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SHORT COMMUNICATION

Effect of daily fiber intake on luteinizing hormone levels in reproductive-aged women

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Abstract
Purpose To evaluate whether the association between fiber intake and LH levels is driven by the association between fiber and estradiol, or whether there is an independent association.
Methods A prospective cohort of 259 premenopausal women were followed for up to 2 menstrual cycles. Estrogen and LH were measured ≤8 times per cycle at visits scheduled using fertility monitors. Diet was assessed ≤4 times per cycle by 24-h recall. Linear mixed models on the log scale of hormones were utilized to evaluate the total effects of fiber intake. Inverse probability weights were utilized to estimate the independent effect of fiber on LH levels.
Results In unweighted analyses, we observed a significant, inverse association between fiber intake (in 5 g/day increments) and log LH levels (β, −0.051, 95% confidence interval (CI), −0.100, −0.002). No association was observed in the weighted analyses, after estradiol levels were taken into account (β, −0.016, 95% CI, −0.060, 0.027).
Conclusions The decreased levels of LH associated with high fiber intake were attenuated after taking estradiol levels into account, suggesting that the association between fiber and LH is most likely a consequence of fiber's impact on estradiol and not due to an independent mechanism.

Keywords Dietary fiber · Luteinizing hormone · Estradiol · Women

Introduction

Vegetarian diets have been associated with a higher incidence of reproductive disorders and depressed levels of luteinizing hormone (LH) and estrogens [1]. Specifically, a higher frequency of menstrual cycle irregularities has been observed among vegetarians compared to non-vegetarians, as well as in omnivorous women who were placed on a vegetarian diet for 2 months or longer [1, 2]. It hypothesized that the decline in reproductive hormone levels is a result of the high fiber intakes characteristic of vegetarian diets. While several studies among premenopausal women have confirmed an inverse association between fiber intake and estrogen, only three studies have evaluated fiber's association with LH [3–5], and only one study has observed a significant inverse association [4]. While the lack of association in the other studies could be explained by limited sample sizes [3, 5], poor timing of serum collection [5], and/or low range of fiber consumption [3, 5], there is little biological rationale to support an independent association between fiber intake and LH levels. Some epidemiological studies have linked high fiber diets with decreased leptin concentrations [6] that in turn have been shown to suppress gonadotropin-releasing hormone (GnRH) [7]; however. no experimental studies have

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confirmed a direct association. Conversely, experimental studies provide strong biological rationale to support the inverse association between fiber intake and estrogen via a decrease of β-glucuronidase activity in feces [8].

When we previously reported a significant inverse association between fiber intake and LH [4], we maintained that it was most likely a consequence of fiber's influence on estrogens due to the lack of biological rationale. While this is plausible given that estrogen affects levels of other reproductive hormones via feedback mechanisms under regulation of the hypothalamic-pituitary-ovarian axis, at the time this was not tested. Therefore, we aim to evaluate whether the association between fiber intake and LH levels is driven by the association between fiber and estradiol, or whether there is an independent association.

Subjects and methods

Healthy, regularly menstruating, and premenopausal volunteers ($n = 259$) were recruited to participate in the BioCycle Study, a prospective cohort study of menstrual cycle function. These women, aged 18–44 years, were recruited from western New York and followed for one ($n = 9$) or two ($n = 250$) menstrual cycles. Details of the study design and exclusion criteria are described elsewhere [9]. In brief, exclusion criteria included current use of oral contraceptives, vitamin and mineral supplements, or prescription medications; pregnancy or breastfeeding in the past 6 months; recent history of infections or diagnosis of chronic conditions, including history of menstrual and ovulation disorders and gastrointestinal conditions (e.g., Crohn's Disease). Women planning to restrict their diet for weight loss or medical reasons were excluded as well as those consuming a diet high in phyto-estrogens (e.g., soy-based diet). The University at Buffalo Health Sciences Institutional Review Board approved the study and all participants provided written informed consent.

Fasting morning blood draws were collected between 7 and 8:30 a.m. at clinic visits scheduled on approximately the 2nd day of menstruation, mid and late follicular phase, 2 days around expected ovulation, and early, mid, and late luteal phase in each cycle. Fertility monitors (Clearblue Easy^{TM} Fertility Monitor, Inverness Medical, Waltham, MA), which measure estrone-3-glucuronide and LH in urine, were used in this study to time mid-cycle visits [10]. Women began using the fertility monitors on day 6 after menses and continued using them for 10–20 days, depending on whether the woman reached peak levels on the monitor. When the monitor indicated "peak fertility", the women were asked to come in that morning for their late follicular phase visit and the following two mornings for their two visits around expected ovulation. Blood 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 was measured by radioimmunoassay (coefficient of variation (CV) <10%), and follicle-stimulating hormone (FSH), LH, and progesterone were measured by Specialty Laboratories, Inc. (Valencia, CA) by solid phase competitive chemiluminescent enzymatic immunoassays on the DPC Immulite 2000 analyzer (Siemens Medical Solutions Diagnostics, Deerfield, IL) (CV <4% for FSH and LH; CV <14% for progesterone). Overall, compliance was high with 94% of women completing at least 7 clinic visits per cycle and 100% completing 5 visits per cycle.

Dietary intake was assessed on the same days as serum collection using a 24-h dietary recall conducted 4 times per cycle (corresponding to visits during menses, mid follicular phase, ovulation, and mid luteal phase). Dietary intake data were collected and analyzed using the Nutrition Data System for Research (NDSR) software version 2005 developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. Total dietary fiber was defined as unavailable carbohydrates (cellulose, hemi-cellulose, pectins, gums, and muscilages) and lignin. No significant differences were found in total dietary fiber intakes across each cycle, and thus average daily intakes were calculated per cycle. The majority of women completed 4 dietary recalls per cycle (87%), and all participants completed at least 2 recalls per cycle.

Linear mixed models on the log scale of the hormones were utilized to evaluate the total effects of fiber intake (in 5 g/day increments, i.e., a medium sized apple, 1 cup of broccoli, or 2 slices of whole grain bread) on LH and other reproductive hormone levels, adjusting for age, race, energy intake, and vitamin E but not adjusting for other reproductive hormones. To determine whether the association between fiber and LH was independent of estradiol, we further adjusted for estradiol and other reproductive hormones. However, since estradiol levels change over the cycle and are strongly associated with changing LH levels, traditional regression adjustment is inadequate. Therefore, adjustment for age, race, energy intake, vitamin E, as well as FSH and estradiol levels was made through the use of inverse probability weights [11]. Weights were based on the probability of an individual consuming the amount of fiber they actually consumed, conditional on their observed covariates (including measurements of hormone levels from previous visits). In order to estimate the stabilized weights for each cycle visit under study, the conditional density of estradiol levels at each cycle visit while adjusting for other factors was obtained by ordinary least-squares regression and estimated by the normal distribution.

All linear mixed models included random intercepts to account for the variability in baseline hormone levels between women, and for the correlation between cycles of the same woman. Models utilized data throughout the cycle, which included up to 8 measurements per cycle unless otherwise indicated. For LH, only measurements around the predicted day of ovulation (days 12, 13, and 14 of a standardized 28-day cycle) were included for consistency. Potential confounding factors were evaluated using a hybrid approach that combined prior knowledge and a statistical approach on the basis of change in point estimates. Covariates were included in the model if they changed the exposure coefficient by more than 15% and were significant at the $\alpha = 0.10$ level. Factors shown to have an effect on the point estimates were included in the final adjusted models and included age, race, energy intake, and vitamin E. Various anthropometric (such as BMI) and dietary factors (such as fat intake) were also considered but were not found to influence the results. All analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC).

Results

On average, this cohort of women were young (mean age 27.5 year ± 8.2 SD), of healthy weight (mean BMI 24.1 ± 3.9 SD), had moderate to high physical activity (90.5%), were mostly non-smokers (82%), and were omnivorous (100%) and non-consumers of soy protein (90%). Fiber intake varied according to age ($P = 0.01$) and race ($P < 0.001$), with younger and minority women tending to consume less fiber (Table 1). BMI and physical activity were not significantly associated with fiber intake. Higher fiber intake was also associated with higher energy intake ($P < 0.001$) and lower fat intake ($P = 0.002$). Highly correlated nutrients such as magnesium, potassium, folate, and vitamin E were positively associated with fiber intake ($P < 0.001$). Fiber intakes were inversely associated with estrogen and progesterone levels (Table 1), with increasing fiber intake associated with decreased levels of estrogen in a dose–response manner and with decreased levels of progesterone at the highest levels of intake.

The associations between daily mean fiber intake and reproductive hormone levels in models that do and do not take other reproductive hormone levels into account are presented in Table 2. In the original analysis, we estimated the total effects of fiber intake on LH and observed a significant inverse association (β, −0.051, 95% CI, −0.100, −0.002). However, in the model that estimated the independent effects of dietary fiber on LH where hormone levels were taken into account (specifically estradiol), we observed no association (β, −0.016, 95% CI, −0.060, 0.027). Estradiol, progesterone, and FSH maintained similar associations in both models but were slightly attenuated in the models that accounted for other reproductive hormones. In particular, higher fiber intake was also associated with decreased levels of progesterone, consistent with our previous findings of increased odds of anovulation corresponding to high fiber intakes [4].

Discussion

Our results suggest that the effect of fiber on LH is most likely due to fiber's effect on estrogen. While higher fiber intakes tend to be associated with decreased levels of LH, this does not seem to be due to an independent mechanism between fiber and LH.

This is the first study, to our knowledge, to address the association between dietary fiber and reproductive hormones independent of the other reproductive hormones. It is also the largest study to date that included multiple reproductive hormone measurements timed with the use of a fertility monitor. Additionally, since our study sample population was composed of women not on a restricted diet, many potential confounders were excluded by design. Fiber intake promotes both satiation and satiety that can lead to decreased energy intake; however, higher fiber intake in our cohort of women was associated with higher energy intake, not lower intake. Since dietary restriction has been shown to reduce LH pulse frequency [12], possibly through a decrease in leptin that modulates GnRH production, it is possible that previous studies were observing this negative energy balance effect, and not an effect of dietary fiber. Additionally, due to the low intake of soy in our population, this hormonal active nutrient seemed to have little effect on the association between fiber and hormones in this study.

There are several limitations. Residual confounding is a possibility since it can be very difficult to capture a participant's true dietary intake. While the use of four 24-h recalls per cycle was a vast improvement over previous observational studies, misclassification is still possible. Further, dietary recalls are inadequate measures of nutrient and caloric absorption. Additionally, it is difficult to establish an association between health outcomes and intake of a single nutrient due to the strong correlations between food constituents. While we attempted to control for many of these factors, by design or adjustment, it is hard to exclude the possibility that other food components associated with high fiber intake may mediate part of this association. The use of a fertility monitor to time mid-cycle visits helped decrease the variability of LH; however, due to its episodic secretion, misclassification of the LH surge is possible especially given that serum levels of LH can

Table 1 Demographic, hormonal, and dietary characteristics of BioCycle participants

	Total cohort	Average fiber intake (g/day)				*P* value[b]
		<10	10.01–16	16.01–21.99	≥22	
Number of menstrual cycles	509	155	228	85	41	
Demographics						
Age, years; mean (SD)	27.5 (8.2)	25.9 (8.0)	27.9 (8.4)	29.5 (8.0)	27.0 (8.0)	0.01
BMI, kg/m^2; mean (SD)	24.1 (3.9)	24.5 (3.8)	24.0 (3.9)	24.0 (3.8)	22.8 (4.3)	0.09
Physical activity, *n* (%)[a]						0.19
Low	48 (9.4)	17 (11.9)	24 (10.5)	3 (3.6)	4 (9.8)	
Moderate	182 (35.8)	51 (33.6)	74 (32.5)	38 (45.2)	19 (46.3)	
High	278 (54.7)	86 (55.3)	130 (57.0)	44 (51.2)	18 (43.9)	
Race, *n* (%)						<0.001
Caucasian	302 (59.3)	63 (40.8)	142 (62.3)	64 (75.0)	33 (80.5)	
African-American	101 (19.8)	57 (36.8)	34 (14.9)	7 (8.3)	3 (7.3)	
Other	106 (20.8)	35 (22.4)	52 (22.8)	14 (16.7)	5 (12.2)	
≤High school education, *n* (%)	64 (12.6)	26 (17.1)	31 (13.6)	6 (7.1)	2 (4.9)	0.02
Nulliparous, *n* (%)	367 (74)	120 (81)	161 (71)	55 (66)	31 (76)	0.07
Never smoker, *n* (%)	415 (81.5)	133 (85.8)	185 (81.1)	65 (77.4)	32 (80.5)	0.40
Past oral contraceptive user, *n* (%)	276 (54.2)	73 (48.7)	123 (54.0)	52 (61.9)	27 (65.9)	0.05
Cycle length, days; mean (SD)	28.8 (4.1)	28.7 (4.0)	28.9 (4.4)	28.9 (3.8)	28.9 (3.2)	0.27
Reproductive hormones; median (*Q*1, *Q*3)						
Estrogen, pg/mL	84.6 (65.4, 108.6)	95.2 (71.3, 116.4)	82.2 (64.3, 108.2)	78.5 (65.2, 105.0)	65.6 (51.7, 89.3)	<0.001
LH, ng/mL	10.3 (7.2, 14.7)	10.4 (7.0, 14.5)	10.7 (7.5, 15.4)	10.0 (7.2, 13.4)	8.2 (6.1, 12.5)	0.26
FSH, mIU/mL	6.8 (5.3, 8.5)	6.9 (5.4, 8.5)	6.8 (5.3, 8.7)	7.2 (5.3, 8.7)	6.0 (5.0, 7.4)	0.77
Luteal progesterone, ng/mL	5.6 (3.3, 8.2)	5.3 (3.6, 8.1)	5.9 (3.8, 8.4)	5.6 (2.9, 8.3)	4.1 (1.7, 7.1)	0.05
Dietary variables; mean (*SD*)						
Calories, kcal	1,608.1 (405.0)	1,381.7 (318.2)	1,615.2 (356.7)	1,840.2 (415.6)	1,943.3 (426.9)	<0.001
Carbohydrate, %	50.9 (8.2)	49.1 (7.8)	50.5 (8.2)	52.4 (8.2)	56.8 (7.1)	<0.001
Protein, %	15.7 (3.4)	15.9 (3.6)	16.0 (3.3)	15.4 (3.7)	14.6 (2.7)	0.08
Total fat, %	33.9 (6.3)	35.1 (6.3)	33.7 (6.1)	33.4 (6.7)	30.9 (5.6)	0.002
MUFA, g/day	23.1 (8.4)	20.8 (6.8)	22.8 (7.8)	25.8 (9.7)	27.1 (11.3)	<0.001
PUFA, g/day	12.8 (5.3)	10.9 (4.1)	12.8 (4.7)	14.8 (6.3)	16.3 (7.5)	<0.001
SFA, g/day	21.3 (8.5)	19.0 (6.5)	21.4 (8.1)	24.8 (10.6)	21.5 (9.7)	<0.001
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
Total fiber, g/day	13.6 (6.0)	8.0 (1.4)	12.9 (1.7)	18.5 (1.6)	28.1 (5.6)	<0.001
Insoluble fiber, g/day	9.6 (4.7)	5.4 (1.1)	9.0 (1.5)	13.4 (1.8)	21.0 (4.6)	<0.001
Soluble fiber, g/day	3.8 (1.4)	2.5 (0.6)	3.7 (0.7)	4.9 (0.9)	6.8 (1.4)	<0.001
Vegetable fiber, g/day	4.9 (3.0)	3.3 (1.5)	4.5 (2.1)	6.5 (2.5)	9.9 (4.6)	<0.001
Fruit fiber, g/day	2.3 (1.9)	1.1 (1.0)	2.4 (1.7)	3.1 (2.1)	4.6 (2.2)	<0.001
Grain fiber, g/day	5.6 (3.1)	3.4 (1.3)	5.4 (2.0)	7.5 (2.5)	10.7 (5.1)	<0.001
Soy protein, g/day	21.4 (8.1)	14.8 (3.8)	20.8 (4.5)	27.8 (6.7)	36.2 (9.7)	<0.001

BMI body mass index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *SD* standard deviation, *SFA* saturated fatty acids

[a] One missing value

[b] Two-sided *P* values were calculated using generalized linear mixed models. All comparisons take repeated measures and correlations between cycles into account

Table 2 Dietary fiber intake (5-g increments) and log serum concentrations of reproductive hormones

	β (95% confidence interval)	
	Total effects[a]	Associations independent of other reproductive hormones[b]
Estrogen (pg/mL)	−0.049 (−0.087, −0.011)	−0.044 (−0.078, −0.009)
LH (ng/mL)	−0.051 (−0.100, −0.002)	−0.016 (−0.060, 0.027)
FSH (mIU/mL)	−0.034 (−0.068, 0.005)	−0.013 (−0.043, 0.018)
Progesterone (ng/mL)	−0.117 (−0.198, −0.037)	−0.074 (−0.148, −0.001)

FSH follicle-stimulating hormone, *LH* luteinizing hormone

[a] Linear mixed model with random intercepts adjusted for energy intake (continuous), race (white, black, other), age (continuous), and vitamin E (continuous)

[b] Linear mixed model with random intercepts adjusted for energy intake (continuous), race (white, black, other), age (continuous), vitamin E (continuous), and the other reproductive hormone levels (continuous) through the use of inverse probability weights

vary tenfold in a couple of hours. Finally, the strict inclusion criteria of our study could also limit the generalizability of our findings.

In conclusion, the significant, inverse total effect of dietary fiber on LH levels seems to be completely explained by the effect of fiber intake on estradiol levels, as there was no observed independent effect of fiber on LH levels (after taking estradiol levels into account). These findings put into perspective the direct role of fiber on estradiol as it appears to have cascading effects on additional pathways including other reproductive hormones. We hope that these findings provide new insight into dietary fiber's biological effects on reproductive hormones and the menstrual cycle.

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Conflict of interest The authors declare that they have no conflict of interest.

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