[a] *Janet W. Rich-Edwards, Sc.D., M.P.H.,*[b,c,d] *Bernard A. Rosner, Ph.D.,*[d,e] *and Walter C. Willett, M.D., Dr.P.H.*[a,c,d]

[a] Department of Nutrition, [c] Department of Epidemiology, and [e] Department of Biostatistics, Harvard School of Public Health, Boston; [b] Connors Center for Women's Health and Gender Biology, and [d] Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

Objective: To examine whether use of multivitamins and intake of specific nutrients in multivitamins is associated with ovulatory infertility.
Design: A prospective cohort study.
Setting: The Nurses' Health Study II.
Patient(s): Eighteen thousand five hundred fifty-five married, premenopausal women without a history of infertility who attempted a pregnancy or became pregnant between 1991 and 1999.
Intervention(s): None, observational study.
Main Outcome Measure(s): Incident reports of infertility caused by anovulation.
Result(s): During 8 years of follow-up, 438 women reported infertility caused by ovulatory disorder. There was an inverse association between frequency of multivitamin use and ovulatory infertility. The multivariate-adjusted relative risk (95% confidence interval) of ovulatory infertility was 0.88 (0.60, 1.28) for women consuming two tablets per week or less, 0.69 (0.51, 0.95) for women consuming three to five tablets per week, and 0.59 (0.46, 0.75) for women consuming six or more tablets per week, when compared with women who did not use these supplements (*P*, trend $<$.001). Folic acid appeared to explain part of the association between multivitamin supplement use and risk of ovulatory infertility.
Conclusion(s): Regular use of multivitamin supplements may decrease the risk of ovulatory infertility. (Fertil Steril® 2008;89:668–76. ©2008 by American Society for Reproductive Medicine.)

Key Words: Infertility, ovulation, multivitamins, folic acid, epidemiology, reproductive medicine

Infertility is a common condition, affecting as many as one of six couples during their lifetimes (1). Some investigators have proposed that the already high frequency of this disorder is likely to rise as the postponement of childbearing increases, particularly in developed regions of the world (2, 3). Assisted reproductive technologies are the main strategy used to control the burden of infertility. However, their large costs (4, 5) and frequency of adverse events (6–10) warrant the consideration of alternative approaches to control infertility including prevention.

The role of dietary factors in human fertility has not been investigated in detail, but intake of some micronutrients may enhance female fertility. Studies published elsewhere have documented higher pregnancy rates among users of micronutrient supplements who either have (11) or do not have (12) fertility disorders. Although these studies could not identify specific nutrients or mechanisms explaining the beneficial effect of these supplements, recent findings suggest that folate status may be important in the ovarian response to FSH (13). Therefore, we evaluated whether use of multivitamin supplements was associated with the incidence of ovulatory disorder infertility and explored which nutrients could explain the association if it exists.

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Reprint requests: Jorge E. Chavarro, M.D., Sc.D., Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115 (FAX: 617-432-2435; E-mail: jchavarr@hsph.harvard.edu).

MATERIALS AND METHODS

Study Population

The Nurses' Health Study II is a prospective cohort study that is designed to investigate the role of diet and other lifestyle factors in common chronic diseases. In 1989, 116,671 female registered nurses aged 24 to 42 years were enrolled in the study, and they have been followed every 2 years since then with mailed questionnaires. Here we present a prospective analysis of incident ovulatory infertility among participants of this cohort. The study was approved by the institutional review board of Brigham and Women's Hospital.

Follow-up for the current study started in 1991, when diet was first measured, and was concluded in 1999. On biennial questionnaires, participants were asked whether they had

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tried without success to become pregnant for >1 year since the previous questionnaire administration and to indicate whether their inability to conceive was caused by tubal blockage, ovulatory disorder, endometriosis, cervical mucus factor, or spousal factor; was not found; was not investigated; or was caused by another reason. In a validation study among members of this cohort who were not included in the present analyses, self-reported diagnosis of ovulatory infertility was confirmed by review of medical records in 95% of the cases (14). Self-reports of infertility of women in the current analysis were not confirmed by review of medical records. Women also were asked whether they became pregnant during the preceding 2-year period, including pregnancies resulting in miscarriages or induced abortions. By using this information, we simulated a cohort of women who were attempting to become pregnant. Only married women, with available dietary information and without a history of infertility, were eligible to enter the analysis. These women contributed information to the analysis during each 2-year period in which they reported a pregnancy or a failed pregnancy attempt, and they were followed until they reported an infertility event from any cause, reached menopause, or underwent a sterilization procedure (themselves or their partner), whichever came first. Only 10 diabetic women met these criteria. Because insulin resistance and hyperinsulinemia, hallmark characteristics of type 2 diabetes, may affect ovulatory function (15), we excluded these 10 women from the analysis. After exclusions, we identified 18,555 women without a history of infertility who tried to become pregnant or who became pregnant during the 8-year follow-up period. In each 2-year period, women who met these criteria and who reported infertility caused by ovulatory disorder were considered cases, and the remaining women were considered noncases.

Dietary information was collected in 1991 and 1995 by using a food-frequency questionnaire validated elsewhere (16–18). Participants were asked to report how often, on average during the previous year, they had consumed each of the foods and beverages that were included in the food-frequency questionnaire. The questionnaire offered nine options for frequency of intake, ranging from never or less than once per month to six or more times per day. Participants also were asked whether they used multivitamin supplements and other nutrient supplements. Multivitamin users were asked to specify the brand of the multivitamin and their frequency of use. Women also were asked to report the use of several commonly used supplements of specific vitamins; those reporting use were asked to specify the daily dose. Nutrient intakes were estimated by summing the nutrient contribution of all food items in the questionnaire, taking into consideration the brand, type, and dose of dietary supplements used. The nutrient content of each food and specified portion size was obtained from a nutrient database that was derived from the US Department of Agriculture (19) and from additional information that was obtained from food manufacturers. To reduce extraneous variation in nutrient intakes, nutrient intakes were adjusted for total energy intake by using the nutrient residual method (20).

Statistical Analyses

The relative risk (RR; calculated as an odds ratio) of ovulatory infertility according to categories of multivitamin supplement use and intake of B vitamins was estimated by using logistic regression. The generalized estimating equation approach (21), with an exchangeable working correlation structure, was used to account for the within-person correlation in outcomes at different time periods. Women were initially divided into users and nonusers of multivitamins according to their most recent dietary assessment. Multivitamin users were further divided into four categories according to their frequency of use: two or less tablets per week, three to five tablets per week, six or more tablets per week, and no frequency of use provided. The RR of ovulatory infertility was computed as the risk among women who were at a specific level of supplement use, divided by the risk among nonusers. Women also were divided into five groups according to quintiles of intake of individual B vitamins (B_1, B_2, B_6, B_{12}, folic acid, niacin, and pantothenic acid). In these models, the RR was computed as the risk of infertility in a specific quintile of cumulative averaged intake (22), compared with the risk in the lowest quintile. Tests for linear trend were conducted by using the median values of intake in each category as a continuous variable.

To control for confounding by age and to take into account potential time trends in infertility (23), all models were adjusted for age in years at the beginning of each mailing cycle and calendar time of the current questionnaire cycle. Multivariate models included additional terms for body mass index, parity, smoking history, physical activity, history of oral contraceptive use, dietary factors found to be related to infertility in preliminary analyses (intakes of alcohol, coffee, protein and major types of fatty acids), and total energy intake. Multivariate models for intake of B vitamins were adjusted further for iron intake. The values of the dietary and nondietary variables were updated as new data became available.

We performed additional analyses to assess which components of multivitamins were responsible for the association between this supplement and ovulatory infertility. First, we identified the nutrients for which multivitamins are the main source in this population and introduced them, one at a time, into the multivariate models including multivitamins. Then, we examined the association between long-term intake of B vitamins and ovulatory infertility, with and without adjustment for multivitamin use, and among nonusers of multivitamins.

We examined whether the association between the use of multivitamin supplements and ovulatory infertility was modified by subject characteristics (age, parity, and body mass index), the presence of long menstrual cycles ($\geq$40 d), alcohol intake, or use of iron supplements by introducing cross-product terms between use of multivitamin supplements and the variable of interest.

The population-attributable risk and its 95% confidence interval (CI) (24) were used to estimate the proportion of

ovulatory infertility cases within this cohort that could have been avoided, had all women consumed multivitamins at certain frequencies, assuming that the association between multivitamins and ovulatory infertility is causal. Analyses were performed in SAS, version 9.1 (SAS Institute, Cary, NC).

RESULTS

During 8 years of follow-up, 26,971 eligible pregnancies and pregnancy attempts were accrued among 18,555 women. Of these events, 3,430 were incident reports of infertility from any cause, of which 2,165 were of women reporting at least one diagnosis for infertility and 438 (20% of women that reported a specific diagnosis) were incident reports of ovulatory infertility. Women reporting ovulatory infertility were more than four times more likely to report long and irregular menstrual cycles or clinical signs of excess androgens when compared with women reporting infertility from other causes (OR [95% CI] = 4.15 [2.98–5.76]) or when compared with women who became pregnant during follow-up (OR [95% CI] = 4.43 [3.35–5.86]).

At baseline, women differed on several characteristics on the basis of their use of multivitamin supplements (Table 1). Multivitamin users tended to consume less alcohol and coffee, to smoke less, and to be more physically active than nonusers. Also, multivitamin users were less likely to be users of hormonal contraception or intrauterine devices at the beginning of the first 2-year period during which they reported an eligible event. Frequency of multivitamin use was strongly correlated with the total intake (diet plus supplements) of specific B vitamins. The Spearman correlation coefficients between intake of B vitamins and multivitamin use frequency were 0.79 for B_1, 0.78 for B_2, 0.77 for B_6, 0.67 for B_{12}, 0.81 for folic acid, 0.76 for niacin, and 0.58 for pantothenic acid.

In analyses that were adjusted for age and calendar time, use of multivitamin supplements was associated with a decreased risk of ovulatory infertility in a dose-dependent manner (Table 2). Multivitamin users had approximately one third lower risk of developing ovulatory infertility when compared with nonusers ($P<.001$). When multivitamin users were grouped by their frequency of use, those consuming no more than two multivitamin tablets per week did not have a significantly different risk compared with women who did not use multivitamins, whereas women who consumed three or more tablets per week had a significantly reduced risk of ovulatory infertility. In addition, there was a linear trend toward decreased ovulatory infertility risk with increasing frequency of multivitamin supplement use. We considered multiple known and suspected risk factors for infertility to be potential confounders for the observed association. Simultaneously adjusting for these factors changed the age-adjusted results minimally (Table 2). We estimate that 20% of ovulatory infertility cases could be avoided if women consumed three or more multivitamins per week (population-attributable risk [95% CI] = 20% [11%–28%]), assuming this association is causal.

TABLE 1

Baseline characteristics[a] of users and nonusers of multivitamin supplements.

Characteristic	Multivitamins: Users (n = 10,451)	Multivitamins: Nonusers (n = 8,104)
Age (y)	32.5	32.6
Alcohol intake (g/d)	2.7	3.1
Coffee intake of ≥2 cups per d (%)	21	28
Current smoker (%)	6	9
Body mass index (kg/m^2)	23.9	23.9
Physical activity (METs per wk)	22	20
Cycles ≥40 d (%)	3	3
Hyperandrogenism (%)	0.3	0.3
Nulliparous (%)	23	24
Contraceptive use[b] (%)		
Oral contraceptives	12	22
Other hormonal contraceptive[c]	0.1	0.3
Intrauterine devices	1	1.6
Two or more contraceptives	12	12

Note: MET = metabolic equivalents per hour.

[a] Baseline refers to the year of entry into the study for each individual. Values are presented as age-standardized means and proportions with the exception of values for age.

[b] Two years before the first event was reported.

[c] Injectable or implantable progestins.

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Because multivitamin use was associated with lower use of contraception, and multivitamin use may be higher among women who are planning a pregnancy, we performed two sensitivity analyses. We first restricted the analysis to noncontracepting women and then conducted a case–control analysis, using as controls women who were diagnosed with infertility from other causes. Among women who were not using any type of contraception, the multivariate-adjusted RR and 95% CI, comparing all users of multivitamins with nonusers, was 0.46 (0.35, 0.61). Among these women, there also was a linear trend toward decreasing ovulatory infertility with increasing frequency of multivitamin use (Table 3). The results were similar when the analyses were restricted to women not using specific types of contraception. In the case–control analysis, the multivariate-adjusted RR (95% CI) of ovulatory infertility comparing all users of multivitamins with nonusers was 0.77 (0.62, 0.95), and there was a linear trend toward decreased risk of ovulatory infertility with increased frequency of multivitamin use (P, trend = .04).

TABLE 2

Relative risks and 95% CIs for ovulatory infertility by category of multivitamin supplement use.

Parameter	Cases/noncases[a]	Age-adjusted[b] RR (95% CI)	Multivariate-adjusted[c] RR (95% CI)
Multivitamin use			
Nonusers	224/10,926	1.00 (referent)	1.00 (referent)
Users	214/15,607	0.67 (0.55, 0.80)	0.65 (0.53, 0.80)
Frequency of use			
Nonusers	224/10,926	1.00 (referent)	1.00 (referent)
≤2 tablets per wk	32/1,808	0.84 (0.58, 1.23)	0.88 (0.60, 1.28)
3–5 tablets per wk	52/3,796	0.66 (0.49, 0.90)	0.69 (0.51, 0.95)
≥6 tablets per wk	127/9,783	0.63 (0.51, 0.79)	0.59 (0.46, 0.75)
P trend[d]		<.0001	<.001

[a] Two hundred twenty-three multivitamin users (3 cases and 220 noncases) did not provide information about the frequency of multivitamin use.

[b] Adjusted for age (continuous) and calendar time (four 2-y intervals).

[c] Age-adjusted model further adjusted for total energy intake (continuous); body mass index (<20, 20–24.9, 25–29.9, ≥30, and missing); parity (0, 1, ≥2, and missing); smoking history (never, past 1–4 cigarettes per d, past 5–14 cigarettes per d, past 15–24 cigarettes per d, past ≥ 25 cigarettes per d or unknown amount, current 1–4 cigarettes per d, current 5–14 cigarettes per d, current 15–24 cigarettes per d, and current ≥ 25 cigarettes per d or unknown amount); physical activity (<3 metabolic equivalent [MET]-h/wk, 3–8.9 MET-h/wk, 9–17.9 MET-h/wk, 18–26.9 MET-h/wk, 27–41.9 MET-h/wk, ≥42 MET-h/wk, and missing); contraceptive use (current user, never user, past user 0–23 mo ago, past user 24–47 mo ago, past user 48–71 mo ago, past user 72–95 mo ago, past user 96–119 mo ago, past user ≥120 mo ago, and missing); and intakes of alcohol (no intake, <2 g/d, 2–4.9 g/d, and ≥5 g/d), coffee (<1 serving per mo, 1 serving per mo, 2–6 servings per wk, 1 serving per d, 2–3 servings per d, ≥4 servings per d), major types of fatty acids, and protein.

[d] Calculated by using the median frequency of use in each category as a continuous variable.

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Because multivitamins are the most important contributor to the intake of numerous micronutrients in the study population, we evaluated which of these nutrients might be responsible for the observed association. First, we added one at a time to the total intake (diet and supplements) of each of these nutrients (iron, magnesium, zinc, copper, manganese, folic acid, niacin, pantothenic acid, retinol, and vitamins B_1, B_2, B_6, B_{12}, C, D, and E) to the final multivariate-adjusted models for multivitamin supplements among the entire study population and observed whether their inclusion attenuated the association between multivitamins and ovulatory infertility. In the models comparing multivitamin nonusers with users according to frequency of use, adding intakes of folic acid, iron, vitamins B_1, B_2, and D attenuated the association between multivitamins and ovulatory infertility. The multivariate-adjusted RRs (95% CIs; *P*, trend) comparing women using six or more multivitamins per week with nonusers were 0.59 (0.46, 0.75; <.001) in the initial model without added nutrients, 0.74 (0.54, 1.00; .05) after adjusting for iron intake, 0.70 (0.48, 1.02; .08) after adjusting for vitamin D intake, 0.70 (0.44, 1.09; .11) after adjusting for vitamin B_1 intake, 0.68 (0.45, 1.03; .08) after adjusting for vitamin B_2 intake, and 0.88 (0.58, 1.32; .59) after adjusting for folic acid intake. Adjustment for the remaining micronutrients considered did not affect the association (data not shown).

Because the previous analysis suggested that some B vitamins may mediate the association between multivitamin supplements and ovulatory infertility, we then examined whether long-term intake of individual B vitamins was associated with the risk of developing ovulatory infertility. In age- and energy-adjusted analyses, intakes of vitamins B_1, B_2, B_6, B_{12}, folic acid, and niacin were inversely related to the risk of ovulatory infertility, whereas the intake of pantothenic acid was unrelated to ovulatory infertility (Table 4). Adjustment for known and suspected risk factors for infertility, particularly adjustment for iron intake, attenuated these associations. After adjustment, only intake of folic acid was associated with a reduced risk of ovulatory infertility (Table 4). When this analysis was restricted to non-contracepting women, there was a strong inverse association between folic acid intake and ovulatory infertility (*P*, trend =.007; Fig. 1). Additional adjustment for multivitamin supplement use attenuated the association between folic acid intake and risk of ovulatory infertility in the entire study population. In this model, the RRs (95% CI) of ovulatory infertility for women in successively higher quintiles of folic acid intake were 0.83 (0.61, 1.13), 0.90 (0.61, 1.31), 0.63 (0.39, 1.02), and 0.64 (0.36, 1.14), compared with women in the lowest quintile of folic acid intake (*P*, trend =.17). A similar pattern was observed when folic acid intake was examined among nonusers of multivitamin supplements only. The corresponding multivariate-

TABLE 3

Multivariate-adjusted RRs of ovulatory infertility by frequency of multivitamins among nonusers of contraception.

Subgroup	Frequency of multivitamin use			
	Never	≤2 per wk	3–5 per wk	≥6 per wk
All participants				
Cases/noncases	224/10,926	32/1,808	52/3,796	127/9,783
RR (95% CI)	1.00 (referent)	0.88 (0.60, 1.28)	0.69 (0.51, 0.95)	0.59 (0.46, 0.75)
Not using any contraceptive method				
Cases/noncases	128/3,872	16/667	25/1,548	71/4,997
RR (95% CI)	1.00 (referent)	0.72 (0.42, 1.24)	0.50 (0.32, 0.78)	0.41 (0.29, 0.56)
Not using hormonal contraception[a]				
Cases/noncases	184/8,760	25/1,524	49/3,251	113/8,822
RR (95% CI)	1.00 (referent)	0.80 (0.52, 1.22)	0.75 (0.54, 1.04)	0.57 (0.44, 0.73)
Not using barrier methods[b]				
Cases/noncases	172/6,355	23/998	29/2,201	88/6,190
Relative risk (95% CI)	1.00 (referent)	0.82 (0.53, 1.29)	0.49 (0.33, 0.73)	0.49 (0.37, 0.65)

Note: P values were calculated by using the median frequency of use in each category as a continuous variable. *P* values for each subgroup were <.001 across all frequencies. Relative risks were adjusted for age (continuous); calendar time (four 2-y intervals); total energy intake (continuous); body mass index (<20, 20–24.9, 25–29.9, ≥30, and missing); parity (0, 1, ≥2, and missing); smoking history (never, past 1–4 cigarettes per d, past 5–14 cigarettes per d, past 15–24 cigarettes per d, past ≥ 25 cigarettes per d or unknown amount, current 1–4 cigarettes per d, current 5–14 cigarettes per d, current 15–24 cigarettes per d, and current ≥25 cigarettes per d or unknown amount); physical activity (<3 metabolic equivalent [MET]-h per wk, 3–8.9 MET-h per wk, 9–17.9 MET-h per wk, 18–26.9 MET-h per wk, 27–41.9 MET-h per wk, ≥42 MET-h per wk, and missing); and intakes of alcohol (no intake, <2 g/d, 2–4.9 g/d, ≥5 g/d), coffee (<1 serving per mo, 1 serving per mo, 2–6 servings per wk, 1 serving per d, 2–3 servings per d, ≥4 servings per d), major types of fatty acids, and protein. The model including all participants regardless of recent contraception also includes terms for recency of contraceptive use (current user, never user, past user 0–23 mo ago, past user 24–47 mo ago, past user 48–71 mo ago, past user 72–95 mo ago, past user 96–119 mo ago, past user ≥120 mo ago, and missing).

[a] Oral contraceptives and injected or implantable progestins.

[b] Condoms, diaphragms, or cervical caps.

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adjusted RRs (95% CIs) among non-multivitamin users were 0.88 (0.57, 1.36), 1.06 (0.68, 1.65), 0.78 (0.48, 1.27), and 0.83 (0.49, 1.40) in relation to women not using multivitamins in the lowest quintile of intake (*P*, trend = .43).

We evaluated the possibility that personal characteristics or other dietary behaviors may modify the association between use of multiple vitamin supplements and ovulatory infertility. We found no evidence of interaction between use of multivitamin supplements and age (*P*=.18) and presence of long menstrual cycles (*P*=.56). Similarly, the association between multivitamins and ovulatory infertility did not differ significantly between nulliparous and parous women (*P*=.50), alcohol drinkers and nondrinkers (*P*=.17), users and nonusers of iron supplements (*P*=.83), or lean and overweight or obese women (*P*=.46).

DISCUSSION

We examined the association between use of multivitamin supplements and risk of ovulatory infertility and found that using these supplements at least three times per week was associated with a reduced risk of ovulatory infertility. Our results suggest that B vitamins, particularly folic acid, explain some of the association between multivitamin supplements and ovulatory infertility.

Only two studies published elsewhere have evaluated whether supplements containing multiple micronutrients may have an impact on fertility (11, 12). The first study was a double-blind, randomized, controlled trial that was designed to evaluate the efficacy of folic acid–containing multivitamin–multimineral supplement in reducing the occurrence of neural tube defects and other congenital malformations as part of a Hungarian family-planning program. More than 7,900 women without a history of infertility entered the trial. A secondary analysis of this trial revealed that after 1 year, 71% of the women assigned to the multivitamin–multimineral arm of the trial became pregnant, whereas 68% of the women in the placebo arm became pregnant (*P*<.01) (12). The second study was a 3-month controlled pilot trial that was conducted among 30 women who had not been able to become pregnant after 6 to 36 months of unprotected intercourse. During the trial, four women in its

TABLE 4

Relative risks (95% confidence intervals) for ovulatory infertility by total intake quintiles of B vitamins.

Nutrient	Quintile of total intake (diet and supplements) 1	2	3	4	5	*P* trend[a]
Thiamin (B_1)						
Median intake (mg/d)	1.3	1.7	2.3	3.1	4.8	
Cases/noncases	117/5,239	96/5,330	83/5,316	64/5,345	78/5,303	
Age and energy adjusted[b]	1.00 (referent)	0.87 (0.67, 1.15)	0.76 (0.57, 1.02)	0.58 (0.43, 0.80)	0.67 (0.50, 0.90)	.005
Multivariate adjusted[c]	1.00 (referent)	0.93 (0.70, 1.26)	0.87 (0.62, 1.21)	0.77 (0.52, 1.14)	0.84 (0.58, 1.22)	.44
Riboflavin (B_2)						
Median intake (mg/d)	1.6	2.1	2.9	3.9	5.8	
Cases/noncases	104/5,291	104/5,317	88/5,280	72/5,304	70/5,341	
Age and energy adjusted[b]	1.00 (referent)	1.07 (0.81, 1.42)	0.92 (0.68, 1.23)	0.74 (0.55, 1.01)	0.69 (0.50, 0.93)	.002
Multivariate adjusted[c]	1.00 (referent)	1.07 (0.80, 1.44)	1.02 (0.73, 1.43)	0.99 (0.67, 1.46)	0.86 (0.58, 1.28)	.32
Vitamin B_6						
Median intake (mg/d)	1.8	2.3	3.3	4.7	11.4	
Cases/noncases	99/5,350	106/5,151	95/5,418	75/5,249	63/5,365	
Age and energy adjusted[b]	1.00 (referent)	1.17 (0.89, 1.54)	1.03 (0.77, 1.37)	0.81 (0.60, 1.09)	0.66 (0.47, 0.90)	.001
Multivariate adjusted[c]	1.00 (referent)	1.06 (0.79, 1.45)	0.96 (0.68, 1.34)	0.89 (0.60, 1.32)	0.78 (0.51, 1.18)	.15
Vitamin B_{12}						
Median intake (μg/d)	4	6	9	12	18	
Cases/noncases	101/5,624	112/5,203	94/5,679	64/5,024	67/5,003	
Age and energy adjusted[b]	1.00 (referent)	1.27 (0.96, 1.67)	0.98 (0.71, 1.31)	0.74 (0.54, 1.01)	0.76 (0.55, 1.03)	.002
Multivariate adjusted[c]	1.00 (referent)	1.34 (1.01, 1.77)	1.07 (0.79, 1.45)	0.88 (0.61, 1.26)	0.94 (0.65, 1.37)	.24
Folic acid						
Median intake (μg/d)	243	337	495	726	1,138	
Cases/noncases	113/5,310	99/5,254	101/5,300	68/5,332	57/5,337	
Age and energy adjusted[b]	1.00 (referent)	0.91 (0.69, 1.20)	0.94 (0.71, 1.24)	0.62 (0.45, 0.84)	0.51 (0.37, 0.71)	$<$.001
Multivariate adjusted[c]	1.00 (referent)	0.82 (0.60, 1.11)	0.85 (0.61, 1.18)	0.60 (0.40, 0.89)	0.61 (0.37, 1.00)	.04
Niacin						
Median intake (mg/d)	20	25	32	40	51	
Cases/noncases	98/5,276	108/5,309	78/5,287	68/5,373	86/5,288	
Age and energy adjusted[b]	1.00 (referent)	1.15 (0.87, 1.51)	0.86 (0.64, 1.16)	0.74 (0.54, 1.02)	0.86 (0.64, 1.16)	.05
Multivariate adjusted[c]	1.00 (referent)	1.10 (0.81, 1.48)	0.89 (0.64, 1.25)	0.87 (0.60, 1.30)	0.91 (0.63, 1.31)	.39
Pantothenic acid						
Median intake (μg/d)	4.0	5.0	6.0	10.6	17.1	
Cases/noncases	96/5,375	92/5,263	75/5,283	86/5,340	89/5,272	

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TABLE 4

Continued.

Nutrient	Quintile of total intake (diet and supplements)					*P* trend[a]
	1	2	3	4	5	
Age and energy adjusted[b]	1.00 (referent)	1.05 (0.78, 1.41)	0.89 (0.65, 1.22)	0.99 (0.73, 1.35)	0.96 (0.71, 1.28)	.79
Multivariate adjusted[c]	1.00 (referent)	0.98 (0.71, 1.33)	0.77 (0.55, 1.09)	0.91 (0.65, 1.28)	0.89 (0.64, 1.25)	.85

[a] Calculated by using median nutrient intake in each quintile as a continuous variable.

[b] Adjusted for age (continuous), calendar time (four 2-y intervals), and total energy intake (continuous).

[c] Age and energy-adjusted model further adjusted for body mass index (<20, 20–24.9, 25–29.9, ≥30, and missing); parity (0, 1, ≥2, and missing); smoking history (never, past 1–4 cigarettes per d, past 5–14 cigarettes per d, past 15–24 cigarettes per d, past ≥25 cigarettes per d or unknown amount, current 1–4 cigarettes per d, current 5–14 cigarettes per d, current 15–24 cigarettes per d, and current ≥25 cigarettes per d or unknown amount); physical activity (<3 metabolic equivalent [MET]-h/wk, 3–8.9 MET-h/wk, 9–17.9 MET-h/wk, 18–26.9 MET-h/wk, 27–41.9 MET-h/wk, ≥42 MET-h/wk, and missing); contraceptive use (current user, never user, past user 0–23 mo ago, past user 24–47 mo ago, past user 48–71 mo ago, past user 72–95 mo ago, past user 96–119 mo ago, past user ≥120 mo ago, and missing); and intake of alcohol (no intake, <2 g/d, 2–4.9 g/d, ≥5 g/d), coffee (<1 serving per mo, 1 serving per mo, 2–6 servings per wk, 1 serving per d, 2–3 servings per d, ≥4 servings per d), major types of fatty acids, protein, and iron (quintiles).

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FIGURE 1

Multivariate-adjusted (see Table 4, footnote c) relative risk of ovulatory infertility by total intake of folic acid among noncontracepting women (women who did not report recent use of hormonal contraception, barrier methods, intrauterine devices, or spermicides).

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supplement arm (27%) and none in its placebo arm became pregnant (11).

An important difference between our study and the previous ones is the type of outcome assessed. Because previous studies have used pregnancies, rather than specific infertility diagnoses, as their outcome, it is possible that the effects observed by those investigators were not a result of improved ovulatory function. Nevertheless, analyses from the Hungarian trial found that multivitamin supplementation improved menstrual cycle regularity (25), suggesting that a decrease in the frequency of ovulatory problems may account for the beneficial effects of multivitamins. Our findings are in agreement with the previous two studies, although the magnitude of the association across studies varies widely. It is possible that the difference in magnitude between our results and those from the Hungarian trial stem from a dilution of the actual multivitamin effect in the trial as a result of not assessing specific outcomes that are likely to be influenced by multivitamins, but rather a composite of infertility types that were influenced and not influenced by the intervention. However, the difference in magnitude between our study and the smaller trial may reflect the small number of outcomes observed because of the limited size of the study.

Our data suggest that folic acid may be responsible for part of the association between multivitamin use and ovulatory infertility. Although, to our knowledge, there have not been other studies of folic acid intake and risk of infertility in general or risk of ovulatory infertility in particular, our findings are in agreement with previous clinical observations and

animal studies. In women undergoing controlled ovarian hyperstimulation with recombinant FSH, carriers of the T allele in position 677 of the MTHFR gene (which leads to decreased enzyme activity and 5-methyltetrahydrofolate concentrations) have a decreased ovarian responsiveness to this hormone (13). Although currently unknown, it is possible that ovarian response to endogenous FSH pulses also is decreased in low folate conditions, which can be overcome by greater intake of folic acid. Our findings are in agreement with this hypothesis. Folic acid supplementation has been found to increase litter size in pigs (26). In addition, experimentally induced folate deficiency has resulted in decreased ovulation in rats (27).

Our study has some limitations that need to be considered in the interpretation of our results. First, in reconstructing this cohort, we assumed that pregnancies occurring among married women were planned. Cases, who were clearly attempting to conceive, may have been more health conscious than some pregnancy noncases, who may have conceived accidentally. However, this situation would have resulted in a positive association between multivitamin use, or any other health-conscious behavior, and ovulatory infertility, rather than in the strong inverse association we observed. In addition, we restricted the study to married women, whose pregnancies are more likely to be intentional than those of unmarried women (28), and included in the noncase group women who had been diagnosed with infertility from other causes, making it less likely that pregnancy intention affected our findings. Also, the association between multivitamins and ovulatory infertility persisted when we restricted our analyses to women who had not recently used contraceptives and when women with ovulatory infertility were compared with infertile women who received other diagnoses, supporting the notion that pregnancy intention did not have an important impact in this study. Second, because this was an observational study, we cannot completely rule out the possibility that our findings may be a result in part of unmeasured characteristics associated with both ovulatory infertility and use of multivitamin supplements. Nevertheless, we considered the potential confounding effects of many variables meeting these characteristics, as well as of recognized risk factors for infertility, and found that statistical adjustment for these variables had minimal impact on our results. Last, because of the high correlation between multivitamin supplement use and folic acid intake, it is difficult to distinguish their independent effects. This issue would be better addressed by larger studies or by studying populations with a wider range of folic acid intake from sources other than multivitamins.

In conclusion, in this prospective study, we found that consuming multivitamin supplements at least three times per week was associated with a reduced risk of ovulatory infertility. This association appeared to be mediated in part by folic acid. Because there are very few studies exploring this relationship, it is desirable that our results are confirmed or refuted, preferably in large randomized trials. However, because supplementation with folic acid by itself or as part of a multivitamin has been shown to reduce the risk of neural tube defects (29, 30) and may prevent other congenital malformations (31), women planning to become pregnant should consider taking a multivitamin because this also may help them to become pregnant.

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