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Dietary fat intake, erythrocyte fatty acids, and risk of uterine fibroids

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Abstract

Objective: To prospectively evaluate the association between dietary fat intake and risk of uterine fibroids; and to evaluate the association between erythrocyte membrane fatty acid (FA) levels and fibroid risk.

Design: Prospective cohort study. Cox proportional hazard models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI). In a subset of participants 34 individual FAs were measured and logistic regression analysis was used to estimate odds ratios (ORs) and 95% CIs for the association between FA tertiles and fibroids.

Setting: Not applicable.

Patients: 81,590 premenopausal U.S. women in the Nurses' Health Study II, ages 25-42 at enrollment in 1989 for whom diet was assessed via a food frequency questionnaire (FFQ). 553 participants with erythrocyte FA measurements.

Intervention(s): Not applicable.

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Main Outcome Measure: Cases of fibroids were defined on the basis of self-reported ultrasound or hysterectomy-confirmation.

Results: Eight thousand one hundred forty-two cases of ultrasound or hysterectomy-confirmed UL were diagnosed over an 18-year period (1991-2009). No associations were observed between intake of any dietary fats and fibroids in the multivariable models. However, when erythrocyte FAs were examined, an inverse association was observed between total n-3 polyunsaturated FAs and likelihood of fibroids (OR for 3rd versus 1st tertile=0.41; 95% CI=0.19-0.89; p_{trend}=0.02). In addition, total *trans* FAs were associated with greater odds of fibroids (OR for 3rd tertile=3.33; 95% CI=1.50-7.38; p_{trend}=0.002).

Conclusion: Our findings provide preliminary suggestions that n-3 polyunsaturated FAs and *trans* FAs may play a role in fibroid etiology; however, these results should be confirmed in future studies.

Capsule:

N-3 polyunsaturated FAs and *trans* FAs, which can be influenced by dietary intake, may play a role in the incidence of clinically relevant uterine fibroids.

Keywords

uterine fibroid; diet; fats; fatty acids; erythrocyte

INTRODUCTION

Uterine fibroids are the most common pelvic tumor in reproductive age women.(1) They are the primary indication for hysterectomies in the United States with over 200,000 procedures annually, and are a leading cause of hospitalizations for gynecologic conditions unrelated to pregnancy.(2) While non-malignant, fibroids are frequently associated with pelvic pain, abnormal uterine bleeding, infertility, and adverse pregnancy outcomes. Despite the high morbidity and health care costs associated with fibroids, the etiology is not fully understood, and few modifiable risk factors have been identified.

Dietary factors may play a role in fibroid etiology due to their potential to modify endogenous hormones as well as their inflammatory effects. For example, *trans* fat intake influences circulating levels of interleukin (IL)-6 and other inflammatory markers(3-5) and a chronic inflammatory milieu has been hypothesized to promote fibroid development.(6) However, to our knowledge, only one prospective study, the Black Women's Health Study (BWHS) has examined the association between dietary fat intake and fibroid risk, observing a small increased risk with intake of several specific omega-3 fatty acids (FAs) and no clear associations with total fat or other fat subtypes.(7) Fish consumption was a large contributor to omega-3 fatty acid consumption in this cohort, thus environmental contaminants through fish intake could explain this increased risk. BWHS did not measure circulating fatty acids that capture both dietary intake and fatty acid metabolism, more precisely reflecting the internal dose. Further, the fatty acid composition of the erythrocyte membrane reflects dietary intake over months, in contrast to serum/plasma which may only reflect dietary

intake over days to weeks.(8) Examination of these biomarkers may provide additional insight into fibroid etiology.

In this study we used data from the prospective Nurses' Health Study II to investigate whether intake of dietary fats was associated with ultrasound or hysterectomy-confirmation uterine fibroids over an 18-year follow-up period. In a subset of women, we also examined the association between fatty acids measured in erythrocyte membranes and subsequent fibroid risk.

METHODS

Study population

The Nurses' Health Study II (NHSII) is an ongoing prospective cohort study established in 1989. At baseline, 116,429 U.S. female registered nurses aged 25-42 years completed a questionnaire that collected information on demographic and lifestyle factors, anthropometric variables, and disease history. Follow-up questionnaires are sent biennially to update information on exposures and disease status. Additional study details have been provided elsewhere.(9) The cumulative follow-up for NHSII is >95%. Ongoing consent was assumed upon return of the completed questionnaire. This study was approved by the institutional review boards of the Harvard T.H. Chan School of Public Health and the Brigham and Women's Hospital, Boston, Massachusetts.

Between 1996-1999, 29,611 NHSII participants ages 32-54 years, provided blood samples and answered a short questionnaire at blood collection including information on date, time, and number of hours since last food intake. After overnight shipment all samples were processed into plasma, white blood cell, and red blood cell components and have been stored at <=−130 degrees C in continuously monitored liquid nitrogen freezers. Further details of the blood collection procedure for NHSII have been described previously.(10) NHSII participants who provided a blood sample were similar to the total cohort.(10)

Analytic populations

Follow-up for the analyses that utilized the diet data began in 1991 when 97,813 NHSII participants first returned the food frequency questionnaire (FFQ) and concluded in 2009, the last year uterine fibroid incidence was assessed on the biennial questionnaire. Criteria for exclusion were: implausible total energy intake (<600 kcal/day or >3,500 kcal/day), blank entries for more than 70 food items on the 1991 FFQ, or a diagnosis of uterine fibroid or cancer diagnosis (other than nonmelanoma skin cancer) prior to June 1991. The analytical cohort was limited to those who were premenopausal and had intact uteri. After these exclusions, 81,590 premenopausal women with dietary information remained.

Participants in the erythrocyte membrane fatty acid analysis were selected from 794 control participants in a previous nested case-control study that examined erythrocyte membrane FAs and breast cancer risk.(11) From the original 794 controls, we excluded those with pre-blood draw fibroid diagnosis (n=65), pre-blood draw hysterectomy (n=87), and those who were postmenopausal or missing menopausal status (n=89). After these exclusions 553 women with previously measured erythrocyte membrane FAs levels remained.

Dietary assessment

Diet was assessed in 1991, 1995, 1999, 2003, and 2007 using an FFQ that listed over 130 food items. Participants were asked how often, on average, they consumed each type of food or beverage during the previous year. For each food item, nine responses were possible, ranging from never or less than once per month to 6 or more times per day. Nutrient intakes were calculated by first multiplying the portion size of a single serving of each food by its reported frequency of intake for the total amount of food consumed, and then multiplying the total amount consumed by the nutrient content of the food, and then summing across all food items. The questionnaire included information about specific types of margarine and fats used for baking and frying and this was incorporated into the nutrient calculations. Nutrient values in foods were obtained from the US Department of Agriculture (Nutrient Data Laboratory), food manufacturers, independent academic sources, and our own fatty acid analyses of commonly used margarines, cooking oils, and baked foods.(12-14) The food composition database has been updated every four years to account for changes in the food supply including updated fatty acid analyses. The reproducibility and validity of the NHSII FFQ has been previously reported(15-17) with de-attenuated correlation coefficients between the FFQ and 7-day diet records of 0.67 for total fat, 0.69 for saturated fat, 0.57 for polyunsaturated fat, 0.56 for monounsaturated fat, 0.69 omega-3 fatty acids, 0.65 for cholesterol,(17) and 0.66 for fish intake.(15)

Laboratory assays

Erythrocyte FA concentrations were assayed in Dr. Hannia Campos' laboratory at the Harvard T.H. Chan School of Public Health using gas-liquid chromatography. A detailed description of the laboratory process has been published elsewhere.(18) Masked replicates from pooled specimens were included for quality control. Out of the 34 FAs included in our analysis, 8 FAs with levels close to the detection limit had coefficients of variation between 20% to 95% (lauric acid, mystristic acid, pentadecanoic acid, mysristoleic acid, docosadienoic acid, palmitelaidic acid, linolelaidic acid, and octadecadienoic acid). Additional details are available elsewhere.(11)

Fatty acids

We examined erythrocyte FAs individually and in the following groups by type; saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), n-3 polyunsaturated fatty acids (PUFA), n-6 PUFA, and *trans* FA. In addition, we calculated the ratio of total n-6 PUFA to total n-3 PUFA, as this ratio has been hypothesized to predict several chronic inflammatory diseases.(19) The saturation indices, SI_{n-7} (palmitic/palmitoleic acid) and SI_{n-9} (stearic/oleic acid), were considered as indicators of the steroyl coenzyme-A desaturase activity.(20, 21) We also examined SFA and *trans* FA primarily from milk or meat from cattle or other ruminants (15:0+17:0+16:1n–7t), termed dairy derived fatty acids, and *trans* FA from partially hydrogenated oils (18:1 *trans*+18:2 *trans*), termed industrial *trans* for this analysis. Previous work has shown that erythrocyte content of fatty acids are closely correlated with plasma content with an average correlation of 0.72.(22)

Ascertainment and definition of uterine fibroids

Starting in 1993, participants were asked on each biennial questionnaire if they ever received a physician diagnosis of uterine fibroids, and if so, the date of diagnosis and whether the diagnosis was confirmed by pelvic exam, ultrasound, or hysterectomy. Cases were defined on the basis of self-reported ultrasound or hysterectomy-confirmation. Participants who reported fibroids not confirmed by ultrasound or hysterectomy did not contribute person-time to that study period but were allowed to reenter the analysis if confirmed by ultrasound or hysterectomy in the future.

In a previous validation study, a subset of newly diagnosed cases confirmed by ultrasound or hysterectomy (N=243, 100 white and 143 African-American) were mailed a questionnaire on symptoms and requested a review of their medical records.(23) Of the 216 who responded (89%), 6% denied the diagnosis and 34% confirmed the diagnosis but did not release their medical records. Among the cases in which medical records could be obtained, 93% were confirmed. The proportion diagnosed by hysterectomy, myomectomy, examination under anesthesia or ultrasound did not differ between those who did and did not give permission for medical record release. The proportion confirmed by medical record did not differ comparing white (94%) and African-American (92%) participants.

Statistical analysis

Dietary intake analysis.—In the analysis examining dietary intake assessed from the FFQ, participants contributed follow-up time from the return of the 1991 questionnaire until self-report of a uterine fibroid, diagnosis of any cancer (except non-melanoma skin cancer), death, loss to follow-up, hysterectomy, menopause, or until return of the 2009 questionnaire (the last year uterine fibroids incidence was assessed on the biennial questionnaire – at which time the youngest participant was age 45) – whichever occurred first. Cox proportional hazards regression models were used with age and the questionnaire period as the time scale to estimate hazard ratios (HR) and 95% confidence intervals (CI). The lowest category of intake of each nutrient or food was used as the reference group. We examined associations with total fat, vegetable fat, animal fat, saturated fat, *trans*-unsaturated fat, monounsaturated fat, polyunsaturated fat, long-chain omega-6 fatty acids, long-chain omega-3 fatty acids, EPA, DHA, and DPA. We also examined dietary cholesterol which comes from intake of animal products that are also high in fats. In addition, we examined the association with dark fish intake as it is the top contributor long-chain omega-3 fatty acid intake in the NHSII, has also been associated with environmental pollutants (e.g., polychlorinated biphenyls) that have been proposed to increase fibroid risk,(24) and was suggestively associated with fibroid risk in the BWHS.(7)

Cumulative average consumption is reported, as this method captures long-term dietary intake and minimizes measurement error due to within-person variation over time.(25) Covariate adjusted models included the following potential confounders that were chosen a priori due to their association with fibroids or dietary factors: total calories, race/ethnicity, age at menarche, infertility, parity, age at first birth, time since last birth, age first oral contraceptive use (, menstrual cycle length, body mass index, smoking, recent gynecologic/breast exam, and use of anti-hypertensive medications/diastolic blood pressure. Covariates

were updated throughout the analysis as new information became available for the biennial questionnaires. Tests for linear trend of the exposures of interest were performed by assigning the median value of each category to all participants in that group.

Erythrocyte fatty acids analysis.

Tertiles of FAs were determined by the distribution among the controls. Logistic regression analysis was used to estimate odds ratios (ORs) and 95% CIs for the association between FA tertiles and fibroids. The final covariate adjusted models included characteristics of the blood draw as well as the following potential confounders that were associated with FA levels in this dataset: age (continuous), blood draw time (1a-8, 9a- 12p, 1-mid), fasting status (y/n), blood draw season (Nov-Apr, May-Oct), race (white, nonwhite), parity (nulliparous, 1, 2, 3, 4+), age at first birth (<25, 25-30, 31+), time since last birth (<6, 6-12, 13+ years), age at menarche (<12, 12-13, 14+), menstrual cycle length (<26, 26-31, 32+ days), and body mass index (<20, 20-21.9, 22-23.9, 24-24.9, 25-26.9, 27-29.9, 30+). In addition, we examined age at first oral contraceptive use, smoking, recent gynecologic/breast exam, and use of anti-hypertensive medications/diastolic blood pressure as potential confounders but none were associated with FAs in this dataset and thus were not included in the final model. Tests for linear trend of the exposures of interest were performed by assigning the median value of each category to all participants in that group and additional by examining the fatty acids as continuous variables. All statistical analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, North Carolina) and all tests of statistical significance were two-sided.

RESULTS

During 1,536,355 person-years of follow-up contributed by 81,590 women, 8,142 incident cases of ultrasound or hysterectomy-confirmed uterine fibroids were reported. Women with a higher intake of total fat were more likely to be a current smoker or parous, had a higher mean body mass index, and were less likely to have had a recent gynecologic exam (Table 1).

Total fat intake was not associated with risk of fibroids (Table 2). When specific types of fat were examined there was the suggestion of a higher risk of fibroids with both *trans*-unsaturated fat intake and cholesterol intake in the age-adjusted analyses (HR [95% CI] for 5^{th} quintile vs 1^{st} quintile of 1.07 [1.00-1.15], p_{trend}=0.02 and 1.10 [1.03-1.18], p_{trend}=0.003, respectively); however the associations were attenuated after adjustment for covariates (HR [95% CI] for 5^{th} quintiles=1.00 [0.93-1.07], p_{trend}=0.99 and 1.04 [0.96-1.11], p_{trend}=0.34, respectively). No other dietary fats were associated with fibroids risk (Table 2) nor was dark fish intake (results not shown).

Characteristics of the subset of women who had erythrocyte fatty acids levels measured are presented in Table 3 by fibroids status. The median time between blood draw and fibroids diagnosis was 5.5 years, with an interquartile range of 3.3-9.3 years. Results were similar between age-adjusted (data not shown) and covariate-adjusted models with results for covariate-adjusted models presented in Table 4. We observed an inverse association between erythrocyte levels of total n-3 PUFA and odds of fibroids (OR [95% CI] for 3^{rd} vs 1^{st}

tertile=0.41 [0.19-0.89], p_{trend}=0.02). Of the individual n-3 PUFAs examined, the inverse association was strongest for EPA (OR [95% CI] for 3rd tertile=0.42 [0.19-0.90], p_{trend}=0.03).

Total *trans* FA was associated with greater odds of fibroids (OR [95% CI] for 3rd tertile=3.33 [1.50-7.38], p_{trend}=0.002) with the largest magnitude of individual *trans* FA associations with linolelaidic acid and 18:1 *trans* (OR [95% CI]=2.87 [1.30-6.34], p_{trend}=0.008 and OR=3.10 [1.42-6.77], p_{trend}=0.003, respectively). A significant positive association was also observed with industrial *trans* fatty acids (Table 4). No associations were observed for total or individual SFA, MUFA, or n-6 PUFA.

DISCUSSION

In this large, prospective cohort study we observed no associations between intake of dietary fats (total or specific types) and risk of uterine fibroids. However, in a subset of women with erythrocyte fatty acid measurements, we observed a lower odds of fibroids among women with higher n-3 PUFA erythrocyte levels and a greater odds among those with higher *trans* FA erythrocyte levels. These findings suggest n-3 PUFAs and *trans* FAs may be associated with fibroid risk.

Dietary fat intake may influence the etiology of fibroids through estrogenic or inflammatory effects. A meta-analysis of 13 intervention studies reported that reducing fat consumption among both pre- and post-menopausal women resulted in lower serum estradiol levels.(26) More recently it has been hypothesized that systemic chronic inflammation, marked by increased T helper cytokines and decreased functional regulatory T cells, may lead to the development of fibroids through the formation of fibrous tissue and smooth muscle proliferation.(6) In addition, women with fibroids may be at increased risk of atherosclerosis and hypertension,(27-29) and inflammation has been identified as playing an important role in atherosclerosis development.(30) *Trans* fat intake influences circulating levels of IL-6, IL-1β, tumor necrosis factor-α (TNF-α) and other inflammatory markers,(3-5) and markers including IL-6, IL-1, and TNF-α have been reported to influence the secretion of enzymes that digest endometrial extracellular matrices.(31) In contrast, dietary intake and plasma levels of omega-3 FAs has been inversely associated with inflammatory cytokines, including IL-6, TNF-α and TNF-α receptors.(32, 33) Our results among women with erythrocyte FA levels which indicated a decreased risk with higher n-3 PUFAs concentrations and an increased risk with higher *trans* FAs concentrations are consistent with these observations.

To our knowledge, only three studies, two case-control and one prospective cohort study, have examined the association between dietary fat intake and fibroid risk. An Italian hospital-based case-control study of 843 histologically-confirmed fibroid cases and 1557 controls with acute non-gynecologic, non-hormonal, and non-neoplastic conditions (e.g., traumatic injury, non-traumatic orthopedic disorders, surgical conditions, eye disorders) reported no associations between butter, margarine, or oil intake in the year before the study and fibroids risk.(34) This study was not able to adjust for total caloric intake and categorization based on tertiles (i.e., low, intermediate, and high) were used for the three sources of fat intake. Consistent with these null results, a cross-sectional study of Japanese

women enrolled through a health check-up program (54 fibroids cases and 234 non-cases) reported no association between total fat or specific subtypes (i.e., SFA, MUFA, PUFA) assessed with a 169-item FFQ and fibroids identified through transvaginal ultrasound.(35)

Most recently, the prospective BWHS followed over 12,000 African American women for eight years identifying 2695 fibroid cases diagnosed by ultrasound or hysterectomy/surgery. In this analysis, Wise, et al. reported increased risks of fibroids with intake of specific n-3 PUFAs but no consistent associations with total fat or other fat subtypes.(7) They further reported a greater risk with dark meat fish, which was the main source of n-3 PUFAs in this population. These results are intriguing given that long-chain omega-3 fatty acids exhibit anti-inflammatory properties(32, 33) and have previously been associated with positive health benefits such as reduced risk of coronary heart disease(36) and endometriosis.(37) However, it is consistent with a modest increased risk of prostate cancer observed with higher EPA and DPA blood levels.(38) In addition, consumption of fish that contain persistent organic pollutants has been previously associated with fibroids incidence.(24) In our FFQ-based analysis among a predominantly white population we observed no significant associations between any dietary fats, including long-chain omega-3 fatty acids, or dark fish, and risk of uterine fibroids. Although in both the NHSII and BWHS dark fish was the largest contributor to n-3 PUFAs, differences in types of dark fish consumed, other differing sources of n-3 PUFAs, and/or differing exposures to environmental contaminants through these sources could have contributed to the disparate results.

To our knowledge, we are the only study to have examined the association between erythrocyte FAs and fibroid risk. The differing results observed between dietary FAs assessed with FFQs compared to erythrocyte FA levels in this analysis deserves further discussion. Circulating FAs originate from a variety of sources, including dietary intake as well as being synthesized and/or transformed *in vivo.* The latter of these sources cannot be assessed by dietary intake. Thus, the FA composition of the erythrocyte membrane likely represents an integrated measure of the interactions between dietary fatty acid intake, other dietary factors, and internal transformation of fatty acids which may explain the association observed with the erythrocyte FAs and not with the dietary fat intake assessed with FFQs. In a similar population in the Nurses' Health Study, moderate to strong correlations were observed between erythrocyte FAs and FA intake assessed via the cumulative average method from three FFQs with adjusted Spearman correlation coefficients of 0.54 ($p<0.01$) for the n-3 PUFA DHA and 48 ($p<0.01$) for *trans* FAs,(22) indicating that erythrocyte concentrations of these specific fatty acids, which are largely of exogenous origin, are suitable biomarkers for long-term FA dietary intake.

This is the largest study to date to examine the association between dietary fat intake and fibroid risk with 18 years of follow-up and multiple dietary assessments. While the FFQ has been previously validated(15-17) some error in self-report is expected. Thus our assessment of erythrocyte membrane fatty acid levels on a subset of women complemented the FFQ data, allowing us to consider both dietary intake and endogenous synthesis and transformation of fatty acids providing new insight into the potential association between dietary fats and fibroids risk. The use of erythrocyte measures of FAs instead of plasma was

a further strength as erythrocytes are likely to represent long-term intake better than plasma due to their longer half-life.(39)

Fibroid diagnosis was collected through self-report, which includes the potential for outcome misclassification. To address this we restricted our case definition to those reporting a diagnosis that was confirmed with ultrasound or hysterectomy. Based on the results from the previous validation study conducted in this cohort, we are confident that women reporting a fibroid diagnosis have been diagnosed with fibroids.(23) However, we cannot quantify how many women with undiagnosed fibroids are present in this cohort. Baird et al., have reported that 43% of white women of reproductive age showed ultrasound evidence of having an undiagnosed fibroid.(40) The presence of undiagnosed disease usually leads to bias to the null, i.e. it reduces the probability of observing true associations rather than generating false positive associations. However, it is difficult to design studies of fibroids to prevent this phenomenon. Even among studies in which controls or non-cases have an ultrasound to confirm that they do not have fibroids, the time of fibroid onset among the cases is unknown and exposure status before fibroid onset is difficult to determine. In addition, these cross-sectional studies do not have ability to prospectively collect dietary data over many years or blood samples prior to fibroid diagnosis as is possible with our study design. We observed statistically significant results despite this potential misclassification, suggesting that the associations observed with erythrocyte fatty acid levels may be stronger than we observed. In regards to generalizability, while our study participants were limited to U.S. female registered nurses at study enrollment, there is no strong rationale as to why the association between erythrocyte fatty acid levels and UL incidence would differ in this population compared to women in the general population. However, as the NHSII is a predominantly white cohort, we had insufficient numbers to examine these associations by race. This is an important limitation as black women are disproportionately impacted by fibroids and some risk factors for fibroids could differ between racial/ethnic groups.

Finally, we must acknowledge limitations of the erythrocyte analyses. With the limited sample size and multiple fatty acids examined in our biomarker analyses we may have observed significant associations due to chance. If we account for multiple comparison using a Bonferroni correction none of the observed associations would be statistically significant, thus our results should be interpreted with caution. Further, it is currently not clear whether our erythrocyte fatty acid measures capture the relevant etiologic window for fibroid development. However, erythrocytes likely reflect longer term dietary intake given their half-life of 120 days compared to serum measures which reflect intake over days.

In conclusion, our prospective analysis suggests that n-3 PUFAs and *trans* FAs may play a role in the incidence of clinically relevant uterine fibroids. As these factors are influenced by dietary intake, increasing long-chain omega-3 FA intake and decreasing *trans* FA intake should be further examined as potentially modifiable risk factors in the etiology of uterine fibroids.

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Table 1.

Distribution of potential risk factors for uterine fibroids according to total fat intake among women in the Nurses' Health Studv II at baseline in 1991[1]

	Total fat intake quintile				
	1	2	3	4	5
No. of Women	15,223	14,780	14,949	16,162	19,856
Age (years), Mean (Standard deviation [SD])	36.1 (4.6)	36.0 (4.6)	36.0 (4.6)	36.1 (4.6)	36.4 (4.6)
Caucasian, %	90.0	92.6	92.8	93.3	93.6
Body Mass Index (kg/m^2), Mean (SD)	23.4 (4.5)	24.0 (4.7)	24.3 (5.0)	24.8 (5.3)	25.6 (6.0)
Cigarette smoking, %					
Never	67.2	67.2	66.9	65.9	64.4
Past	23.3	22.5	22.1	22.1	20.3
Current	9.5	10.2	11.0	12.1	15.3
Age at menarche, %					
< 12 years	23.8	23.4	22.7	24.1	24.5
12 years	30.0	29.8	30.7	30.3	30.8
13 years	27.3	28.2	28.6	27.9	27.1
≥ 14 years	18.8	18.6	18.1	17.7	17.6
Menstrual cycle length, %					
< 26 days	16.6	15.8	16.7	16.7	17.3
26-31 days	68.2	69.1	68.0	68.3	67.8
32-50 days	11.2	11.0	11.1	11.1	10.5
51+ or irregular cycles	3.9	4.1	4.1	3.9	4.3
Ever use of oral contraceptives, %	82.0	83.8	83.6	84.6	85.1
Age at first use of oral contraceptives, %					
Never	18.5	16.6	16.7	15.8	15.2
13-16	5.5	5.0	5.1	5.1	5.4
17-20	38.6	40.1	40.1	41.7	42.0
21-24	26.9	28.6	28.6	28.6	28.8
25+	10.6	9.8	9.5	8.8	8.6
Nulliparous, %	35.6	27.7	24.6	23.6	24.7
Infertility diagnosis, %	6.9	6.5	5.9	6.0	6.0
Time since last birth (years), %					
< 1	6.8	6.7	6.4	5.9	5.4
1-3	29.6	30.3	30.5	29.1	27.5
4-5	15.4	14.7	15.1	15.2	15.0
6-7	10.9	11.8	11.9	11.8	12.3
8-9	9.3	9.0	9.7	10.5	10.0
10+	28.0	27.5	26.4	27.5	29.8
Recent gynecologic exam, %					
No exam	12.6	12.7	13.4	14.6	16.6
Anti-hypertensive medication use, %	2.6	2.4	2.7	2.7	3.2

	Total fat intake quintile				
	1	**2**	**3**	**4**	**5**
Diastolic blood pressure, %					
<65	25.9	23.0	22.4	21.4	20.0
65-74	48.3	49.5	49.0	47.0	46.4
75-84	21.0	22.1	22.9	25.2	25.8
85-89	2.9	3.4	3.7	4.0	4.8
90 +	2.0	2.0	2.1	2.4	2.9
Total Calories (kcal), Mean (SD)	1778 (547)	1794 (534)	1814 (541)	1814 (550)	1780 (564)
Total fat intake (grams), Mean (SD)	47.0 (5.5)	56.6 (1.7)	62.0 (1.5)	67.5 (1.7)	77.4 (5.9)

[1]All data shown are standardized to the age distributions of the 1991 cohort

Table 2.

Hazard ratios and 95% confidence intervals for uterine fibroids according to quintiles of dietary fat intake (energy-adjusted g/day) in the Nurses' Health Study II, 1991-2009

	Cases	Age-adjusted HR (95% confidence interval)		MV[1] HR (95% confidence interval)	
Total fat					
1	1558	1.00	Referent	1.00	Referent
2	1663	1.04	0.97, 1.12	1.04	0.97, 1.11
3	1697	1.07	0.99, 1.14	1.06	0.98, 1.13
4	1707	1.09	1.02, 1.17	1.08	1.00, 1.15
5	1517	1.03	0.96, 1.10	0.98	0.91, 1.06
P_{trend}[2]			0.21		0.92
Vegetable fat					
1	1470	1.00	Referent	1.00	Referent
2	1667	1.03	0.96, 1.11	1.03	0.96, 1.11
3	1723	1.05	0.97, 1.12	1.03	0.96, 1.11
4	1667	1.00	0.93, 1.08	0.99	0.92, 1.06
5	1615	1.00	0.93, 1.07	0.98	0.91, 1.05
P_{trend}[2]			0.63		0.22
Animal fat					
1	1658	1.00	Referent	1.00	Referent
2	1629	0.99	0.92, 1.06	0.97	0.91, 1.04
3	1694	1.05	0.98, 1.12	1.03	0.97, 1.11
4	1647	1.05	0.98, 1.13	1.03	0.96, 1.10
5	1514	1.02	0.95, 1.10	0.99	0.92, 1.06
P_{trend}[2]			0.19		0.83
Saturated fat					
1	1680	1.00	Referent	1.00	Referent
2	1716	1.02	0.95, 1.09	1.01	0.95, 1.08
3	1683	1.04	0.97, 1.11	1.04	0.97, 1.11
4	1610	1.03	0.96, 1.10	1.02	0.95, 1.09
5	1453	1.00	0.93, 1.07	0.98	0.91, 1.05
P_{trend}[2]			0.95		0.64
Trans-unsaturated fat					
1	1573	1.00	Referent	1.00	Referent
2	1674	1.03	0.96, 1.10	1.02	0.95, 1.09
3	1634	1.00	0.93, 1.07	0.97	0.91, 1.04
4	1724	1.08	1.01, 1.16	1.03	0.96, 1.11
5	1537	1.07	1.00, 1.15	1.00	0.93, 1.07

	Cases	Age-adjusted HR (95% confidence interval)		MV[1] HR (95% confidence interval)	
P_{trend}[2]			0.02		0.99
Monounsaturated fat					
1	1492	1.00	Referent	1.00	Referent
2	1643	1.06	0.98, 1.13	1.05	0.98, 1.13
3	1682	1.06	0.99, 1.14	1.05	0.98, 1.13
4	1745	1.12	1.04, 1.20	1.10	1.02, 1.17
5	1580	1.05	0.98, 1.13	1.00	0.93, 1.08
P_{trend}[2]			0.07		0.67
Polyunsaturated fat					
1	1505	1.00	Referent	1.00	Referent
2	1674	1.02	0.95, 1.10	1.01	0.94, 1.09
3	1721	1.04	0.97, 1.12	1.02	0.95, 1.09
4	1645	1.00	0.93, 1.07	0.96	0.89, 1.03
5	1597	1.04	0.96, 1.11	0.98	0.92, 1.06
P_{trend}[2]			0.58		0.31
Long-chain omega-6 fatty acids					
1	1413	1.00	Referent	1.00	Referent
2	1645	1.02	0.95, 1.09	1.01	0.94, 1.08
3	1727	1.02	0.95, 1.09	0.99	0.93, 1.07
4	1713	0.99	0.93, 1.07	0.96	0.90, 1.04
5	1644	1.00	0.93, 1.08	0.96	0.89, 1.03
P_{trend}[2]			0.84		0.13
Long-chain omega-3 fatty acids					
1	1424	1.00	Referent	1.00	Referent
2	1673	1.05	0.98, 1.13	1.04	0.97, 1.11
3	1683	1.01	0.94, 1.08	0.98	0.91, 1.05
4	1689	1.02	0.95, 1.10	0.99	0.92, 1.06
5	1673	1.05	0.98, 1.13	1.01	0.94, 1.08
P_{trend}[2]			0.37		0.81
EPA					
1	1245	1.00	Referent	1.00	Referent
2	1835	1.02	0.95, 1.10	1.03	0.96, 1.10
3	1723	1.09	1.02, 1.18	1.08	1.00, 1.16
4	1652	1.07	0.99, 1.15	1.06	0.98, 1.14
5	1687	1.05	0.98, 1.13	1.04	0.96, 1.12
P_{trend}[2]			0.27		0.51
DHA					
1	1487	1.00	Referent	1.00	Referent

	Cases	Age-adjusted HR (95% confidence interval)		MV[1] HR (95% confidence interval)	
2	1631	1.02	0.95, 1.10	1.02	0.95, 1.09
3	1669	1.08	1.01, 1.16	1.08	1.00, 1.15
4	1739	1.05	0.89, 1.12	1.03	0.96, 1.11
5	1616	1.10	1.02, 1.18	1.08	1.00, 1.16
P_{trend}[2]			0.01		0.06
DPA					
1	1483	1.00	Referent	1.00	Referent
2	1882	0.93	0.87, 0.99	1.02	0.95, 1.10
3	1453	0.91	0.85, 0.97	1.06	0.99, 1.14
4	1970	0.94	0.88, 1.00	1.03	0.96, 1.11
5	1354	0.85	0.80, 0.92	1.06	0.98, 1.14
P_{trend}[2]			0.006		0.20
Marina fatty acids (EPA, DHA, and DPA)					
1	1511	1.00	Referent	1.00	Referent
2	1582	1.01	0.94, 1.08	1.00	0.93, 1.08
3	1641	1.06	0.99, 1.14	1.04	0.97, 1.12
4	1756	1.09	1.02, 1.17	1.08	1.01, 1.16
5	1652	1.06	0.99, 1.14	1.03	0.96, 1.11
P_{trend}[2]			0.04		0.20
Cholesterol					
1	1556	1.00	Referent	1.00	Referent
2	1657	1.03	0.96, 1.11	1.02	0.96, 1.10
3	1677	1.05	0.98, 1.12	1.03	0.96, 1.10
4	1668	1.08	1.00, 1.15	1.04	0.97, 1.11
5	1584	1.10	1.03, 1.18	1.04	0.96, 1.11
P_{trend}[2]			0.003		0.34

Abbreviations: HR, hazard ratio; CI, confidence interval

[1]Adjusted for age (continuous), total calories (continuous), race/ethnicity (Caucasian, Black, Hispanic, Asian, other), age at menarche (<11, 11, 12, 13, 14-15, >15), infertility (yes, no), parity (nulliparous, 1, 2, 3, 4+), age at first birth (<25, 25-30, >30), time since last birth (<1, 1-3, 4-5, 6-7, 8-9, 10-12, 13-15, 16+), age first oral contraceptive use (13-16, 17-20, 21-24, 25+), menstrual cycle length (<26, 26-31, 32-50, and >50 days), body mass index (<20, 20-21.9, 22-23.9, 24-24.9, 25-26.9, 27-29.9, 30+), smoking (never, past, current), recent gynecologic/breast exam (no recent exam, recent exam), and use of anti-hypertensive medications/diastolic blood pressure (no meds <65, no meds 65-74, no meds 75-84, no meds 85-89, no meds 90+, meds <65, meds 65-74, meds 75-84, meds 85-89, meds 90+).

[2]Determined using category medians.

Table 3.

Characteristics at blood collection (1996-1999) of uterine fibroids 56 cases and 497 controls in the Nurses' Health Study II, 1996-2009

	Controls		Cases	
	(n=497)		(n=56)	
Age at blood draw (range), Mean (standard deviation [SD])	43 (33-52)	(4.0)	43 (33-50)	(3.5)
Caucasian, n (%)	471	(94.8)	54	(96.4)
Parity, n (%)				
Nulliparous	97	(19.5)	8	(14.3)
1	65	(13.1)	5	(8.9)
2	177	(35.5)	20	(35.7)
3	113	(22.7)	18	(32.1)
4+	45	(9.0)	5	(8.9)
Age at first birth, n (%)				
<25	141	(35.3)	13	(27.1)
25-30	187	(46.8)	31	(64.6)
31+	72	(18.0)	4	(8.3)
Time since last birth, n (%)				
<6 years	102	(25.5)	14	(29.2)
6-12 years	181	(45.3)	18	(37.5)
13+ years	117	(29.3)	16	(33.3)
Aqe at menarche, n (%)				
<12	96	(19.3)	12	(21.4)
12-13	315	(63.3)	35	(62.5)
14+	86	(17.3)	9	(16.1)
Menstrual cycle length, n (%)				
<26 days	93	(19.2)	9	(17.0)
26-31 days	348	(71.9)	35	(66.0)
32+ days	43	(8.9)	9	(17.0)
Body mass index (kq/m^2), Mean (SD)	25.2	(6.0)	26.2	(6.4)

Table 4.

Multivariable-adjusted[1] odds ratio (95% confidence interval) of fibroids according to tertiles of erythrocyte fatty acid concentration among 553 participants (56 fibroids cases, 497 non-cases), Nurses' Health Study II

	T1	T2	T3	P_{trend}[2]	$P_{continuous}$[3]
Total Saturated Fatty Acids	1.00 (Referent)	0.94 (0.45-1.95)	1.12 (0.55-2.27)	0.70	0.51
Lauric acid (12:0)	1.00 (Referent)	0.98 (0.48-2.00)	0.92 (0.45-1.87)	0.81	0.66
Mystristic acid (14:0)	1.00 (Referent)	0.83 (0.40-1.74)	1.05 (0.52-2.10)	0.82	0.88
Pentadecanoic acid (15:0)	1.00 (Referent)	0.62 (0.29-1.32)	1.07 (0.54-2.15)	0.78	0.39
Palmitic acid (16:0)	1.00 (Referent)	1.15 (0.58-2.30)	0.92 (0.44-1.93)	0.83	0.68
Margric acid (17:0)	1.00 (Referent)	1.60 (0.75-3.43)	1.85 (0.85-4.03)	0.15	0.07
Stearic acid (18:0)	1.00 (Referent)	1.02 (0.49-2.12)	1.22 (0.59-2.49)	0.56	0.08
Nonadecanoic acid (19:0)	1.00 (Referent)	1.27 (0.57-2.81)	2.08 (0.97-4.46)	0.05	0.19
Arachidic acid (20:0)	1.00 (Referent)	1.03 (0.50-2.15)	1.17 (0.57-2.40)	0.66	0.46
Behenic acid (22:0)	1.00 (Referent)	0.94 (0.46-1.92)	1.01 (0.49-2.07)	0.96	0.55
Tricosanoic acid (23:0)	1.00 (Referent)	0.96 (0.48-1.90)	0.63 (0.29-1.35)	0.22	0.50
Lignoceric acid (24:0)	1.00 (Referent)	0.59 (0.29-1.19)	0.68 (0.34-1.36)	0.26	0.21
Total Monounsaturated Fatty Acids	1.00 (Referent)	0.78 (0.39-1.57)	0.64 (0.30-1.36)	0.24	0.12
Mysristoleic acid (14:1n-5c)	1.00 (Referent)	0.96 (0.46-2.02)	1.22 (0.60-2.47)	0.53	0.54
Pentadecenoic acid (15:1n-5c)	1.00 (Referent)	0.83 (0.42-1.64)	0.55 (0.25-1.23)	0.15	0.06
Palmitoleic acid (16:1n-7c)	1.00 (Referent)	0.92 (0.47-1.80)	0.48 (0.21-1.10)	0.08	0.05
Oleic acid (18:1n-9c)	1.00 (Referent)	0.39 (0.18-0.84)	0.60 (0.30-1.23)	0.11	0.46
Octadecenoic acid (18:1n-7c)	1.00 (Referent)	2.24 (1.09-4.63)	1.19 (0.53-2.71)	0.77	0.94
Gondoic acid (20:1n-9c)	1.00 (Referent)	2.03 (0.99-4.18)	0.96 (0.43-2.15)	0.88	0.58
Nervonic acid (24:1n-9c)	1.00 (Referent)	1.01 (0.52-1.99)	0.65 (0.30-1.38)	0.28	0.18
n-3 Polyunsaturated Fatty Acids	1.00 (Referent)	0.49 (0.24-0.97)	0.41 (0.19-0.89)	0.02	0.02
Alpha-linolenic acid (ALA, 18:3n-3c)	1.00 (Referent)	1.69 (0.84-3.40)	0.75 (0.34-1.69)	0.40	0.74
Eicosapentaenoic acid (EPA, 20:5n-3c)	1.00 (Referent)	0.64 (0.32-1.25)	0.42 (0.19-0.90)	0.03	0.01
Docosapentaenoic acid (DPA, 22:5n-3c)	1.00 (Referent)	0.60 (0.29-1.22)	0.73 (0.36-1.48)	0.34	0.11
Docosahexaenoic acid (DHA, 22:6n-3c)	1.00 (Referent)	0.40 (0.19-0.85)	0.57 (0.28-1.15)	0.09	0.03
n-6 Polyunsaturated Fatty Acids	1.00 (Referent)	0.93 (0.44-1.92)	1.14 (0.57-2.29)	0.74	0.81
Linoleic acid (18:2n-6cc)	1.00 (Referent)	0.94 (0.45-1.93)	1.09 (0.53-2.23)	0.81	0.58

	T1	T2	T3	p_{trend} [2]	$p_{continuous}$ [3]
Gamma-linolenic acid (18:3n-6c)	1.00 (Referent)	0.85 (0.42-1.73)	0.83 (0.40-1.75)	0.65	0.29
Eicosadienoic acid (20:2n-6c)	1.00 (Referent)	1.05 (0.51-2.13)	1.04 (0.51-2.15)	0.92	0.95
Dihomogammalinolenic acid (20:3n-6c)	1.00 (Referent)	0.60 (0.29-1.23)	0.70 (0.34-1.42)	0.33	0.11
Arachidonic acid (20:4n-6c)	1.00 (Referent)	0.62 (0.30-1.29)	0.90 (0.45-1.80)	0.76	0.79
Docosadienoic acid (22:2n-6c)	1.00 (Referent)	0.85 (0.38-1.89)	1.86 (0.90-3.84)	0.05	0.05
Aolrenic acid (22:4n-6c)	1.00 (Referent)	1.12 (0.55-2.29)	1.03 (0.50-2.12)	0.93	0.42
Total *Trans* Fatty Acids	1.00 (Referent)	1.23 (0.52-2.92)	3.33 (1.50-7.38)	0.002	0.004
Palmitelaidic acid (16:1n-7t)	1.00 (Referent)	0.90 (0.42-1.92)	1.42 (0.68-2.99)	0.30	0.10
Linolelaidic acid (18:2n-6t)	1.00 (Referent)	1.87 (0.81-4.29)	2.87 (1.30-6.34)	0.008	0.04
Octadecadienoic acid (18:2n-7c)	1.00 (Referent)	0.86 (0.41-1.81)	1.26 (0.63-2.52)	0.48	0.59
18:1 *trans*	1.00 (Referent)	1.14 (0.48-2.71)	3.10 (1.42-6.77)	0.003	0.002
18:2 *trans*	1.00 (Referent)	1.31 (0.61-2.81)	1.64 (0.78-3.46)	0.19	0.34
Dairy-derived Fatty Acids	1.00 (Referent)	1.26 (0.58-2.72)	1.68 (0.79-3.59)	0.17	0.10
Industrial *trans*	1.00 (Referent)	1.08 (0.46-2.56)	3.06 (1.41-6.66)	0.002	0.003
Total n-6/n-3 Ratio	1.00 (Referent)	1.23 (0.55-2.74)	1.94 (0.91-4.16)	0.08	0.03
SI $ratio_{n-7}$	1.00 (Referent)	2.03 (0.90-4.56)	2.10 (0.90-4.86)	0.10	0.05
SI $ratio_{n-9}$	1.00 (Referent)	1.19 (0.57-2.46)	1.21 (0.58-2.52)	0.62	0.09

[1] Adjusted for age (continuous), blood draw time (1a-8, 9a-12p, 1-mid), fasting status (yes/no), blood draw season (Nov-Apr, May-Oct), race (white, nonwhite), parity (nulliparous, 1, 2, 3, 4+), age at first birth (<25, 25-30, 31+), time since last birth (<6, 6-12, 13+ years), age at menarche (<12, 12-13, 14+), menstrual cycle length (<26, 26-31, 32+ days), and body mass index (<20, 20-21.9, 22-23.9, 24-24.9, 25-26.9, 27-29.9, 30+).

[2] Determined using category medians.

[3] Determined using a continuous variable.