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Dietary Fat Intake and Reproductive Hormone Concentrations and Ovulation in Regularly Menstruating Women

Sunni L. Mumford
National Institute of Child Health and Human Development

Jorge E. Chavarro
Harvard University

Cuilin Zhang
National Institute of Child Health and Human Development

Neil J. Perkins
National Institute of Child Health and Human Development

Lindsey A. Sjaarda
National Institute of Child Health and Human Development

See next page for additional authors

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Authors
Sunni L. Mumford, Jorge E. Chavarro, Cuilin Zhang, Neil J. Perkins, Lindsey A. Sjaarda, Anna Z. Pollack, Karen C. Schliep, Kara A. Michels, Shvetha M. Zarek, Torie C. Plowden, Rose G. Radin, Lynne C. Messer, Robyn A. Frankel, and Jean Wactawski-Wende

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Dietary fat intake and reproductive hormone concentrations and ovulation in regularly menstruating women[1,2]

Sunni L Mumford,[3] Jorge E Chavarro,[5] Cuilin Zhang,[3] Neil J Perkins,[3] Lindsey A Sjaarda,[3] Anna Z Pollack,[6] Karen C Schliep,[3] Kara A Michels,[3] Shvetha M Zarek,[3,4] Torie C Plowden,[3,4] Rose G Radin,[3] Lynne C Messer,[8] Robyn A Frankel,[3] and Jean Wactawski-Wende[7]*

[3]Division of Intramural Population Health Research and [4]Program of Reproductive and Adult Endocrinology, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Bethesda, MD; [5]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA; [6]Department of Global and Community Health, George Mason University, Fairfax, VA; [7]Department of Epidemiology and Environmental Health, University at Buffalo, State University of New York, Buffalo, NY; and [8]School of Community Health, Portland State University, Portland, OR

ABSTRACT

Background: Emerging evidence suggests potential links between some dietary fatty acids and improved fertility, because specific fatty acids may affect prostaglandin synthesis and steroidogenesis.

Objective: The objective of this exploratory study was to evaluate associations between total and specific types of dietary fat intake and *1*) hormone concentrations and *2*) the risk of sporadic anovulation in a cohort of 259 regularly menstruating women in the BioCycle Study.

Design: Endogenous reproductive hormones were measured up to 8 times/cycle for up to 2 cycles, with visits scheduled with the use of fertility monitors. Dietary intake was assessed with up to four 24-h recalls/cycle. Linear mixed models and generalized linear models were used to evaluate the associations between dietary fatty acids and both reproductive hormone concentrations and ovulatory status. All models were adjusted for total energy intake, age, body mass index, and race.

Results: Relative to the lowest levels of percentage of energy from total fat, the highest tertile was associated with increased total and free testosterone concentrations (total: percentage change of 4.0%; 95% CI: 0.7%, 7.3%; free: percentage change of 4.1%; 95% CI: 0.5%, 7.7%). In particular, the percentage of energy from polyunsaturated fatty acids (PUFAs) in the highest tertile was associated with increases in total and free testosterone (total: percentage change of 3.7%; 95% CI: 0.6%, 6.8%; free: percentage change of 4.0%; 95% CI: 0.5%, 7.5%). The PUFA docosapentaenoic acid (22:5n–3) was not significantly associated with testosterone concentrations (P-trend = 0.86 in energy substitution models) but was associated with increased progesterone and a reduced risk of anovulation (highest tertile compared with the lowest tertile: RR: 0.42; 95% CI: 0.18, 0.95). Fat intakes were not associated with other reproductive hormone concentrations.

Conclusions: These results indicate that total fat intake, and PUFA intake in particular, is associated with very small increases in testosterone concentrations in healthy women and that increased docosapentaenoic acid was associated with a lower risk of anovulation. *Am J Clin Nutr* doi: 10.3945/ajcn.115.119321.

Keywords: dietary fats, estradiol, menstrual cycle, ovulation, testosterone

INTRODUCTION

A growing body of literature suggests that dietary and lifestyle factors play an important role in various biological processes involved in human reproduction. Dietary fat intakes in particular are hypothesized to affect fertility and pregnancy outcomes, likely through prostaglandin synthesis and steroidogenesis (1, 2).

Although animal studies have shown the importance of dietary fatty acid composition on ovulation and fertility (3–5), only a few studies to date have evaluated the association between fat intake and menstrual cycle function in humans (6–11). Some evidence suggests that increases in total and specific types of dietary fatty acid intake improve menstrual cycle function (6–11), although other studies observed no associations between fat intakes and estradiol concentrations (12–20). Less is known, however, with regard to the association between fat intakes and androgen concentrations and ovulation, although a previous study showed improvements in metabolic and endocrine characteristics in response to changes in dietary macronutrient intake (lowering carbohydrates and increasing fat intake) in women with polycystic ovarian syndrome (21), which may point to potential dietary interventions to improve fertility. Previous studies have typically been limited by small sample sizes, inadequate timing of sample collection to cycle phase, and failure to evaluate ovulatory function among healthy women. Moreover, the evaluation of specific types of fatty acids may be relevant given that other reproductive outcomes have been differentially influenced by type of fatty acids; for example, PUFAs have been shown to

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[2] Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

*To whom correspondence should be addressed. E-mail: mumfords@mail.nih.gov.

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reduce preterm birth (1), whereas *trans* fats have been associated with ovulatory infertility (22) and endometriosis (23). Therefore, the objective of this exploratory study was to evaluate associations between total and specific types of dietary fat intake and both hormone concentrations and sporadic anovulation in a cohort of 259 regularly menstruating women in the BioCycle Study.

METHODS

Design and study population

The BioCycle Study (2005–2007) was a prospective cohort of 259 regularly menstruating, healthy volunteers, aged 18–44 y, recruited from western New York. Details of the study design are described elsewhere (24). Exclusion criteria included the following: current use of hormonal contraceptives [or use during the previous 3 mo for short-acting (e.g., oral contraceptive pills) and 12 mo for longer-acting hormonal contraceptives (e.g., Norplant (Wyeth-Ayerst), Depo-Provera (Pfizer), or hormonal intrauterine device)], pregnancy or breastfeeding in the past 6 mo, and diagnosis of certain chronic conditions, including a history of menstrual and ovulation disorders or uterine abnormalities, such as uterine fibroids. Women with a self-reported BMI (kg/m^2) of <18 or >35 at screening were excluded, as were women who were planning to restrict their diet over the study period or who consumed a diet high in phytoestrogens. The University at Buffalo Health Sciences Institutional Review Board approved the study, and served as the institutional review board designated by the NIH under a reliance agreement. All of the participants provided written informed consent.

Participants were followed for 1 ($n = 9$) or 2 ($n = 250$) menstrual cycles (**Supplemental Figure 1**). Blood samples were collected for hormonal assessment during the following phases: second day of menstruation, mid- and late follicular phase, luteinizing hormone (LH)[9] and follicle stimulating hormone (FSH) surges and predicted ovulation, and early, mid-, and late luteal phase. Fertility monitors (Clearblue Easy Fertility Monitor; Inverness Medical) were used to determine the timing of midcycle visits, with other visits scheduled according to an algorithm that took each woman's reported cycle length history into consideration (25). Women were highly compliant with the study protocol; 94% of participants completed at least 7 clinic visits/cycle.

Dietary assessment

Dietary intake was assessed 4 times/cycle by using a 24-h dietary recall on the same days as the sample collection for the menstruation, midfollicular, ovulation, and midluteal phases, for up to 8 recalls over 2 cycles. 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, Minnesota. The percentage of energy from total fats, MUFAs, PUFAs [including ω-3s and ω-6s, and specifically the PUFAs α-linolenic acid (18:3n–3), EPA (20:5n–3), docosapentaenoic acid (22:5n–3), and DHA (22:6n–3)], SFAs, and *trans* fats were calculated from the 24-h dietary recalls. Because the consumption of total fat and specific types of fat did not significantly change across the cycle, we calculated average fat intakes/cycle for this analysis (including up to 4 recalls/cycle). The majority of women completed 4 dietary recalls/cycle (87%). For all statistical models, we used the average cycle-specific fat intake as the exposure of interest.

Reproductive hormones

Reproductive hormones were measured in fasting serum blood samples collected at each cycle visit (8 visits/cycle for 2 cycles) at the Kaleida Health Center for Laboratory Medicine (Buffalo, New York). Total estradiol, FSH, LH, progesterone, and sex hormone–binding globulin (SHBG) were measured by using solid-phase competitive chemiluminescent enzymatic immunoassays by Specialty Laboratories on a DPC Immulite 2000 analyzer (Siemens Medical Solutions Diagnostics). Total testosterone concentrations (ng/dL) were determined by liquid chromatography/tandem mass spectrometry with the use of a Shimadzu Prominence Liquid Chromatogram (Shimadzu Scientific Instruments) with an ABSceix 5500 tandem mass spectrometer (Sciex). Increased sensitivity was obtained by the use of mobile phase B (100% acetonitrile) with a low standard of 4 ng/dL added to the standard curve. Calculations of free (i.e., bioavailable) estradiol and testosterone, and the free androgen index (the ratio of total testosterone to SHBG, multiplied by 100), were performed via standardized methods (26, 27). Across the study period, the CVs for these tests reported by the laboratory were $<10\%$ for estradiol and SHBG, $<5\%$ for LH and FSH, $<14\%$ for progesterone, and $<7\%$ for total testosterone. Sporadic anovulatory cycles were defined as cycles with progesterone concentrations ≤ 5 ng/mL and no observed serum LH peak during the later cycle visits. Specifically, the timing of the LH peak in relation to the peak progesterone measurements was used to inform whether an adequate number of serum samples during the last half of the cycle were obtained to assess an increase in progesterone and evidence of ovulation. On the basis of this algorithm, 42 of the 509 cycles (8.3%) in this study were classified as anovulatory (28 women had a single anovulatory cycle and 7 women had 2 anovulatory cycles during the study period) (28).

Covariate assessment

Participants completed questionnaires and provided information on age, race, lifestyle, and reproductive and health history at baseline. Weight and height were measured by trained research staff using standardized protocols and were used to calculate BMI. In addition, women completed the International Physical Activity Questionnaire long-form 2002, from which high, moderate, and low physical activity categories were calculated on the basis of standard International Physical Activity Questionnaire cutoffs (29). All of the covariates assessed had at least a 94% response rate.

Statistical analysis

Descriptive statistics were calculated for demographic and lifestyle characteristics, for mean dietary intakes across the study

[9] Abbreviations used: FSH, follicle stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone–binding globulin.

(including up to 8 recalls/participant), and mean hormone concentrations across the study (including up to 16 measurements/participant) and compared between tertiles of percentage of energy from total fat intake averaged over the study period. Exact chi-square tests and ANOVAs were used to test for associations between demographic variables and tertiles of percentage of energy from total fat intake.

Linear mixed models with random intercepts were used to evaluate the association between average dietary fat intake (as a percentage of energy for each type of fat) across the cycle (averages calculated on the basis of up to 4 recalls/cycle) and log serum concentrations of estradiol, free estradiol, FSH, LH, luteal progesterone, SHBG, testosterone, free testosterone, and the free androgen index at each cycle visit. The models took repeated measures into account and included up to 16 hormone concentrations/participant (up to 8/cycle). Fat intake was evaluated in tertiles, and trend tests were calculated by using the median intake of fat in each tertile as a continuous variable. Results are presented as percentage changes with 95% CIs. The first model was adjusted for age, total energy intake, BMI, and race and represents a total energy substitution model. This model can be interpreted as the effect of increasing the percentage of energy from fat (or each type of fat) and reducing the intake from all other sources of energy by the same amount while keeping calories constant. To evaluate the association of the substitution of carbohydrates with intake of fat (or each type of fat), we further fit a carbohydrate substitution model. Specifically, this model additionally adjusted for the remaining types of fat (SFAs, MUFAs, PUFAs, and *trans* fats) and protein intake. This model can be interpreted as the effect of increasing the percentage of energy intake from fat (or each type of fat) in place of carbohydrates while keeping total calories constant. We further evaluated the association between substituting one type of fat for another by modeling the intake of specific fats as continuous variables and calculating the difference in coefficients and the covariance matrix to estimate CIs (30). Multivariable RR estimation by Poisson regression with robust error variance was used to estimate RRs and 95% CIs for the associations between average dietary fat intake across the cycle and anovulation (cycle was the unit of analysis). The total energy substitution, carbohydrate substitution, and fat-by-fat substitution models were likewise evaluated. Physical activity, smoking, and additional measures of dietary intake, including fiber, were considered as potential covariates but did not appreciably alter the estimates. SAS version 9.4 (SAS Institute) was used for all statistical analyses.

RESULTS

Overall, women in the BioCycle Study were young (mean age: 27.3 y), of healthy weight (mean BMI: 24.1), physically active (moderate to high physical activity: 90.3%), and nonsmokers (96.1%) (**Table 1**). Smokers were more likely to be in the middle or upper tertile of total fat intake. Total fat intake as a percentage of energy was not associated with age, BMI, physical activity, race, education, marital status, parity, or past oral contraceptive use. Women in the highest tertile of percentage of energy from fat also had a significantly higher mean percentage of energy from SFAs, MUFAs, PUFAs, ω-3s, ω-6s, *trans* fat, and total energy, but lower mean percentage of energy from carbohydrates and fiber intake. Women in this study had, on average, 33.9%, 15.7%, 50.8%, and 1.0% of energy from fat, protein, carbohydrates, and alcohol, respectively. Within-woman CVs for PUFAs, ω-3s, marine ω-3 PUFAs, and the PUFAs α-linolenic acid, EPA, docosapentaenoic acid, and DHA were 77%, 59%, 37%, 61%, 34%, 45%, and 36%, respectively.

Total fat intake, in particular of PUFAs, was significantly and positively associated with total and free testosterone concentrations, and α-linolenic acid was positively associated with free testosterone and the free androgen index (P-trend < 0.05 for both total energy substitution and carbohydrate substitution models for each association listed; **Table 2**). These associations were consistent for both the total energy substitution model (total fat and total testosterone association: highest compared with lowest tertile: 4.0% change; 95% CI: 0.7%, 7.3%) and the carbohydrate substitution model (total fat and total testosterone association: highest compared with lowest tertile: 4.2% change; 95% CI: 0.9%, 7.6%). Thus, increases in total fat or PUFAs, either in place of all other energy sources or carbohydrates in particular, were associated with increases in testosterone.

Marine ω-3 PUFAs (EPA, docosapentaenoic acid, and DHA) were associated with increases in luteal-phase progesterone concentrations. Specifically, intakes of these fatty acids in the third tertile compared with the first tertile were associated with significant increased progesterone concentrations in the carbohydrate substitution models (P-trend < 0.05 for all models; Table 2). Similar results were obtained in the energy substitution models, with significant trends observed between each marine ω-3 PUFA and progesterone, although the association between the third and first tertile was not significant for DHA. There was also a significant trend observed between increasing DHA intakes and increased total estradiol in the energy substitution model (P-trend = 0.04; Table 2), and similarly, between ω-3s and LH concentrations in the energy substitution model (P-trend = 0.04; Table 2). No other associations were observed between fat intakes and estradiol, progesterone, LH, or FSH concentrations. Overall, the substitution of *trans* fat or ω-3s for other types of fat was not associated with reproductive hormone concentrations (**Table 3**).

Total fat, SFA, MFA, and PUFA intakes when considered as percentage of energy from fat were not associated with the risk of a sporadic anovulatory cycle in this study in the total energy substitution, carbohydrate substitution, or fat-by-fat substitution models (**Table 4**). However, we did observe that cycles in which women consumed docosapentaenoic acid in the second and third tertiles of intake had a significantly reduced risk of being anovulatory (RR for tertile 3 compared with tertile 1: 0.42; 95% CI: 0.18, 0.95) in the carbohydrate substitution models. This association was marginally significant in the total energy substitution models (RR for tertile 3 compared with tertile 1: 0.53; 95% CI: 0.27, 1.05; $P = 0.07$).

DISCUSSION

We observed that percentages of energy from total fat, total PUFAs, and α-linolenic acid were associated with modest increases in testosterone concentrations but not with estradiol or anovulation in a cohort of regularly menstruating women. In addition, marine ω-3 fatty acids were associated with increased progesterone concentrations and docosapentaenoic acid was

TABLE 1
Description of the study cohort by tertile of average percentage of energy from total fat intake across the study period[1]

		Tertile of total fat intake			
	Overall	1: 18.3–31.8% of energy	2: >31.8–36.0% of energy	3: >36.0–49.2% of energy	P^2
Women, *n*	259	86	87	86	
Demographic characteristics					
Age, y	27.3 ± 8.2[3]	26.2 ± 7.9	27.3 ± 8.0	28.5 ± 8.6	0.17
BMI, kg/m^2	24.1 ± 3.9	23.9 ± 3.9	23.6 ± 3.7	24.8 ± 4.0	0.11
Physical activity, *n* (%)					0.71
Low	25 (10)	9 (11)	8 (9)	8 (9)	
Moderate	92 (35)	31 (36)	35 (40)	26 (30)	
High	142 (55)	46 (53)	44 (51)	52 (60)	
Race, *n* (%)					0.15
White	154 (60)	45 (52)	51 (58)	58 (67)	
Black	51 (20)	16 (19)	19 (22)	16 (19)	
Other	54 (20)	25 (29)	17 (20)	12 (14)	
High school education or less, *n* (%)	33 (13)	11 (13)	10 (11)	12 (14)	0.87
Current smoker, *n* (%)	10 (4)	0 (0)	4 (5)	6 (7)	0.03
Married, *n* (%)	66 (25)	23 (27)	21 (24)	22 (26)	0.92
Nulliparous, *n* (%)	187 (72)	63 (73)	62 (71)	62 (72)	0.89
Past OC use, *n* (%)	140 (54)	43 (50)	51 (59)	46 (53)	0.79
Dietary intake					
Percentage of energy					
Fat	33.9 ± 5.4	28.0 ± 3.1	33.8 ± 1.1	39.8 ± 3.0	<0.0001
Saturated fat	11.6 ± 2.5	9.3 ± 1.9	11.7 ± 1.6	13.6 ± 2.0	<0.0001
Monounsaturated fat	12.6 ± 2.4	10.3 ± 1.6	12.5 ± 1.0	14.9 ± 1.6	<0.0001
Polyunsaturated fat	7.0 ± 1.6	6.1 ± 1.3	6.9 ± 1.5	8.1 ± 1.5	<0.0001
ω-3s	0.8 ± 0.3	0.7 ± 0.3	0.8 ± 0.2	0.8 ± 0.2	<0.0001
ω-6s	6.3 ± 1.5	5.4 ± 1.1	6.2 ± 1.3	7.2 ± 1.3	<0.0001
trans Fat	2.3 ± 0.8	1.8 ± 0.6	2.3 ± 0.7	2.7 ± 0.8	<0.0001
Protein	15.7 ± 2.9	15.4 ± 2.7	15.9 ± 3.3	15.9 ± 2.7	0.51
Carbohydrates	50.8 ± 7.1	57.8 ± 4.8	50.5 ± 4.2	44.3 ± 4.5	<0.0001
Alcohol	1.0 ± 2.0	0.6 ± 1.3	1.3 ± 2.5	1.2 ± 1.8	0.03
Total energy intake, kcal	1607.4 ± 354.3	1512.9 ± 311.6	1634.6 ± 341.6	1674.6 ± 389.4	0.007
Fiber, g/d	13.6 ± 5.6	15.2 ± 6.9	13.3 ± 4.7	12.2 ± 4.3	0.002
ω-3s, g/d	1.4 ± 0.5	1.1 ± 0.5	1.4 ± 0.5	1.6 ± 0.6	<0.0001
α-Linolenic acid, g/d	1.3 ± 0.5	1.0 ± 0.4	1.3 ± 0.5	1.5 ± 0.5	<0.0001
Marine PUFAs, g/d	0.10 ± 0.13	0.10 ± 0.15	0.11 ± 0.12	0.11 ± 0.11	0.92
EPA, g/d	0.02 ± 0.04	0.03 ± 0.05	0.03 ± 0.03	0.02 ± 0.03	0.94
Docosapentaenoic acid, g/d	0.02 ± 0.01	0.01 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	0.37
DHA, g/d	0.06 ± 0.08	0.06 ± 0.10	0.06 ± 0.07	0.06 ± 0.07	0.88
Reproductive hormones					
Estradiol, pg/mL	111.3 ± 39.6	110.0 ± 40.9	113.0 ± 37.0	110.7 ± 41.3	0.88
Free estradiol, pg/mL	1.7 ± 0.6	1.8 ± 0.7	1.7 ± 0.5	1.7 ± 0.6	0.79
FSH, mIU/mL	6.4 ± 2.2	6.1 ± 1.9	6.4 ± 2.3	6.7 ± 2.3	0.11
LH, ng/mL	9.5 ± 3.1	9.1 ± 2.7	9.5 ± 3.0	9.9 ± 3.5	0.19
Luteal progesterone,[4] ng/mL	7.1 ± 3.3	7.0 ± 3.6	7.3 ± 3.1	7.0 ± 3.2	0.52
SHBG, nmol/L	48.0 ± 21.0	45.3 ± 18.3	51.2 ± 22.1	47.5 ± 22.2	0.18
Testosterone, ng/dL	30.2 ± 10.0	29.6 ± 8.6	29.8 ± 10.2	31.4 ± 11.1	0.67
Free testosterone, ng/dL	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.30
Free androgen index	2.7 ± 1.6	2.7 ± 1.3	2.4 ± 1.1	3.0 ± 2.2	0.10

[1]Six women were missing information on parity and 4 were missing information on past OC use. FSH, follicle stimulating hormone; LH, luteinizing hormone; OC, oral contraceptive; SHBG, sex hormone–binding globulin.

[2]*P* values were based on ANOVA or Fisher's exact test, where appropriate, and for reproductive hormones were based on the mean of the log hormone concentrations.

[3]Mean ± SD (all such values).

[4]Mean concentrations of measurements during the luteal-phase visits.

associated with a decreased risk of anovulation. These associations remained significant even after adjustment for other factors related to both hormone concentrations and anovulation. These findings highlight the potential role of fatty acids in androgen synthesis, although the clinical implications of these subtle associations between dietary fat intakes and testosterone concentrations are unknown.

Our findings of increased testosterone in response to higher fat intakes are consistent with a few small studies that found lower testosterone or lower dehydroepiandrosterone sulfate in women

TABLE 2
Association between specific fatty acids and reproductive hormone concentrations in healthy premenopausal women[1]

	Total energy substitution model[2]			Carbohydrate substitution model[3]		
Type of fat	Tertile 2 vs. tertile 1[4]	Tertile 3 vs. tertile 1	*P*-trend[4]	Tertile 2 vs. tertile 1	Tertile 3 vs. tertile 1	*P*-trend[4]
Total fat, % of energy						
Estradiol, pg/mL	−1.9 (−7.6, 4.2)	−3.5 (−9.4, 2.8)	0.27	−2.1 (−7.9, 4.0)	−3.8 (−9.8, 2.5)	0.23
Free estradiol, pg/mL	−2.8 (−8.4, 3.1)	−3.6 (−9.4, 2.5)	0.26	−3.0 (−8.5, 2.9)	−3.8 (−9.7, 2.3)	0.23
FSH, mIU/mL	1.5 (−3.4, 6.7)	2.7 (−2.6, 8.2)	0.32	1.6 (−3.3, 6.9)	2.9 (−2.4, 8.5)	0.29
LH, ng/mL	−2.4 (−8.5, 4.1)	5.0 (−1.9, 12.4)	0.12	−2.4 (−8.5, 4.2)	5.0 (−2.0, 12.4)	0.12
Progesterone,[5] ng/mL	−11.0 (−24.6, 5.0)	−13.7 (−27.3, 2.5)	0.10	−11.5 (−25.0, 4.5)	−14.4 (−28.0, 1.8)	0.09
SHBG, nmol/L	−2.4 (−6.9, 2.3)	−0.3 (−5.3, 4.9)	0.65	−1.6 (−5.4, 2.3)	−0.2 (−4.3, 4.1)	0.99
Testosterone, ng/dL	1.4 (−1.5, 4.4)	4.0 (0.7, 7.3)*	0.01*	1.6 (−1.3, 4.6)	4.2 (0.9, 7.6)*	0.01*
Free testosterone, ng/dL	0.7 (−2.6, 4.0)	4.1 (0.5, 7.7)*	0.02*	0.8 (−2.4, 4.2)	4.3 (0.7, 7.9)*	0.02*
Free androgen index	1.2 (−3.6, 6.3)	4.3 (−1.1, 10.0)	0.11	1.3 (−3.6, 6.5)	4.4 (−1.0, 10.1)	0.10
Saturated fat, % of energy						
Estradiol, pg/mL	−0.2 (−6.1, 6.1)	−3.2 (−9.5, 3.6)	0.27	−0.9 (−7.2, 5.8)	−4.4 (−11.6, 3.4)	0.21
Free estradiol, pg/mL	0.3 (−5.5, 6.5)	−3.6 (−5.5, 6.5)	0.17	0.3 (−6.0, 6.9)	−3.8 (−10.9, 3.8)	0.18
FSH, mIU/mL	−0.7 (−5.6, 4.4)	−0.8 (−6.2, 4.9)	0.87	−0.9 (−6.2, 4.6)	−0.9 (−7.0, 5.8)	0.93
LH, ng/mL	−2.4 (−8.6, 4.2)	2.4 (−4.7, 10.1)	0.24	−1.5 (−8.2, 5.7)	4.4 (−4.0, 13.4)	0.11
Progesterone, ng/mL	0.1 (−15.4, 18.4)	−13.2 (−27.5, 4.1)	0.07	9.6 (−8.3, 31.1)	0.0 (−18.8, 23.1)	0.48
SHBG, nmol/L	−1.1 (−4.9, 2.9)	0.8 (−3.5, 5.4)	0.45	−2.9 (−6.9, 1.3)	−2.0 (−6.8, 3.1)	0.99
Testosterone, ng/dL	−0.2 (−3.1, 2.8)	0.1 (−3.2, 3.6)	0.90	0.1 (−3.0, 3.4)	0.9 (−3.0, 4.8)	0.62
Free testosterone, ng/dL	−0.6 (−3.8, 2.7)	−0.5 (−4.2, 3.4)	0.92	0.0 (−3.4, 3.6)	0.8 (−3.3, 5.2)	0.63
Free androgen index	−0.3 (−5.2, 4.8)	−1.2 (−6.7, 4.6)	0.68	1.4 (−3.8, 7.0)	1.9 (−4.4, 8.8)	0.71
Monounsaturated fat, % of energy						
Estradiol, pg/mL	−0.4 (−6.4, 5.9)	−2.4 (−8.4, 4.1)	0.44	1.3 (−5.4, 8.4)	0.8 (−7.7, 10.0)	0.90
Free estradiol, pg/mL	−1.2 (−6.9, 4.9)	−3.1 (−9.0, 3.0)	0.31	0.3 (−6.1, 7.3)	−0.3 (−8.4, 8.7)	0.94
FSH, mIU/mL	2.9 (−2.2, 8.3)	3.5 (−1.9, 9.1)	0.23	3.4 (−2.3, 9.4)	3.3 (−3.9, 11.1)	0.44
LH, ng/mL	0.4 (−6.1, 7.3)	1.7 (−5.1, 8.9)	0.61	−0.9 (−8.0, 6.6)	−1.9 (−10.7, 7.8)	0.69
Progesterone, ng/mL	4.3 (−11.8, 23.2)	−18.1 (−31.1, −2.6)*	0.01*	9.4 (−9.0, 31.7)	−10.9 (−29.6, 12.9)	0.24
SHBG, nmol/L	0.3 (−3.6, 4.3)	3.1 (−1.0, 7.6)	0.11	0.7 (−3.7, 5.2)	3.7 (−2.2, 9.7)	0.19
Testosterone, ng/dL	1.6 (−1.4, 4.6)	2.5 (−0.7, 5.8)	0.13	1.4 (−2.0, 4.8)	1.1 (−3.1, 5.5)	0.68
Free testosterone, ng/dL	1.5 (−1.8, 4.9)	2.0 (−1.5, 5.7)	0.27	1.6 (−2.1, 5.4)	1.2 (−3.5, 6.2)	0.70
Free androgen index	1.3 (−3.6, 6.5)	0.6 (−4.5, 6.1)	0.86	1.9 (−3.6, 7.8)	0.9 (−6.2, 8.5)	0.87
Polyunsaturated fat, % of energy						
Estradiol, pg/mL	−0.9 (−6.6, 5.1)	−0.2 (−6.2, 6.2)	0.98	−1.6 (−7.3, 4.4)	−2.3 (−8.8, 4.6)	0.52
Free estradiol, pg/mL	−0.3 (−5.9, 5.7)	−0.7 (−6.6, 5.4)	0.81	−0.7 (−6.4, 5.4)	−1.7 (−8.1, 5.2)	0.62
FSH, mIU/mL	3.5 (−1.5, 8.7)	4.1 (−1.2, 9.5)	0.15	3.5 (−1.6, 8.8)	3.8 (−2.1, 9.9)	0.24
LH, ng/mL	1.4 (−4.8, 8.1)	6.4 (−0.4, 13.7)	0.06	1.6 (−4.8, 8.4)	7.3 (−0.4, 15.5)	0.06
Progesterone, ng/mL	8.9 (−7.6, 28.3)	8.8 (−8.2, 28.9)	0.37	12.7 (−4.5, 33.1)	18.8 (−1.5, 43.2)	0.08
SHBG, nmol/L	0.8 (−2.9, 4.7)	2.8 (−1.2, 7.0)	0.16	0.2 (−3.5, 4.1)	1.0 (−3.4, 5.5)	0.65
Testosterone, ng/dL	1.3 (−1.5, 4.3)	3.7 (0.6, 6.8)*	0.02*	1.4 (−1.5, 4.4)	4.2 (0.8, 7.8)*	0.01*
Free testosterone, ng/dL	1.6 (−1.5, 4.8)	4.0 (0.5, 7.5)*	0.02*	1.8 (−1.4, 5.1)	4.7 (0.9, 8.7)*	0.01*
Free androgen index	1.8 (−3.0, 6.8)	2.6 (−2.5, 8.0)	0.32	2.3 (−2.4, 7.5)	4.4 (−1.3, 10.4)	0.14
ω-3s, % of energy						
Estradiol, pg/mL	−0.7 (−6.2, 5.1)	2.7 (−3.4, 9.3)	0.38	0.1 (−5.9, 6.4)	3.0 (−4.4, 11.1)	0.40
Free estradiol, pg/mL	−2.0 (−7.3, 3.7)	2.9 (−3.1, 9.3)	0.32	−1.0 (−6.8, 5.2)	4.3 (−3.1, 12.3)	0.22
FSH, mIU/mL	1.5 (−3.2, 6.4)	1.5 (−3.6, 6.9)	0.58	0.6 (−4.5, 5.9)	−0.4 (−6.5, 6.1)	0.88
LH, ng/mL	7.6 (1.2, 14.3)*	7.5 (0.6, 14.9)*	0.04*	5.7 (−1.1, 12.9)	4.3 (−3.8, 13.1)	0.36
Progesterone, ng/mL	14.2 (−2.7, 34.0)	18.9 (0.4, 40.6)	0.05	15.0 (−2.9, 36.2)	20.1 (−2.2, 47.3)	0.09
SHBG, nmol/L	1.5 (−2.0, 5.2)	−1.6 (−5.5, 2.4)	0.41	1.8 (−2.0, 5.8)	−2.1 (−6.7, 2.7)	0.32
Testosterone, ng/dL	1.7 (−1.0, 4.5)	3.6 (0.5, 6.8)*	0.02*	0.7 (−2.2, 3.7)	2.1 (−1.5, 5.9)	0.25
Free testosterone, ng/dL	1.5 (−1.5, 4.6)	4.9 (1.4, 8.4)*	0.01*	0.4 (−2.8, 3.7)	3.3 (−0.9, 7.5)	0.11
Free androgen index	0.1 (−4.3, 4.8)	5.9 (0.6, 11.4)*	0.02*	−1.1 (−5.8, 3.9)	4.5 (−1.7, 11.1)	0.12
ω-6s, % of energy						
Estradiol, pg/mL	−0.6 (−6.2, 5.4)	0.0 (−5.9, 6.4)	0.96	−1.2 (−7.1, 5.0)	−1.7 (−9.0, 6.3)	0.69
Free estradiol, pg/mL	−0.1 (−5.6, 5.9)	−0.7 (−6.5, 5.5)	0.82	−0.9 (−6.8, 5.2)	−2.7 (−9.8, 5.0)	0.48
FSH, mIU/mL	3.0 (−1.8, 8.2)	3.7 (−1.5, 9.1)	0.19	2.3 (−2.9, 7.7)	1.8 (−4.6, 8.7)	0.65
LH, ng/mL	0.7 (−5.4, 7.1)	6.2 (−0.5, 13.4)	0.06	−0.9 (−7.2, 6.0)	2.7 (−5.5, 11.7)	0.47
Progesterone, ng/mL	10.6 (−6.1, 30.2)	7.9 (−8.9, 27.8)	0.45	13.4 (−4.4, 34.4)	13.3 (−8.2, 40.1)	0.30
SHBG, nmol/L	1.6 (−2.0, 5.4)	2.2 (−1.8, 6.4)	0.30	2.0 (−1.9, 6.1)	2.9 (−2.2, 8.2)	0.29
Testosterone, ng/dL	1.4 (−1.4, 4.3)	3.8 (0.7, 6.9)*	0.01*	1.0 (−2.0, 4.1)	3.1 (−0.7, 7.1)	0.10
Free testosterone, ng/dL	1.5 (−1.5, 4.7)	4.0 (0.6, 7.6)*	0.02*	0.9 (−2.4, 4.3)	2.8 (−1.5, 7.3)	0.19
Free androgen index	1.1 (−3.5, 6.0)	2.7 (−2.4, 8.0)	0.29	0.2 (−4.7, 5.3)	0.9 (−5.4, 7.6)	0.77

(Continued)

TABLE 2 (*Continued*)

	Total energy substitution model[2]			Carbohydrate substitution model[3]		
Type of fat	Tertile 2 vs. tertile 1[4]	Tertile 3 vs. tertile 1	*P*-trend[4]	Tertile 2 vs. tertile 1	Tertile 3 vs. tertile 1	*P*-trend[4]
trans Fat, % of energy						
Estradiol, pg/mL	1.1 (−4.9, 7.5)	−0.5 (−6.6, 6.0)	0.83	1.6 (−4.5, 8.2)	−0.1 (−6.9, 7.3)	0.92
Free estradiol, pg/mL	0.2 (−5.6, 6.4)	−0.5 (−6.5, 5.8)	0.85	1.0 (−5.0, 7.4)	0.7 (−6.1, 7.9)	0.87
FSH, mIU/mL	3.4 (−1.7, 8.8)	0.8 (−4.3, 6.2)	0.90	3.4 (−1.9, 8.9)	0.2 (−5.5, 6.3)	0.93
LH, ng/mL	3.9 (−2.7, 11.0)	1.0 (−5.5, 8.0)	0.88	4.2 (−2.6, 11.3)	1.8 (−5.6, 9.9)	0.71
Progesterone, ng/mL	−7.3 (−21.6, 9.5)	−16.7 (−29.7, −1.2)*	0.03*	−2.8 (−18.0, 15.4)	−6.9 (−23.2, 12.7)	0.46
SHBG, nmol/L	1.1 (−2.8, 5.1)	2.0 (−2.1, 6.3)	0.35	0.4 (−3.5, 4.4)	−0.4 (−4.8, 4.2)	0.83
Testosterone, ng/dL	1.5 (−1.5, 4.6)	2.3 (−0.8, 5.5)	0.15	1.7 (−1.2, 4.8)	3.1 (−0.3, 6.7)	0.08
Free testosterone, ng/dL	1.3 (−2.0, 4.7)	1.5 (−1.9, 5.0)	0.42	1.7 (−1.6, 5.1)	2.7 (−1.1, 6.7)	0.17
Free androgen index	−0.2 (−5.1, 4.9)	−0.4 (−5.4, 4.9)	0.88	0.6 (−4.3, 5.9)	2.1 (−3.6, 8.1)	0.47
α-Linolenic acid, g/d						
Estradiol, pg/mL	−0.4 (−6.3, 5.9)	0.6 (−6.3, 7.9)	0.84	−0.6 (−6.5, 5.8)	0.0 (−6.9, 7.5)	0.96
Free estradiol, pg/mL	1.8 (−4.1, 8.1)	1.8 (−5.0, 9.2)	0.64	1.8 (−4.2, 8.1)	1.8 (−5.2, 9.2)	0.66
FSH, mIU/mL	2.3 (−2.7, 7.7)	2.3 (−3.4, 8.5)	0.50	2.4 (−2.7, 7.9)	2.1 (−3.8, 8.4)	0.57
LH, ng/mL	5.4 (−11.8, 12.6)	7.6 (−0.2, 16.1)	0.08	5.2 (−1.6, 12.4)	7.4 (−0.6, 16.0)	0.09
Progesterone, ng/mL	11.3 (−6.0, 31.8)	−0.8 (−18.3, 20.4)	0.75	12.6 (−4.8, 33.1)	2.3 (−15.8, 24.5)	0.99
SHBG, nmol/L	−4.0 (−7.7, −0.2)*	−3.2 (−7.6, 1.2)	0.25	−4.1 (−7.8, −0.3)*	−4.0 (−8.2, 0.5)	0.14
Testosterone, ng/dL	−0.1 (−3.1, 2.8)	2.8 (−0.6, 6.5)	0.07	−0.1 (−3.1, 2.9)	2.7 (−0.8, 6.4)	0.08
Free testosterone, ng/dL	1.7 (−2.2, 4.4)	5.2 (1.3, 9.3)*	0.01*	1.1 (−2.2, 4.5)	5.3 (1.3, 9.4)*	0.01*
Free androgen index	4.0 (−1.0, 9.3)	8.2 (2.1, 14.6)*	0.01*	4.1 (−1.0, 9.4)	8.8 (2.6, 15.3)*	0.01*
EPA, g/d						
Estradiol, pg/mL	−1.9 (−7.5, 4.1)	5.9 (−0.5, 12.6)	0.02*	−2.0 (−7.8, 4.3)	5.5 (−1.3, 12.9)	0.03*
Free estradiol, pg/mL	−1.5 (−7.0, 4.4)	6.1 (−0.1, 12.6)	0.02*	−1.3 (−7.0, 4.9)	6.4 (−0.4, 13.7)	0.02*
FSH, mIU/mL	−1.2 (−5.9, 3.9)	−1.6 (−6.6, 3.6)	0.60	−1.2 (−6.2, 4.1)	−1.9 (−7.2, 3.9)	0.59
LH, ng/mL	−0.9 (−7.0, 5.7)	0.8 (−5.6, 7.8)	0.70	−1.0 (−7.4, 5.9)	1.2 (−5.9, 8.9)	0.60
Progesterone, ng/mL	−15.2 (−27.9, −0.2)*	19.4 (1.2, 40.9)*	0.002*	−12.8 (−26.2, 3.1)	22.1 (1.9, 46.4)*	0.001*
SHBG, nmol/L	2.0 (−1.8, 5.9)	−0.1 (−4.1, 4.1)	0.62	1.9 (−2.0, 6.0)	−0.3 (−4.6, 4.2)	0.53
Testosterone, ng/dL	1.6 (−1.3, 4.6)	1.1 (−2.0, 4.3)	0.72	2.0 (−1.0, 5.1)	1.9 (−1.5, 5.3)	0.50
Free testosterone, ng/dL	1.4 (−1.8, 4.7)	1.4 (−2.0, 4.9)	0.56	1.8 (−1.5, 5.3)	2.2 (−1.5, 6.2)	0.38
Free androgen index	0.3 (−4.4, 5.3)	1.3 (−3.8, 6.6)	0.62	0.8 (−4.1, 6.1)	2.1 (−3.5, 8.0)	0.48
Docosapentaenoic acid, g/d						
Estradiol, pg/mL	−2.5 (−8.2, 3.6)	2.2 (−4.1, 9.1)	0.33	−3.1 (−8.9, 3.1)	1.1 (−6.1, 8.9)	0.55
Free estradiol, pg/mL	−1.4 (−7.0, 4.6)	0.9 (−5.4, 7.5)	0.68	−1.7 (−7.5, 4.5)	0.1 (−6.9, 7.7)	0.86
FSH, mIU/mL	−0.7 (−5.5, 4.4)	3.3 (−2.2, 9.0)	0.16	−0.1 (−5.2, 5.1)	4.4 (−1.8, 11.1)	0.11
LH, ng/mL	−0.5 (−6.8, 6.2)	0.5 (−6.2, 7.8)	0.83	−0.4 (−6.9, 6.5)	1.1 (−6.8, 9.5)	0.74
Progesterone, ng/mL	10.3 (−6.5, 30.1)	20.0 (0.5, 43.2)*	0.05*	13.7 (−4.2, 34.9)	27.3 (3.8, 55.9)*	0.03*
SHBG, nmol/L	−0.5 (−4.3, 3.5)	1.0 (−3.2, 5.4)	0.56	−0.8 (−4.6, 3.3)	0.6 (−4.2, 5.5)	0.72
Testosterone, ng/dL	−2.7 (−5.4, 0.2)	−1.0 (−4.1, 2.2)	0.86	−2.5 (−5.4, 0.5)	−0.4 (−4.0, 3.4)	0.84
Free testosterone, ng/dL	−2.7 (−5.8, 0.5)	−1.3 (−4.8, 2.2)	0.72	−2.5 (−5.7, 0.8)	−0.8 (−4.8, 3.4)	0.98
Free androgen index	−2.5 (−7.1, 2.4)	−2.3 (−7.4, 3.1)	0.51	−2.3 (−7.1, 2.8)	−1.8 (−7.7, 4.4)	0.67
DHA, g/d						
Estradiol, pg/mL	−1.5 (−7.1, 4.6)	5.4 (−1.1, 12.4)	0.04*	−1.5 (−7.4, 4.8)	4.9 (−2.1, 12.4)	0.07
Free estradiol, pg/mL	−0.8 (−6.5, 5.2)	5.3 (−1.1, 12.1)	0.05*	−0.4 (−6.3, 5.8)	5.4 (−1.5, 12.9)	0.07
FSH, mIU/mL	1.3 (−3.6, 6.5)	0.3 (−4.9, 5.8)	0.96	1.3 (−3.8, 6.7)	0.5 (−5.2, 6.5)	0.99
LH, ng/mL	−1.5 (−7.6, 5.0)	1.3 (−5.4, 8.5)	0.55	−1.6 (−8.0, 5.2)	1.7 (−5.6, 9.6)	0.48
Progesterone, ng/mL	−8.6 (−22.4, 7.6)	18.2 (−0.5, 40.4)	0.01*	−5.0 (−19.7, 12.5)	21.7 (0.7, 46.8)*	0.01*
SHBG, nmol/L	0.0 (−3.8, 3.9)	−1.0 (−5.1, 3.3)	0.59	−0.5 (−4.4, 3.5)	−1.3 (−5.6, 3.3)	0.57
Testosterone, ng/dL	1.7 (−1.3, 4.7)	2.3 (−0.9, 5.7)	0.21	1.9 (−1.1, 5.0)	3.3 (−0.2, 6.8)	0.10
Free testosterone, ng/dL	1.5 (−1.7, 4.9)	2.4 (−1.2, 6.1)	0.24	1.9 (−1.4, 5.4)	3.3 (−0.6, 7.3)	0.13
Free androgen index	1.4 (−3.4, 6.5)	2.4 (−2.9, 8.1)	0.41	2.1 (−3.0, 7.4)	3.4 (−2.5, 9.4)	0.32

[1]Values are percentage changes with 95% CIs in parentheses. Linear mixed models with random intercepts were used to evaluate associations between average dietary fat intake (as percentage of energy for each type of fat) across the cycle (averages calculated on the basis of up to 4 recalls/cycle) and log serum concentrations of reproductive hormones at each cycle visit. The models took repeated measures into account and included up to 16 hormone concentrations/participant (up to 8/cycle) for a total of 3899 study visits with dietary intake and hormone concentrations. *$P < 0.05$. FSH, follicle stimulating hormone; LH, luteinizing hormone; ref, reference; SHBG, sex hormone–binding globulin.

[2]Adjusted for age, race, BMI, and total energy intake.

[3]Adjusted for age, race, BMI, and total energy intake, plus intake for the remaining types of fat (saturated, monounsaturated, polyunsaturated, and *trans* fat) and protein intake.

[4]Calculated with the median intake of fat in each tertile as a continuous variable.

[5]Includes only measurements of progesterone during the luteal phase.

TABLE 3
Association between substitution of specific fatty acids for other types of fats and risk of anovulation and log-transformed reproductive hormone concentrations in healthy premenopausal women[1]

	Substitution of *trans* fat with			Substitution of ω-3s with		
Outcome	SFAs	MUFAs	ω-3s	SFAs	MUFAs	*trans* Fat
Anovulation[2]	1.24 (0.77, 1.97)	1.23 (0.74, 2.02)	0.80 (0.30, 2.14)	1.54 (0.55, 4.33)	1.53 (0.58, 4.06)	1.25 (0.47, 3.34)
Estradiol,[3] pg/mL	−1.0 (−4.4, 2.4)	−3.5 (−7.4, 0.6)	−3.8 (−13.8, 7.5)	2.8 (−7.9, 14.9)	0.3 (−10.1, 11.9)	3.9 (−6.9, 16.1)
FSH,[3] mIU/mL	1.0 (−1.9, 3.9)	0.6 (−2.8, 4.1)	−2.4 (−11.0, 7.1)	3.4 (−5.7, 13.4)	3.0 (−6.0, 12.9)	2.4 (−6.6, 12.3)
LH,[3] ng/mL	−0.8 (−4.4, 2.9)	0.2 (−4.1, 4.7)	−9.7 (−19.8, 1.7)	9.8 (−2.5, 23.8)	10.9 (−1.4, 24.8)	10.7 (−1.7, 24.7)
Progesterone,[3,4] ng/mL	−5.9 (−14.1, 3.2)	−6.6 (−16.4, 4.3)	−22.4 (−42.0, 4.0)	21.3 (−9.6, 62.7)	20.3 (−10.1, 60.9)	28.8 (−3.8, 72.5)
SHBG,[3] nmol/L	0.4 (−1.7, 2.7)	−1.2 (−3.8, 1.5)	6.6 (−0.9, 14.7)	−5.8 (−12.4, 1.4)	−7.3 (−13.7, −0.5)*	−6.2 (−12.8, 0.9)
Free testosterone,[3] ng/dL	0.03 (−1.8, 1.9)	1.1 (−1.1, 3.4)	−2.8 (−9.5, 3.3)	2.9 (−3.1, 9.2)	4.0 (−1.9, 10.3)	2.9 (−3.2, 9.2)
Free androgen index[3]	−0.4 (−3.1, 2.4)	1.4 (−1.9, 4.9)	−6.8 (−15.0, 2.2)	6.9 (−2.5, 17.1)	8.8 (−0.5, 19.0)	7.3 (−2.1, 17.6)

[1]Linear mixed models with random intercepts were used to evaluate associations with reproductive hormones, and multivariable RR estimation by Poisson regression with robust error variance was used to estimate RRs for anovulation induced by substituting 1 type of fat for another by modeling the intake of specific fats as continuous variables and calculating the difference in coefficients and the covariance matrix to estimate CIs. The average dietary fat intake (as a percentage of energy for each type of fat) across the cycle was calculated on the basis of up to 4 recalls/cycle. The models take repeated measures into account and included up to 16 hormone concentrations/participant (up to 8/cycle) for a total of 3899 study visits with dietary intake and hormone concentrations. Models were adjusted for age, race, BMI, and total energy intake. *$P < 0.05$. FSH, follicle stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone–binding globulin.

[2]Values are RRs; 95% CIs in parentheses.

[3]Values are percentage changes; 95% CIs in parentheses.

[4]Includes only measurements of progesterone during the luteal phase.

consuming low-fat and high-fiber diets (17, 31) and with studies that observed increases in testosterone and dehydroepiandrosterone sulfate in women consuming high-fat diets (32, 33). Not all previous studies observed associations with androgens (12, 15, 19, 20, 34) or found associations in the other direction (decreased testosterone with high fat intakes) (35). Our results connecting total fat, PUFAs, and androgens are consistent with hypotheses that fatty acids positively influence steroidogenesis. Specifically, ω-6 fatty acids are precursors for prostaglandin synthesis and can modulate the expression of key enzymes involved in prostaglandin and steroid metabolism (2). A small hospital-based study showed that plasma ω-6 PUFAs were associated with increased androgens, although supplementation with ω-3 PUFAs decreased the concentrations of bioavailable testosterone (36). Although we observed increases in androgens with increasing ω-3 PUFAs, this may suggest that the ratio of these 2 fatty acids is important to consider in this relation. However, when we evaluated the ratio we observed no associations with total testosterone concentrations (third tertile compared with first tertile percentage change: 4.0%; 95% CI: 2.6%, 3.6%; P-trend = 0.65). Furthermore, in women with certain ovulatory disorders, specifically polycystic ovary syndrome, PUFAs have been shown to improve metabolic and endocrine characteristics, although improvements in ovulation have not been evaluated directly (37). The clinical implications of subtle associations between dietary fats and testosterone concentrations remain unknown to date and indeed may be different in other populations, such as women with chronic anovulation or other reproductive disorders. It may also be that these subtle changes in testosterone concentrations may not be clinically relevant, because it has been observed that women with clinical hirsutism may have serum testosterone concentrations within the normal range and conversely that women without a complaint of hirsutism may have elevated testosterone concentrations (38, 39). Thus, relations between fatty acids and androgen synthesis, including the effects in populations with a wider range of hormonal fluctuations or gynecologic disorders, effects of dietary interventions, and Mendelian randomization studies to aid causal inference (40, 41) merit further study and our results highlight the potential for dietary components to modulate hormone metabolism.

The lack of association with estradiol in the present study is consistent with much of the previous literature (12–20). A study in the Nurses' Health Study II cohort found no associations between fat intake and estradiol concentrations in 595 premenopausal women; the researchers also concluded that no strong, consistent associations exist in the literature (19). Typically, intervention trials have evaluated the impact of a diet low in fat and high in fiber and found reduced estradiol concentrations in response (13–15, 17, 18). However, not all of the studies observed significant effects or evaluated fat independent of fiber, and the overall evidence can be considered weak (16, 19). With respect to dietary assessment tools, others relied on food-frequency questionnaires, instead of multiple 24-h recalls as in the present study, and had fewer measures across the cycle (typically 3) and were generally timed by cycle day. Previously, we observed that high-fiber diets were associated with reduced estradiol concentrations in the BioCycle Study (42). Interestingly, in studies that tried to distinguish the effects of fat compared with fiber, it appears that high fiber intake may drive the reduction in estradiol concentrations (17), although others concluded that fat affects estrogen metabolism more than fiber (13). Further supporting the independent role of fiber in altering estradiol concentrations, we found no effects of dietary fatty acid intake on estradiol concentrations, and additional adjustment for fiber intake did not appreciably alter our estimates (results not shown).

The literature that describes relations between dietary fats and hormones, such as LH and FSH, is more limited, although a few studies commented on fat intake and menstrual cycle length and

TABLE 4
Associations between specific fatty acids and risk of anovulation in healthy premenopausal women[1]

			Model 1[2]		Model 2[3]	
Tertile	Ovulatory, *n*	Anovulatory, *n* (%)	RR (95% CI)	*P*-trend[4]	RR (95% CI)	*P*-trend[4]
Total fat, % of energy						
1	157	12 (7)	1 (Reference)		1 (Reference)	
2	154	16 (9)	1.31 (0.61,2.80)	0.41	1.33 (0.62,2.89)	0.38
3	156	14 (8)	1.37 (0.64, 2.92)		1.41 (0.65, 3.04)	
Saturated fat, % of energy						
1	153	16 (9)	1 (Reference)		1 (Reference)	
2	157	13 (8)	0.93 (0.44, 1.97)	0.75	0.82 (0.36, 1.91)	0.94
3	157	13 (8)	1.08 (0.50, 2.33)		0.87 (0.37, 2.04)	
Monounsaturated fat, % of energy						
1	157	12 (7)	1 (Reference)		1 (Reference)	
2	156	14 (8)	1.12 (0.52, 2.43)	0.38	1.18 (0.49, 2.88)	0.46
3	154	16 (9)	1.34 (0.68, 2.67)		1.46 (0.53, 4.04)	
Polyunsaturated fat, % of energy						
1	154	15 (9)	1 (Reference)		1 (Reference)	
2	157	14 (8)	0.87 (0.46, 1.68)	0.64	0.79 (0.42, 1.49)	0.29
3	156	13 (8)	0.84 (0.41, 1.73)		0.63 (0.27, 1.47)	
ω-3s, % of energy						
1	154	15 (9)	1 (Reference)		1 (Reference)	
2	157	13 (8)	0.92 (0.49, 1.72)	0.99	0.91 (0.43, 1.91)	0.85
3	156	14 (8)	0.81 (0.39, 1.67)		1.08 (0.44, 2.65)	
ω-6s, % of energy						
1	154	15 (9)	1 (Reference)		1 (Reference)	
2	156	14 (8)	0.92 (0.49, 1.72)	0.56	0.76 (0.40, 1.41)	0.11
3	157	13 (8)	0.81 (0.39, 1.67)		0.46 (0.18, 1.19)	
trans Fat, % of energy						
1	155	14 (8)	1 (Reference)		1 (Reference)	
2	158	12 (7)	0.99 (0.48, 2.03)	0.65	0.97 (0.47, 1.99)	0.88
3	154	16 (9)	1.18 (0.56, 2.48)		1.06 (0.47, 2.37)	
α-Linolenic acid						
1	153	16 (9)	1 (Reference)		1 (Reference)	
2	163	8 (5)	0.50 (0.22, 1.12)	0.21	0.49 (0.22, 1.13)	0.28
3	151	18 (11)	1.44 (0.73, 2.86)		1.37 (0.67, 2.78)	
EPA						
1	178	17 (9)	1 (Reference)		1 (Reference)	
2	129	14 (10)	1.04 (0.54, 1.97)	0.38	1.01 (0.53, 1.91)	0.38
3	160	11 (6)	0.75 (0.36, 1.57)		0.71 (0.30, 1.67)	
Docosapentaenoic acid						
1	143	19 (12)	1 (Reference)		1 (Reference)	
2	169	12 (7)	0.51 (0.26, 1.00)	0.13	0.48 (0.23, 0.98)*	0.09
3	155	11 (7)	0.53 (0.27, 1.05)		0.42 (0.18, 0.95)*	
DHA						
1	156	18 (10)	1 (Reference)		1 (Reference)	
2	156	11 (7)	0.56 (0.30, 1.04)	0.54	0.53 (0.27, 1.03)	0.52
3	155	13 (8)	0.69 (0.35, 1.39)		0.62 (0.26, 1.50)	

[1]Multivariable RR estimation by Poisson regression with robust error variance was used to estimate RRs and 95% CIs for the associations between average dietary fat intake across the cycle and anovulation (cycle was the unit of analysis with a total of 509 cycles evaluated). *$P < 0.05$.

[2]Adjusted for age, race, BMI, and total energy intake.

[3]Adjusted for age, race, BMI, and total energy intake, plus intake for the remaining types of fat (saturated, monounsaturated, polyunsaturated, and *trans* fat) and protein intake.

[4]Calculated with the median intake of fat in each tertile as a continuous variable.

phase lengths (9, 10). Overall, these small studies generally found that greater fat intakes improved menstrual cycle characteristics, but the relation between fat intake and LH and FSH is inconsistent, making comparisons with our results difficult (9, 10, 34). These previous studies tended to be small and did not control for total energy intake or potential confounding. Larger studies that elucidate the impact of dietary fat intake on the gonadotropin-steroid hormone profile and the consequent effects on menstrual cycle function are warranted.

In general, data on the association between fat intake and ovulation are limited. Here we observed that docosapentaenoic acid was associated with a lower risk of anovulation, although no other associations between fatty acid intakes and risk of anovulation were found. It may be that the associations we observed

with total fat and PUFAs and testosterone concentrations are of insufficient magnitude to affect ovulatory function. Indeed, our observed associations with testosterone concentrations, but not with anovulation, may suggest that these subtle associations do not have clinical implications among healthy women, especially given that the observed percentage changes were only ~4% (in comparison to the CV for testosterone of 33% in this study population). There is strong evidence from animal studies that suggests the importance of ω-3 fatty acids in enhancing ovulation, reducing the incidence of ovarian cysts, and improving fertility, embryo quality, and pregnancy maintenance (3–5). In addition, the Nurses' Health Study II observed that PUFAs tended to provide weak protection against ovulatory infertility but only among women with high iron intakes (22). Their finding that increased *trans* fat was associated with a greater likelihood of developing ovulatory infertility is not inconsistent with our results, because we observed a significant reduction in progesterone concentrations in the total energy substitution models and a nonsignificant trend toward a reduced risk of anovulation when substituting *trans* fats with ω-3s, although we did not observe associations between *trans* fats and the relative risk of anovulation. It may be that the intakes of *trans* fat in this population were not sufficient to affect ovulation because labeling requirements for *trans* fatty acids in the United States resulted in a substantial reduction in these fats in the American food supply by the time the BioCycle Study was conducted.

The BioCycle Study has several strengths, including multiple measures of hormones over 2 menstrual cycles (using standardized methods to time cycle phase) and multiple measures of dietary fat intake among an ethnically diverse group of women. Although self-report of diet is subject to measurement error, our study used multiple validated 24-h dietary recalls to reduce the potential for misclassification. Women were selected into the BioCycle Study only if they were not planning to undertake a special diet, which enhances the generalizability of our findings. Indeed, the reported intake of fatty acids in the BioCycle Study was similar to intakes observed among reproductive-aged women in the United States according to the NHANES that was completed during the same time frame as the present study (mean percentage of energy from fat: NHANES, 33.6%; BioCycle, 33.9%; mean percentage of energy from protein: NHANES, 15.2%; BioCycle, 15.7%; mean percentage of energy from carbohydrates: NHANES, 50.4%; BioCycle, 50.8%). The mean reported total calories consumed in NHANES was 1809, which is slightly higher than reported in the BioCycle Study (43). The prospective design and exclusion criteria followed for the BioCycle Study strengthen the ability to draw inferences by reducing the potential for bias from known risk factors for anovulation. These methods are an important improvement over previous studies.

Nevertheless, the study faced several limitations, including the absence of daily measures of progesterone or transvaginal ultrasounds to confirm ovulation. However, multiple well-timed serum hormone measurements, along with the use of fertility monitors that measured LH daily in urine, were used to aid in classifying ovulatory cycles (28, 44). Last, the small number of anovulatory cycles observed limited our power to detect subtle effects of other dietary fatty acids, if such effects exist. The accurate assessment of dietary exposures is especially challenging, because data are highly subject to measurement error, particularly for long-chain marine PUFA intakes (within-woman CVs ranged from 34% to 61%). However, multiple dietary recalls have been shown to be valid measures of dietary exposures for many nutrients (45–47), and based on the variability observed here, our data suggest that we had a sufficient number of repeats to adequately assess long-term nutrient intakes. It should be noted that this study was exploratory and offers a comprehensive and hypothesis-generating picture of the potential associations between specific types of dietary fat and reproductive hormone concentrations, although we cannot rule out the possibility that some associations may be the result of multiple comparisons and due to type 1 error. This study is also unique in that dietary intake and hormone concentrations were assessed at the same cycle visit, so as to reflect short-term effects of dietary intake on reproductive function, thus limiting our ability to assess effects on long-term outcomes.

In conclusion, we observed that total fat intakes, in particular higher PUFA intakes, were associated with greater testosterone concentrations, although the magnitude of the increase was very small. The PUFA docosapentaenoic acid was not associated with testosterone concentrations but was associated with increased progesterone concentrations and a decreased risk of anovulation. Neither total fat nor specific types of fat were associated with other reproductive hormones or anovulation in this cohort of healthy women without any known infertility. These results suggest a role for fatty acids, specifically PUFAs, in androgen synthesis, although future studies are needed to confirm these findings and to allow us to better understand whether alterations in androgen synthesis have implications for women's fertility and long-term reproductive health.

The authors' responsibilities were as follows—SLM and JW-W: provided the study concept and design; SLM: conceived the project, developed the overall research plan, performed statistical analyses, and had primary responsibility for final content; SLM, JEC, and CZ: wrote the manuscript; and all authors: interpreted the data and critically revised the manuscript for important intellectual content. None of the authors reported a conflict of interest.

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