Effect of daily fiber intake on reproductive function: the BioCycle Study[1-3]

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ABSTRACT

Background: High-fiber diets have been associated with decreased breast cancer risk, likely mediated by the effect of fiber on lowering circulating estrogen concentrations. The influence of fiber on aspects of reproduction, which include ovulation, has not been well studied in premenopausal women.

Objective: The objective was to determine if fiber consumption is associated with hormone concentrations and incident anovulation in healthy, regularly menstruating women.

Design: The BioCycle Study was a prospective cohort study conducted from 2004 to 2006 that followed 250 women aged 18–44 y for 2 cycles. Dietary fiber consumption was assessed ≤4 times/cycle by using 24-h recall. Outcomes included concentrations of estradiol, progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), which were measured ≤8 times/cycle, and incident anovulation.

Results: Dietary fiber consumption was inversely associated with hormone concentrations (estradiol, progesterone, LH, and FSH; $P < 0.05$) and positively associated with the risk of anovulation ($P = 0.003$) by using random-effects models with adjustment for total calories, age, race, and vitamin E intake. Each 5-g/d increase in total fiber intake was associated with a 1.78-fold increased risk (95% CI: 1.11, 2.84) of an anovulatory cycle. The adjusted odds ratio of 5 g fruit fiber/d was 3.05 (95% CI: 1.07, 8.71).

Conclusions: These findings suggest that a diet high in fiber is significantly associated with decreased hormone concentrations and a higher probability of anovulation. Further study of the effect of fiber on reproductive health and of the effect of these intakes in reproductive-aged women is warranted. *Am J Clin Nutr* 2009;90:1061–9.

INTRODUCTION

Increased intake of fiber has been promoted due to fiber's favorable association with certain health outcomes. High-fiber diets have been associated with reduced risks of cardiovascular disease (1), stroke (2), diabetes (3), colon cancer (4), and breast cancer (5–8). Current recommendations from the American Heart Association (9), the US Department of Agriculture (10), and the Institute of Medicine (IOM) suggest that individuals should consume 20–35 g fiber/d depending on caloric intake (11). This is in contrast to the average fiber intake in the United States, which is substantially below these recommendations [13.8 g fiber/d for reproductive-aged women (12)]. Several studies have reported inverse associations between fiber intake and estrogen concen-

trations in older women (13–19), presumably because of a decrease of β-glucuronidase activity in feces that results from high fiber consumption and leads to a decreased reabsorption of estrogen in the colon (20).

Although certain beneficial effects of fiber on chronic diseases have been observed, the effect of intake on endogenous hormones and other reproductive factors (eg, anovulation) in younger women has had limited study (21). The influence of fiber intake on reproductive hormone concentrations and anovulation is of particular interest in reproductive-aged women, given the effect of these hormones on conception and pregnancy maintenance (22, 23). The objective of this study was to evaluate the association between dietary fiber consumption and reproductive hormone concentrations and risk of incident anovulation in the BioCycle Study. The hypothesis was that consumption of dietary fiber at or above the Dietary Reference Intake (DRI) would be associated with lower hormone concentrations and a higher risk of incident anovulation.

SUBJECTS AND METHODS

Study design

The BioCycle Study was a prospective cohort study of menstrual cycle function in 259 regularly menstruating, premenopausal, healthy female volunteers, aged 18–44 y, who were recruited from the Western New York region and followed for ≤2 menstrual cycles. Details of the study design are described

elsewhere (24). Exclusion criteria included current use of oral contraceptives, vitamin and mineral supplements, or prescription medications; pregnancy or breastfeeding in the past 6 mo; and recent history of infections or diagnosis of chronic conditions, which included history of menstrual and ovulation disorders and gastrointestinal conditions (eg, Crohn's disease). Women with a self-reported body mass index (BMI; in kg/m^2) of <18 or >35 at baseline were excluded as were women planning to restrict their diet for weight loss or medical reasons. The University at Buffalo Health Sciences Institutional Review Committee approved the study, and all of the participants provided written informed consent.

Participants were followed for 1 ($n = 9$) or 2 ($n = 250$) menstrual cycles with blood samples collected at the following times: on the second day of menstruation; at mid- and late follicular phase; at luteinizing hormone (LH)/follicle-stimulating hormone (FSH) surge and ovulation; and at early, mid-, and late luteal phase (approximately corresponding to days 2, 7, 12, 13, 14, 18, 22, and 27 of a 28-d cycle) in each cycle, with collection dates adjusted for cycle length. Fertility monitors (Clearblue Easy Fertility Monitor; Inverness Medical, Waltham, MA) assisted in the timing of specimen collection. Monitor indications of low, high, and peak fertility were used to time midcycle visits, with peak day and the following 2 d those that approximately represented late follicular, LH surge, and ovulation dates (standardized days 12, 13, and 14). Women began fertility testing on calendar day 6; if by day 14 there was no positive indication on the monitor, a visit was scheduled the following day while the participant continued daily monitor testing for 10 additional days. Women were highly compliant to the study protocol, with 94% of women completing ≥7 clinic visits/cycle.

Dietary assessment

Dietary intake was assessed on the same days as sample collection by using a 24-h dietary recall conducted 4 times/cycle (corresponding to standardized days 2, 7, 14, and 22), for a total of 8 recalls. Dietary intake data were collected and analyzed by using the Nutrition Data System for Research software version 2005 developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN. This program computed the nutrients (ie, dietary fiber), food components (ie, insoluble and soluble), and food sources (ie, vegetable, fruit, and grain) from the 24-h dietary recalls. The majority of women completed 4 dietary recalls/cycle (87%).

Hormone assessment

Reproductive hormone concentrations were measured in serum collected at each cycle visit (8 visits/cycle for 2 cycles), which included estradiol, progesterone, LH, and FSH. Fasting morning blood draws were collected at clinic visits and processed according to standardized protocols. Samples were processed and frozen at −80°C and sent as complete participant cycle batches for hormone analysis (Kaleida Health Center for Laboratory Medicine, Buffalo, NY). Estradiol concentrations were measured in all of the available serum samples by radioimmunoassay. FSH, LH, and progesterone were measured in all of the available serum samples by Specialty Laboratories Inc

(Valencia, CA) by using solid-phase competitive chemiluminescent enzymatic immunoassays on the DPC Immulite 2000 analyzer (Siemens Medical Solutions Diagnostics, Deerfield, IL).

Classification of anovulation

Menstrual cycles were initially classified as anovulatory if the peak progesterone concentration across the cycle was ≤5 ng/mL ($n = 65$) (25, 26). To minimize misclassification, cycles with progesterone concentrations ≤5 ng/mL and an observed serum LH peak on days 22 or 27 of the standardized 28-d cycle were considered ovulatory cycles. On the basis of this algorithm, 42 of the 509 cycles (8.3%) in this study were classified as anovulatory.

Covariate assessment

Anthropometric measures were taken, which included height and weight by using standardized protocols, which were used to determine eligibility with respect to BMI. Participants were asked to complete questionnaires regarding physical activity [International Physical Activity Questionnaire (IPAQ) long form 2002], lifestyle, and health history (27). High, moderate, and low physical activity categories were formed on the basis of standard IPAQ cutoffs. Cycle length was defined as the number of days between menstrual bleeding. Day 1 of the cycle was defined as menstruating by 1600 on that day; the last day of the cycle was the last day before the next onset of bleeding. All of the covariates assessed had a ≥95% response rate.

Statistical analysis

Repeated-measures analysis of variance was used to compare dietary intake by visit for each participant to determine whether intake changed significantly over the cycle. Descriptive statistics were calculated for demographic characteristics, dietary intake, and hormone concentrations. Exact chi-square tests and analysis of variance were used to test for associations between demographic variables and fiber intake. No significant differences were shown in dietary fiber intakes across each cycle. As such, an average daily intake of fiber and other dietary variables was calculated per cycle. In categorizing fiber, consideration was given to levels relative to the DRI. The average daily calorie intake of women in this study was ≈1600 kcal, corresponding to a DRI of 22 g fiber/d in accordance with the recommendation of 14 g fiber/1000 kcal by the IOM (11). From this, we considered ≥22 g fiber/d intake to fulfill the DRI and based creation of the highest category on this cutoff. The additional cutoffs were chosen as 6 g fiber/d (equivalent to ≈1–2 servings of fiber) lower than the adjacent category. Alternative cutoffs were explored to assess sensitivity of results to this categorization, which included quartiles of fiber intake.

Linear mixed models on the log scale of the hormones were used to evaluate the association between hormone concentrations and average fiber intake per cycle (28). These random-intercept models were chosen to account for the variation between baseline concentrations of hormones in individual women and the correlation between cycles of the same women. The linear and nonlinear mixed models make use of all of the available observations and do not require balanced data. For estrogen, models included concentrations throughout the cycle, which

TABLE 1
Characteristics of participants according to Dietary Reference Intake (DRI) categories of fiber consumption and ovulation status/cycle[1]

| | Total cohort | DRI fiber groups (g/d) | | | | | Ovulatory[3] | | |
		1 (≤10)	2 (10.01–16)	3 (16.01–21.99)	4 (≥22)	P value[2]	Yes	No	P value[2]
No. of cycles	509	155	228	85	41		467	42	
Demographics									
Age (y)	27.5 ± 8.2[4]	25.9 ± 8.0	27.9 ± 8.4	29.5 ± 8.0	27.0 ± 8.0	0.009	27.9 ± 8.3	22.0 ± 5.3	<0.001
BMI (kg/m^2)	24.1 ± 3.9	24.5 ± 3.8	24.0 ± 3.9	24.0 ± 3.8	22.8 ± 4.3	0.08	24.2 ± 3.9	23.1 ± 3.6	0.08
Physical activity [n (%)]						0.15			0.93
Low	48 (9.5)	17 (11.9)	24 (10.5)	3 (3.6)	4 (9.8)		44 (9.6)	4 (8.7)	
Moderate	182 (36.0)	51 (33.6)	74 (32.5)	38 (45.2)	19 (46.3)		168 (36.1)	14 (33.3)	
High	275 (54.5)	84 (55.3)	130 (57.0)	43 (51.2)	18 (43.9)		253 (54.4)	24 (57.1)	
Race [n (%)]						<0.001			0.93
White	300 (59.4)	62 (40.8)	142 (62.3)	63 (75.0)	33 (80.5)		276 (59.4)	25 (59.5)	
African American	100 (19.8)	56 (36.8)	34 (14.9)	7 (8.3)	3 (7.3)		91 (19.6)	9 (21.4)	
Other	105 (20.8)	34 (22.4)	52 (22.8)	14 (16.7)	5 (12.2)		98 (21.1)	8 (19.1)	
Years of education [n (%)]						0.06			0.73
≤High school	65 (12.9)	26 (17.1)	31 (13.6)	6 (7.1)	2 (4.9)		58 (12.5)	6 (14.3)	
Postsecondary	440 (87.1)	126 (82.9)	197 (86.4)	78 (92.9)	39 (95.1)		407 (87.5)	36 (85.7)	
History of smoking [n (%)]						0.41			0.02
No	415 (81.9)	133 (85.8)	185 (81.1)	65 (77.4)	32 (80.5)		375 (80.7)	40 (95.2)	
Yes	92 (18.1)	22 (14.2)	43 (18.9)	19 (22.6)	8 (19.5)		90 (19.3)	2 (4.8)	
Past oral contraceptive use [n (%)]						0.11			0.02
No	228 (45.3)	77 (51.3)	105 (46.1)	32 (38.1)	14 (34.2)		203 (43.8)	25 (62.5)	
Yes	275 (54.7)	73 (48.7)	123 (54.0)	52 (61.9)	27 (65.9)		260 (56.2)	15 (37.5)	
Cycle length (d)	28.9 ± 4.1	28.7 ± 4.0	28.9 ± 4.4	29.9 ± 3.8	28.9 ± 3.2	0.95	28.9 ± 4.0	27.8 ± 5.1	0.13
Menstrual hormones[5]									
Average estradiol (pg/mL)	112.3 ± 91.0	123.7 ± 97.2	109.8 ± 87.3	110.2 ± 94.8	87.4 ± 69.9	<0.001	116.9 ± 92.5	64.9 ± 53.1	<0.001
Average luteal progesterone (ng/mL)	7.3 ± 5.5	7.4 ± 5.5	7.5 ± 5.5	7.4 ± 5.8	5.7 ± 4.9	0.007	7.9 ± 5.4	1.2 ± 1.3	<0.001
LH (ng/mL)	14.8 ± 14.9	14.8 ± 14.9	15.3 ± 15.2	14.6 ± 15.6	12.5 ± 12.1	0.22	15.2 ± 15.2	10.9 ± 10.5	0.003
FSH (mIU/mL)	7.8 ± 4.8	7.7 ± 4.8	7.8 ± 4.5	8.1 ± 5.4	7.5 ± 5.1	0.64	8.0 ± 4.9	6.2 ± 2.6	<0.001
Dietary variables									
Total energy (kcal)	1608.1 ± 405.0	1381.7 ± 318.2	1615.2 ± 356.7	1840.2 ± 415.6	1943.3 ± 426.9	<0.001	1610.3 ± 399.1	1583.3 ± 470.7	0.68
Carbohydrate (%)	50.9 ± 8.2	49.1 ± 7.8	50.5 ± 8.2	52.4 ± 8.2	56.8 ± 7.1	<0.001	50.8 ± 8.3	52.2 ± 7.4	0.30
Protein (%)	15.7 ± 3.4	15.9 ± 3.6	16.0 ± 3.3	15.4 ± 3.7	14.6 ± 2.7	0.07	15.8 ± 3.5	15.5 ± 3.4	0.58
Total fat (%)	33.9 ± 6.3	35.1 ± 6.3	33.7 ± 6.1	33.4 ± 6.7	30.9 ± 5.6	0.001	33.8 ± 6.3	33.9 ± 5.8	0.94
Cholesterol (mg/dL)	209.4 ± 115.7	212.4 ± 102.7	210.2 ± 112.8	210.5 ± 147.7	191.2 ± 105.4	0.77	210.3 ± 115.0	199.9 ± 124.3	0.58
Total fiber (g/d)	13.6 ± 6.0	8.0 ± 1.4	12.9 ± 1.7	18.5 ± 1.6	28.1 ± 5.6	<0.001	13.4 ± 5.5	16.0 ± 9.6	0.007
Insoluble fiber (g/d)	9.6 ± 4.7	5.4 ± 1.1	9.0 ± 1.5	13.4 ± 1.8	21.0 ± 4.6	<0.001	9.4 ± 4.3	11.5 ± 7.7	0.006
Soluble fiber (g/d)	3.8 ± 1.4	2.5 ± 0.6	3.7 ± 0.7	4.9 ± 0.9	6.8 ± 1.4	<0.001	3.7 ± 1.4	4.3 ± 2.0	0.02
Vegetable fiber (g/d)	4.9 ± 3.0	3.3 ± 1.5	4.5 ± 2.1	6.5 ± 2.5	9.9 ± 4.6	<0.001	4.8 ± 2.8	5.5 ± 4.6	0.16
Fruit fiber (g/d)	2.3 ± 1.9	1.1 ± 1.0	2.4 ± 1.7	3.1 ± 2.1	4.6 ± 2.2	<0.001	2.3 ± 1.9	3.1 ± 2.4	0.01

(Continued)

TABLE 1 (Continued)

| | Total cohort | DRI fiber groups (g/d) | | | | P value[2] | Ovulatory[3] | | P value[2] |
		1 (≤10)	2 (10.01–16)	3 (16.01–21.99)	4 (≥22)		Yes	No	
Grain fiber (g/d)	5.6 ± 3.1	3.4 ± 1.3	5.4 ± 2.0	7.5 ± 2.5	10.7 ± 5.1	<0.001	5.5 ± 2.8	6.5 ± 5.5	0.04
Magnesium (mg/d)	222.1 ± 74.3	156.6 ± 34.9	218.8 ± 39.9	280.0 ± 47.4	367.7 ± 77.9	<0.001	220.7 ± 70.9	237.5 ± 104.5	0.16
Calcium (mg/d)	697.9 ± 281.9	533.6 ± 214.8	712.3 ± 255.6	887.8 ± 306.5	845.8 ± 241.9	<0.001	701.7 ± 283.5	656.5 ± 262.2	0.32
Potassium (mg/d)	1917.4 ± 607.0	1421.8 ± 357.4	1921.6 ± 435.2	2385.5 ± 502.4	2797.0 ± 610.2	<0.001	1912.7 ± 600.2	1969.4 ± 684.1	0.56
Caffeine (mg/d)	92.1 ± 98.9	68.0 ± 81.5	98.2 ± 106	118.7 ± 102.0	93.5 ± 97.8	0.002	94.9 ± 100.0	61.0 ± 81.3	0.03
Vitamin C (mg/d)	70.0 ± 43.1	52.4 ± 36.1	70.0 ± 39.5	85.8 ± 46.2	104.0 ± 47.7	<0.001	70.0 ± 42.5	70.4 ± 49.3	0.95
Vitamin E (mg/d)	9.8 ± 7.2	6.76 ± 3.4	9.16 ± 5.6	14.01 ± 11.4	15.8 ± 6.7	<0.001	9.8 ± 7.3	9.7 ± 5.6	0.94
Iron (mg/d)	12.3 ± 5.1	9.03 ± 2.7	12.3 ± 4.0	16.1 ± 6.9	16.7 ± 4.4	<0.001	12.3 ± 5.1	12.0 ± 4.6	0.69

[1] LH, luteinizing hormone; FSH, follicle-stimulating hormone.
[2] Calculated by using ANOVA for continuous variables and exact chi-square tests for categorical variables for associations between fiber intakes or ovulation status.
[3] An ovulatory cycle is defined as progesterone >5 ng/mL and/or serum LH peak on day 22 or 27 of a standardized 28-d cycle.
[4] Mean ± SD (all such values).
[5] P values are based on the log(concentration).

included ≤8 measurements/cycle (each measurement was considered as a separate observation in the analysis). Similarly, for progesterone, only concentrations during the luteal phase (days 18, 22, and 27 of the standardized 28-d cycle) were included because there is minimal variation in progesterone concentrations during the follicular phase. For LH and FSH, models included concentrations around ovulation (days 12, 13, and 14 of the standardized 28-d cycle), because their midcycle peak is the most relevant period for these hormones. Nonlinear mixed models were used to model the association between average fiber intake and the probability of anovulation (29).

The presence of confounding was evaluated by using a hybrid approach that combined prior knowledge by using directed acyclic graphs and a statistical approach on the basis of change in point estimates (30). A set of variables was determined by a review of the prior literature, and a detailed directed acyclic graph was created that identified the variables that should be included in the models. Moreover, an exploratory confounding evaluation was used with covariates included in the model if they changed the exposure coefficient by >15% and were significant at $P = 0.10$. Factors that were shown to have an effect on the point estimates were energy intake (continuous), race (white, black, and other), age (continuous), and vitamin E intake (continuous). Highly correlated variables, such as magnesium, potassium, and folate, were analyzed as collinear variables and potential confounders. Sensitivity analyses were conducted to assess the effect of collinearity, which included the use of propensity score methods for confounding adjustment. SAS version 9.1 (SAS Institute, Cary, NC) was used for all of the statistical analyses.

RESULTS

Fiber consumption

Overall, this cohort of women was young (mean age: 27.5 y), of healthy weight (mean BMI: 24.1), had moderate to high physical activity (90.5%), and mostly comprised nonsmokers (82%; **Table 1**). Fiber intake, grouped by the DRI categories, varied significantly according to age and race-ethnicity, with younger and minority women tending to consume less fiber. BMI and physical activity were not significantly associated with fiber intake. Analysis of fiber intake according to quartiles of intake (as opposed to DRI categories) produced similar results for demographic and dietary characteristics (data not shown).

Ovulation status (ovulatory compared with anovulatory) differed significantly according to age, history of smoking, and past use of oral contraceptives. Anovulatory women were on average younger, less likely to be past or current smokers, and less likely to have used oral contraceptives in comparison with ovulatory women. In the dietary assessment, all of the components and sources of dietary fiber were significantly different according to ovulation status except for vegetable fiber. Caffeine intake was also significantly lower in anovulatory women.

Menstrual hormones

Fiber intake was inversely associated with estradiol concentrations, as shown in **Figure 1,** which displays the unadjusted means of estradiol over the menstrual cycle according to fiber
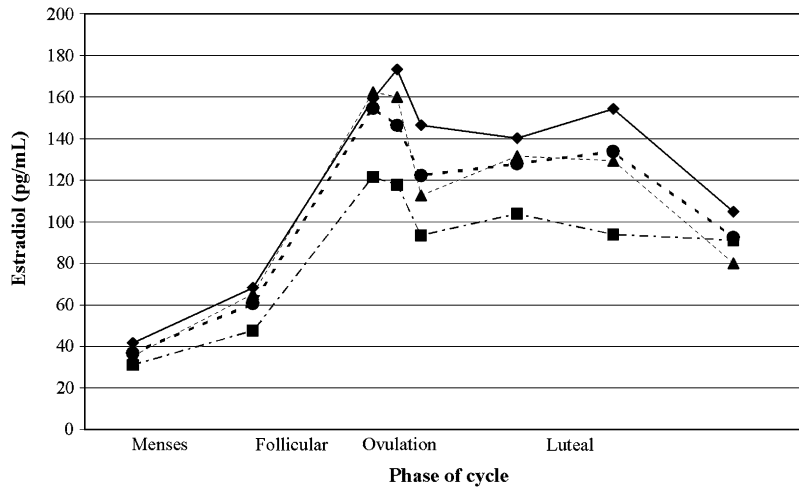
FIGURE 1. Crude concentrations of estradiol across the menstrual cycle according to Dietary Reference Intake categories of fiber consumption: ♦: ≤10 g/d; ●: 10.01–16 g/d; ▲: 16.01–21.99 g/d; ■: ≥22 g/d.

intake categories of DRI. Increased fiber intake was also significantly associated with decreased concentrations of estradiol across the menstrual cycle in models adjusted for total calories and in models adjusted for total calories, age, race, and vitamin E

(**Table 2**). In both models, for each additional 5 g of fiber, log (estradiol) decreased by ≈0.047 pg/mL over the menstrual cycle ($P = 0.01$). Luteal phase progesterone concentrations showed an inverse relation with dietary fiber intake after mixed-model

TABLE 2
Dietary fiber intake (5-g increments) and serum concentrations of menstrual hormones[1]

	β (95% CI)	
	Model 1[2]	Model 2[3]
Total fiber		
Estradiol (pg/mL)	−0.047 (−0.083, −0.011)	−0.049 (−0.087, −0.011)
LH (ng/mL)	−0.035 (−0.080, 0.010)	−0.051 (−0.100, −0.002)
FSH (nIU/mL)	−0.034 (−0.068, −0.001)	−0.034 (−0.068, 0.005)
Progesterone (ng/mL), luteal phase	−0.091 (−0.167, −0.015)	−0.117 (−0.198, −0.037)
Soluble fiber		
Estradiol (pg/mL)	−0.238 (−0.389, −0.087)	−0.222 (−0.377, −0.068)
LH (ng/mL)	−0.178 (−0.372, 0.0161)	−0.232 (−0.437, −0.027)
FSH (nIU/mL)	−0.127 (−0.270, 0.016)	−0.094 (−0.236, 0.049)
Progesterone, luteal phase (ng/mL)	−0.296 (−0.610, 0.017)	−0.328 (−0.650, −0.006)
Insoluble fiber		
Estradiol (pg/mL)	−0.054 (−0.098, −0.009)	−0.057 (−0.104, −0.010)
LH (ng/mL)	−0.029 (−0.086, 0.027)	−0.049 (−0.111, 0.013)
FSH (nIU/mL)	−0.036 (−0.078, 0.006)	−0.035 (−0.078, 0.008)
Progesterone, luteal phase (ng/mL)	−0.111 (−0.205, −0.016)	−0.146 (−0.245, −0.047)
Vegetable fiber		
Estradiol (pg/mL)	−0.024 (−0.087, 0.040)	−0.027 (−0.090, 0.036)
LH (ng/mL)	−0.002 (−0.085, 0.081)	−0.010 (−0.094, 0.074)
FSH (nIU/mL)	0.016 (−0.043, 0.074)	0.008 (−0.049, 0.065)
Progesterone, luteal phase (ng/mL)	0.027 (−0.102, 0.156)	−0.023 (−0.152, 0.107)
Grain fiber		
Estradiol (pg/mL)	−0.068 (−0.133, −0.004)	−0.073 (−0.139, −0.007)
LH (ng/mL)	−0.016 (−0.099, 0.067)	−0.029 (−0.117, 0.058)
FSH (nIU/mL)	−0.059 (−0.119, 0.002)	−0.048 (−0.109, 0.012)
Progesterone (ng/mL), luteal phase	−0.154 (−0.287, −0.021)	−0.163 (−0.300, −0.027)
Fruit fiber		
Estradiol (pg/mL)	−0.128 (−0.225, −0.031)	−0.104 (−0.200, −0.008)
LH (ng/mL)	−0.046 (−0.173, 0.080)	−0.068 (−0.198, 0.061)
FSH (nIU/mL)	−0.019 (−0.110, 0.072)	−0.005 (−0.093, 0.084)
Progesterone, luteal phase (ng/mL)	−0.288 (−0.484, −0.092)	−0.242 (−0.439, −0.045)

[1] LH, luteinizing hormone; FSH, follicle-stimulating hormone. Analyses were performed by using linear mixed models on the log scale of hormones.

[2] Adjusted for energy intake (continuous).

[3] Adjusted for energy intake (continuous), race (white, black, other), age (continuous), and vitamin E (continuous).

analysis ($\beta = -0.117$, $P = 0.004$). LH and FSH concentrations around ovulation both showed significant inverse relations to increasing fiber intake in the fully adjusted models ($\beta = -0.051$, $P = 0.04$ for LH and $\beta = -0.034$, $P = 0.05$ for FSH).

Dietary fiber was stratified into insoluble and soluble components, yielding results similar to those of total dietary fiber. Soluble fiber had a stronger inverse relation with estradiol concentrations ($\beta = -0.222$, $P = 0.01$) than did insoluble fiber ($\beta = -0.057$, $P = 0.02$). Of the 3 sources of fiber, fruit fiber had the strongest association with concentrations of estradiol ($\beta = -0.104$, $P = 0.03$), followed by grain fiber ($\beta = -0.073$, $P = 0.03$), whereas vegetable fiber was not associated ($\beta = -0.027$, $P = 0.40$) with estradiol concentration. Fruit and grain fiber were also associated with statistically significant decreases in concentrations of progesterone ($\beta = -0.242$, $P = 0.02$, for fruit fiber, and $\beta = -0.163$, $P = 0.02$, for grain fiber).

Fiber-rich foods are also typically rich in magnesium, potassium, iron, vitamin C, and vitamin E. Models of fiber intake adjusted for these strongly correlated dietary intake variables by using propensity scores yielded similar results (data not shown). Data from fully adjusted models of magnesium and potassium showed strong associations with estradiol but not with other menstrual hormones (data not shown). The significant associations between estradiol and magnesium and potassium intake, along with strong correlations between fiber and magnesium ($r = 0.87$) and between fiber and potassium ($r = 0.70$), confirmed the possibility of collinearity and limited the ability to discern whether the association of fiber with estradiol is independent of magnesium or potassium.

Anovulation

Results from a nonlinear mixed model adjusting for energy intake, race, age, and vitamin E intake showed that for each 5-g increase in dietary fiber, the adjusted odds ratio (aOR) of anovulation was 1.78 (95% CI: 1.11, 2.84; **Table 3**). Thus, a 5-g/d increase in dietary fiber intake, equivalent to ≈2 slices whole-grain bread (4.6 g/d) or one large apple (4.7 g/d), would result in a 78% elevation in the risk of anovulation. Analysis of continuous soluble and insoluble fiber intake as linear exposures yielded similar results. Soluble fiber had a stronger, positive association with an elevated risk of anovulation (aOR: 6.73; 95% CI: 1.18, 38.26) than did insoluble fiber (aOR: 2.15; 95% CI: 1.22, 3.77). When analyzed by fiber source, a 5-g/d increase in fruit fiber had the strongest association with probability of anovulation (aOR: 3.05; 95% CI: 1.07, 8.71). Grain and vegetable fiber were not significantly associated with anovulation [5-g

grain fiber/d increase (aOR: 1.84; 95% CI: 0.89, 3.78); 5-g/d vegetable fiber increase (aOR: 1.74; 95% CI: 0.87, 3.47)].

Of the 509 menstrual cycles included in this study, 42 (8.3%) were anovulatory. Of the cycles in which women consumed at or above the DRI (≥ 22 g fiber/d; $n = 41$), 22% of cycles were anovulatory compared with 7.1% of cycles in which women consumed ≤ 10 g fiber/d ($n = 155$). Although CIs are wide, after adjustment for energy intake, race, age, and vitamin E intake, category of dietary fiber consumption was positively associated with incident anovulation ($P = 0.004$), with an aOR of 10.98 (95% CI: 1.5, 80.5) for women at or above the DRI compared with the lowest DRI grouping (**Table 4**). Further adjustment for a wide variety of other demographic and dietary characteristics (shown in Table 1) had little effect on these results.

DISCUSSION

Higher consumption of dietary fiber was significantly associated with lower concentrations of reproductive hormones and an increased risk of incident anovulation in this cohort of young, healthy women. The significant association persisted and remained strong, whether fiber intake was considered as a continuous variable or categorized according to groupings on the basis of the DRI. The observed associations between high fiber intake, decreased hormone concentrations, and increased risk of anovulation highlights the potential for reproductive health implications related to fiber intake in young women that to date may not have been recognized.

The role of fiber in lowering estrogen concentrations has previously been shown predominantly in older women. High-fiber diets cause a decrease of β-glucuronidase activity in feces that leads to decreased reabsorption of estrogen in the colon (20). In addition, fiber binds to estrogen in the intestine, increasing its fecal excretion (31, 32). Through fiber's influence on estrogen, fiber subsequently influences other menstrual hormones due to the strict feedback mechanisms, which dictate hormonal fluctuations in the menstrual cycle (33–36). However, fiber also seems to decrease LH and FSH concentrations independent of estradiol. Prior research has confirmed that episodic gonadotropin release and a specific range of concentrations of FSH and LH are required for follicular development and ovulation (22, 37). The biological mechanisms behind the influence of fiber on LH and FSH have yet to be elucidated and require more research.

In women with normal reproductive function, one might anticipate that in response to lower concentrations of estradiol, an intact hypothalamic-pituitary-ovarian axis would respond by

TABLE 3
Dietary fiber consumption (5-g increments) and the risk of anovulation[1]

	Total fiber	Soluble	Insoluble	Vegetable	Fruit	Grain
Range (g/d)	3.46–48.97	1.93–37.91	0.73–9.89	0.18–28.8	0.0–12.54	0.51–27.08
Intake (g/d)	13.57 ± 6.0[2]	9.61 ± 4.71	3.76 ± 1.45	4.90 ± 2.95	2.33 ± 1.94	5.57 ± 3.09
aOR[3]	1.65 (1.08, 2.52)	6.03 (1.11, 32.67)	1.85 (1.12, 3.08)	1.59 (0.79, 3.19)	3.33 (1.08, 10.31)	1.72 (0.85, 3.49)
aOR[4]	1.78 (1.11, 2.84)	6.73 (1.18, 38.26)	2.15 (1.22, 3.77)	1.74 (0.87, 3.47)	3.05 (1.07, 8.71)	1.84 (0.89, 3.78)

[1] aOR, adjusted odds ratio. Analyses were performed by using nonlinear mixed models.
[2] Mean ± SD (all such values).
[3] Adjusted for energy intake (continuous); 95% CIs in parentheses.
[4] Adjusted for energy intake (continuous), race (white, black, other), age (continuous), and vitamin E (continuous); 95% CIs in parentheses.

TABLE 4
Risk of anovulation according to Dietary Reference Intake (DRI) categories[1]

	DRI categories			
	1	2	3	4
Fiber range (g/d)	$\leq$10	10.01–16	16.01–21.99	$\geq$22
Mean fiber (g/d)	8.0 $\pm$ 1.4[2]	12.9 $\pm$ 1.7	18.5 $\pm$ 1.6	28.1 $\pm$ 5.6
Anovulatory/total [n (%)]	11/155 (7.10)	16/228 (7.02)	6/85 (7.06)	9/41 (21.95)
aOR[3]	1.00	1.32 (0.42, 4.29)	1.51 (0.32, 7.12)	11.00 (1.40, 86.69)
aOR[4]	1.00	1.62 (0.54, 4.88)	2.56 (0.56, 11.75)	10.98 (1.50, 80.46)

[1] Analyses were run with nonlinear mixed models. aOR, adjusted odds ratio.
[2] Mean $\pm$ SD (all such values).
[3] Adjusted for energy intake (continuous); 95% CIs in parentheses.
[4] Adjusted for energy intake (continuous), race (white, black, other), age (continuous), and vitamin E (continuous); 95% CIs in parentheses.

increasing FSH and estradiol production from the follicles followed by ovulation. Although this response could explain why some of the women who consumed higher intakes of dietary fiber were ovulatory, the anovulatory women did not exhibit this response; rather, they had consistently lower concentrations of reproductive hormones. Thus, we concluded that the decreased hormone concentrations associated with higher fiber intakes could result in anovulatory cycles due to the close relation between diet and the hypothalamic-pituitary axis (38). However, more research is needed to understand the exact biological mechanisms.

It is also possible that dietary fiber intake is associated with other lifestyle factors related to increased menstrual irregularity in this population, such as intense physical activity, low or high BMI, or low-fat/low-calorie diets. However, the associations in this study were not greatly altered by adjustment for a wide variety of demographic, lifestyle, and dietary characteristics. Due to the homogeneous and healthy nature of the cohort, factors such as BMI and physical activity were not shown to have a significant effect on the findings. Although this may limit generalizability of these findings to all women, it increased the ability to reduce potential confounding and improved the study's internal validity.

The observed association between fiber intake and estradiol concentration is consistent with several observational studies and randomized trials (13–19) and at odds with several studies that did not observe a significant effect (39–42). In addition, previous studies failed to observe associations with other reproductive hormones. The past research studies were limited by sample size (15, 42), only taking measurements during the follicular phase (14, 16, 19, 39), issues with serum collection timing (13, 14, 16, 17, 19, 39, 40, 42), and low ranges of fiber consumption (13–19, 39–42).

A large percentage of prior studies based the timing of visits on an ≈28-d cycle measured post menses (13, 18, 39, 41, 42) despite the evident difficulty in timing visits during critical windows of the menstrual cycle on the basis of participant-reported cycle length or a standardized cycle length (43). Several studies also relied on only one serum measurement in either the follicular or luteal phase of the menstrual cycle and allowed several days for women to come into the clinic for serum collection despite the extreme variability of hormones by day (13, 18, 39, 41, 42). Furthermore, compared with the fiber intakes in this cohort, most of the previous studies had a much higher average fiber intake in the experimental groups (13, 14, 16) and in the

control groups and overall population (15, 18, 39, 41). The narrower range in other studies lessened their ability to perceive the dose-response relation that our results suggested. Importantly, the high fiber intakes evaluated in those studies (22.9 $\pm$ 8.9 g/d) were substantially higher than the average intake of most Americans (12), calling into question the applicability of their findings. Our study had a wider range of intake (3.46–48.97 g/d), which increased the ability to observe an effect according to dose.

Although the observed association with estradiol concentrations was consistent with previous research, the effect of fiber on anovulation has not been observed. Only 4 studies to date have evaluated this association, but none showed an effect (15, 18, 40, 42). In previous studies, ovulation was not assessed on an individual cycle basis but rather by whether there was a significant change in mean progesterone concentrations between the high-fiber and low-fiber groups. In addition to the crude methods for defining ovulation, these studies suffered from the methodologic problems discussed previously, which limited their ability to detect an association with ovulation.

Intensive monitoring of a large number of young, ethnically diverse women throughout 2 menstrual cycles, with multiple clinic visits timed with fertility monitors, was a significant improvement and helped distinguish the BioCycle Study from previous studies (43). Individual cycle assessment of ovulation on the basis of multiple hormone measurements was an important advancement over past studies on anovulation. The prospective design and exclusion criteria at baseline of the BioCycle Study strengthen the ability to draw inference, having reduced the potential for bias from known risk factors for anovulation. In addition, standardized assessment of a wide variety of participant and dietary characteristics increased the ability to adjust for confounding. Collectively, these unique aspects of the study design allowed us to improve and expand on previous studies of fiber and hormonal outcomes, which included anovulation.

Nevertheless, the study faced several limitations, which included the small number of women consuming at or above the DRI ($\geq$22 g dietary fiber/d; n = 41 cycles) and the small number of anovulatory cycles (n = 42), which limited the power of the findings and resulted in wide CIs due to imprecision. In addition, women were only followed for 2 menstrual cycles. Because anovulation occurs commonly in many women, long-term findings may be different. In absence of a daily transvaginal ultrasound or daily first morning urine measurements, the direct

detection of ovulation has some degree of misclassification. We assessed the effect of misclassification through a sensitivity analysis by comparing the results of a commonly used classification for anovulation (≤ 5 ng progesterone/mL; $n = 65$ cycles) with a more conservative classification ($n = 22$ cycles). The effect of fiber on anovulation was strong and consistent regardless of the definition used for classification (data not shown). Although the study included use of a fertility monitor to help time visits, bias could have been introduced through mistimed sample collection. However, various indicators of successfully timed visits were shown to be unrelated to fiber consumptions; thus, any misclassification is likely nondifferential (44). Last, due to the strong correlations between fiber and magnesium and other nutrients, it is difficult to discern the independent effects of these nutrients on hormone concentrations and anovulation. However, the biological mechanism linking dietary fiber to estradiol concentrations is the most plausible, and models that adjusted for highly correlated dietary variables produced similar results.

In conclusion, we observed that fiber consumption at or above the recommended intakes was significantly associated with decreased reproductive hormone concentrations and a substantially elevated probability of anovulatory cycles in women of reproductive age. Although this is a single study, these findings call into question whether current DRIs are applicable to women of reproductive age who are trying to conceive. Further studies are needed to confirm these findings and elucidate the role of fiber intake on reproductive health to inform current recommendations for adequate fiber intake in young women.

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