Dietary fatty acid intakes and the risk of ovulatory infertility[1–3]

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ABSTRACT

Background: Pharmacologic activation of the peroxisome proliferator–activated receptor γ (PPAR-γ) improves ovulatory function in women with polycystic ovary syndrome, and specific dietary fatty acids can affect PPAR-γ activity.

Objective: The objective of the study was to assess whether the intakes of total fat, cholesterol, and major types of fatty acids affect the risk of ovulatory infertility.

Design: We conducted a prospective cohort study of 18 555 married, premenopausal women without a history of infertility who attempted a pregnancy or became pregnant between 1991 and 1999. Diet was assessed twice during follow-up by using a food-frequency questionnaire.

Results: During follow-up, 438 incidents of ovulatory infertility were reported. In logistic regression analyses, intakes of total fat, cholesterol, and most types of fatty acids were not related to ovulatory infertility. Each 2% increase in the intake of energy from *trans* unsaturated fats, as opposed to that from carbohydrates, was associated with a 73% greater risk of ovulatory infertility after adjustment for known and suspected risk factors for this condition [relative risk (RR) = 1.73; 95% CI: 1.09, 2.73]. Obtaining 2% of energy intake from *trans* fats rather than from n−6 polyunsaturated fats was associated with a similar increase in the risk of ovulatory infertility (RR = 1.79; 95% CI: 1.11, 2.89). In addition, obtaining 2% of energy from *trans* fats rather than from monounsaturated fats was associated with a more than doubled risk of ovulatory infertility (RR = 2.31; 95% CI: 1.09, 4.87).

Conclusion: *trans* Unsaturated fats may increase the risk of ovulatory infertility when consumed instead of carbohydrates or unsaturated fats commonly found in nonhydrogenated vegetable oils. *Am J Clin Nutr* 2007;85:231–7.

KEY WORDS Diet, dietary fatty acids, infertility, ovulation, reproductive medicine, nutritional epidemiology

INTRODUCTION

Infertility, defined as the inability to conceive after 12 mo of unprotected intercourse (1), is a common problem affecting 10–15% of couples (2). More than 7 million women in the United States have an impaired ability to bear children (3), and, by 2025, as many as 7.7 million women are expected to face this problem (4). Assisted reproduction technologies have been developed to overcome infertility, but their costs (5–7) make them a less-than-ideal option for tackling infertility at a population level (8). Thus, identifying modifiable risk factors to prevent infertility is important.

The role of diet and other modifiable lifestyle practices in infertility is largely unexplored. However, considerable evidence suggests that dietary factors affecting insulin sensitivity may have an important role in the etiology of some forms of infertility. Factors known to increase insulin resistance, such as increased body weight and decreased physical activity, have been associated with an increased risk of infertility due to ovulatory dysfunction (9, 10). In addition, biochemical markers of sustained hyperglycemia, such as high concentrations of glycated hemoglobin, have been prospectively linked to decreased fertility (11). Moreover, in clinical trials of insulin sensitizers, including those that activate the peroxisome proliferator–activated receptor γ (PPAR-γ), these medications have improved reproductive metabolic profiles and ovulatory function in women with polycystic ovary syndrome (PCOS; 12–17).

Specific dietary unsaturated fatty acids can bind PPAR-γ (18), but their effects appear to differ for *cis* and *trans* isomers (19). Higher intake of *cis* unsaturated fatty acids (commonly found in nonhydrogenated vegetable oils and salad dressings) has been associated with lower concentrations of inflammatory markers (20, 21) and risk of type 2 diabetes (22), as well as with improved metabolic and endocrine characteristics in women with PCOS (23). Conversely, the consumption of *trans* fats (commonly found in commercially fried and baked products) instead of other macronutrients has been associated with greater inflammation (21, 24), insulin resistance (25), and risk of type 2 diabetes (22). Thus, we decided to test the hypotheses that *trans* unsaturated fatty acids (TFAs) increase the risk of ovulatory infertility whereas polyunsaturated fatty acids (PUFAs) reduce this risk.

SUBJECTS AND METHODS

Subjects

The Nurses' Health Study II is a prospective cohort study of 116 671 female registered nurses who were 24–42 y old at study inception in 1989. The current study is a prospective analysis of

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incident ovulatory infertility among married women who provided dietary information as part of their participation in the Nurses' Health Study II. The study was approved by the Institutional Review Board of Brigham and Women's Hospital.

Follow-up for the current analysis started in 1991, when diet was first measured. Every 2 y, participants were asked if they had tried for >1 y to become pregnant and to indicate whether their inability to conceive was cause by tubal blockage, ovulatory disorder, endometriosis, cervical mucous factor, or spousal factor or was not found, was not investigated, or was due to another condition. In a validation substudy of women who reported ovulatory infertility in 1989, self-reported ovulatory infertility was confirmed by review of medical records in 95% of the cases (9). Participants were also asked if they became pregnant—including pregnancies resulting in live births, miscarriages, or induced abortions—during the preceding 2-y period. With this information, we reconstructed a cohort of women who were trying to become pregnant. Only married women [whose pregnancies are more likely to be intentional than those of unmarried women (3)] with available dietary information and without a history of infertility were eligible to enter the analysis. These women contributed information to the analysis during each 2-y period in which they reported a pregnancy or a failed pregnancy attempt, and they were followed until they reported an infertility event from any cause, reached menopause, or underwent a sterilization procedure (themselves or their partner), whichever came first.

Type 2 diabetes has been associated with the intake of some fatty acids (22) and may affect ovulatory function. Of the 1987 women diagnosed with type 2 diabetes through 1999, 886 were unmarried, 408 had a history of infertility, 308 had undergone sterilization, 256 had reached menopause, 116 did not become pregnant or attempt a pregnancy during follow-up, and 3 did not have dietary data; thus, 10 diabetic women remained who met the selection criteria. Because this small number of diabetic subjects would preclude meaningful statistical adjustment for diabetes, these 10 women with diabetes were excluded from analysis. After exclusions, we identified 18 555 women without a history of infertility who tried to become pregnant or became pregnant between 1991 and 1999.

Women who met the selection criteria and reported infertility due to ovulatory disorder during follow-up, including those reporting multiple infertility diagnoses, were considered cases. All other events (pregnancies—whether resulting in live births, miscarriages, or induced abortions—and infertility due to other causes) were considered noncases.

Dietary assessment

Dietary information was collected in 1991 and 1995 by using a semiquantitative food-frequency questionnaire (FFQ) with 133 and 142 food items, respectively. Participants were asked to report how often during the previous year, on average, they had consumed each of the foods and beverages included in the FFQ. The questionnaire had 9 options for frequency of intake, ranging from never or <1 time/mo to ≥6 times/d. Nutrient intakes were estimated by summing the nutrient contribution of all food items in the questionnaire and taking into consideration the brand and type of margarine and the types of fat used in cooking and baking. The nutrient contents of each food and specified portion size were obtained from a nutrient database derived from the US Department of Agriculture (26) and additional information obtained from food manufacturers. The percentage of energy contributed by each energy-bearing nutrient was calculated as the intake of energy from each nutrient divided by total energy intake. To reduce extraneous variation in nonenergy-bearing nutrient intakes, these intakes were adjusted for total energy intake with the use of the nutrient residual method (27).

The FFQ has been previously found to be reproducible and valid for the measurement of fat intake. In a validation study, the deattenuated correlation coefficients between FFQ estimates of nutrient intakes and the estimated intake from the average of repeated dietary records were 0.68 for saturated fatty acids (SFAs), 0.48 for PUFAs, and 0.58 for monounsaturated fatty acids (MUFAs) (28). In another study, the correlation between calculated *trans* unsaturated fat intake from FFQ and TFAs in subcutaneous fat aspirates was 0.51 (29).

To determine whether recent or long-term diet was more relevant in the pathogenesis of ovulatory infertility, we defined dietary intakes in 2 ways. First, we used the most recent intakes, whereby the 1991 diet was used for the 1991–1995 follow-up period and the 1995 diet was assigned to the 1995–1999 follow-up. Second, in separate analyses we calculated cumulative averaged intakes to represent long-term diet. Specifically, the 1991 intakes were used to represent diet during the 1991–1995 follow-up period and the average of the 1991 and 1995 intakes was used for the 1995–1999 period.

Assessment of covariates

We collected information about nondietary covariates known or suspected to be related to ovulatory infertility including age, body mass index (BMI), parity, smoking and physical activity. Data were updated as follow-up questionnaires became available. In addition, we identified women with phenotypical features of PCOS: hyperandrogenism (defined as a history of physician-diagnosed severe teenage acne or use of isotretinoin during adolescence and a history of physician-diagnosed hirsutism) and a lifetime pattern of long menstrual cycles (≥40 d at ages 18–22 y and in 1993).

Statistical analysis

The relative risk of infertility according to dietary fat intake was estimated by using proportional hazards regression. Participants contributed 2 person-years of follow-up for each eligible pregnancy or pregnancy attempt. Because dates of ovulatory infertility diagnosis were not available, all events within each 2-y period were coded as having occurred simultaneously, and the exact method of handling ties (30) was used to account for this.

To assess the shape of the relation between the intake of specific types of fat and ovulatory infertility, we modeled these exposure in 3 ways. First, we divided women into 5 groups by quintile of the percentage of energy obtained from each type of fat. In these models, the relative risk (RR) was computed as the rate of infertility in a specific quintile of intake compared with that in the lowest quintile. Tests for linear trend were conducted by using the median values of intake in each category as a continuous variable. With the available sample size and number of cases, the statistical power to detect significant associations was >80% when the RR comparing extreme quintiles of fatty acid intake was >1.54 or <0.57. Second, we modeled fat intake as a continuous variable by using a linear term to achieve maximum

TABLE 1
Baseline characteristics of the study population by quintile (Q) of energy intake from total and *trans* unsaturated fats[1]

	Total fat				*trans* Unsaturated fat			
	Q1 (n = 3392)	Q3 (n = 3762)	Q5 (n = 3931)	P^2	Q1 (n = 3365)	Q3 (n = 3705)	Q5 (n = 4058)	P^2
Age (y)	33.1 ± 3.7[3]	32.5 ± 3.6	32.3 ± 3.6	< 0.001	33.4 ± 3.8	32.5 ± 3.6	32.0 ± 3.5	< 0.001
Alcohol intake (g/d)	3.1 ± 5.9	2.9 ± 5.4	2.4 ± 4.0	< 0.001	3.3 ± 5.9	2.9 ± 5.3	2.3 ± 4.2	< 0.001
BMI (kg/m^2)	23.2 ± 4.0	23.9 ± 4.3	24.7 ± 5.1	< 0.001	23.2 ± 3.8	23.9 ± 4.5	24.6 ± 4.9	< 0.001
Physical activity (METs/wk)	28.9 ± 35.2	20.4 ± 23.6	16.9 ± 22.3	< 0.001	29.9 ± 35.0	21.0 ± 25.6	20.0 ± 15.7	< 0.001
Cycles ≥40 d (%)	3.1	2.8	3.1	0.51	2.6	3.1	3.1	0.51
Hyperandrogenism (%)	0.2	0.3	0.4	0.17	0.2	0.2	0.3	0.88
Multivitamin use (%)	62	58	48	< 0.001	62	58	49	< 0.001
Nulliparous (%)	33	20	20	< 0.001	32	20	20	< 0.001
Current smoker (%)	6	7	10	< 0.001	6	7	9	< 0.001
Oral contraceptive use (%)[4]	14	16	19	< 0.001	13	16	20	< 0.001

[1] n = 18 555. The numbers of subjects in categories do not add up to 18 555 because information for quintiles 2 and 4 is not included in the table.
[2] From the Kruskal-Wallis test across the 5 quintiles of intake for continuous variables and the chi-square test across the 5 quintiles of intake for categorical variables.
[3] $\bar{x}$ ± SD (all such values).
[4] At the beginning of the mailing cycle, ie, 2 y before the first pregnancy or infertility report.

statistical power. Third, intake was modeled by using a restricted cubic spline (31) to evaluate the potentially nonlinear relation between the intake of specific types of fat and ovulatory infertility without the imposition of a priori assumptions about the shape of these relations. In this analysis, piecewise polynomials across the range of intake for each specific fatty acid (rather than a single linear term) were used to describe their association to ovulatory infertility. Nonlinearity was evaluated by using the likelihood ratio test, in which the model with only the linear term was compared with the model with the linear and the cubic spline terms.

To control for confounding by age, calendar time, and the interaction between them, all models were jointly stratified by age in years at the beginning of each mailing cycle and calendar time of the current questionnaire cycle. All models were adjusted for total energy intake. Multivariate models included additional terms for BMI, parity, smoking history, physical activity, history of contraceptive use, and dietary factors found to be related to infertility in preliminary analyses (ie, multivitamin use and intakes of alcohol, coffee, retinol, iron, and α-carotene). A second set of multivariate models simultaneously included terms for the percentages of energy derived from protein and specific types of fat. When intake is modeled as a continuous variable, the coefficients from this model have the interpretation of substituting a specific percentage of energy from fat for the same percentage of energy from carbohydrates. We estimated the effects of substituting one type of fat for another as the difference between their regression coefficients in the same model and calculated the 95% CIs by using the estimates of the covariance between the regression coefficients (32). No departures from the proportional hazards assumption were found. To explore whether the association between fatty acid intake and ovulatory infertility was modified by other predictors of this condition, we introduced cross-product terms between fat intake (as a linear term) and levels of the variable of interest. The likelihood ratio test was used to test the significance of the interactions. Results were considered to be significant when P was < 0.05 (2-sided). Analyses were performed by using SAS software (version 8.2; SAS Institute, Cary, NC).

RESULTS

During 8 y of follow-up, 26 971 eligible pregnancies or pregnancy attempts were identified in 18 555 women, infertility from any cause was reported for the first time by 3430 women, of whom 2165 underwent an investigation of the cause of infertility, and 438 were incident ovulatory infertility cases. As compared with women with lower total fat intake, women with a higher intake of total fat were younger and consumed less alcohol (**Table 1**); they also were heavier, less physically active, more likely to smoke, and more likely to report use of oral contraception at the beginning of the mailing cycle in which they entered the study. Moreover, women with higher fat intake were less likely to use multiple vitamin supplements and to be nulliparous than were those with lower fat intake. The associations between individual characteristics and intakes of specific types of fat were similar to those described for total fat intake.

We initially explored the relation between recent dietary fat intake and ovulatory infertility. In age and energy-adjusted analyses in which dietary fat was modeled by quintiles of intake (**Table 2**), total fat intake was inversely related to the risk of ovulatory infertility ($RR_{Q5\ versus\ Q1} = 0.80$; 95% CI: 0.60, 1.06; P for trend = 0.02). This association appeared to be driven by intake of SFAs ($RR_{Q5\ versus\ Q1} = 0.69$; 95% CI: 0.52, 0.94; P for trend < 0.01) and MUFAs ($RR_{Q5\ versus\ Q1} = 0.82$; 95% CI: 0.62, 1.08; P for trend = 0.03). After adjustment for potential confounders, these associations were considerably weaker and no longer significant. Simultaneous introduction of all major types of fat and protein intake into the multivariate adjusted models did not change the results for SFAs or MUFAs. However, a weak nonsignificant trend toward increasing risk of ovulatory infertility with increasing TFA intake was observed. Intakes of cholesterol and PUFAs were unrelated to ovulatory infertility in these analyses.

Recent fat intake was subsequently modeled as a continuous variable (**Table 3**). When each of the types of fat was analyzed separately (to estimate the effect of the isocaloric substitution of fat for the average macronutrient mixture in the study population), total fat intake and intakes of saturated and MUFAs were

TABLE 2
Relative risks (95% CIs) of ovulatory infertility by quintile (Q) of recent dietary fat intake[1]

	Fat intake					
Type of fat	Q1	Q2	Q3	Q4	Q5	*P* for trend[2]
Total fat						
Median intake (% of calories)	23.5	27.8	30.6	33.4	37.5	
Case/noncases (*n*)	111/5283	95/5300	78/5316	67/5327	87/5307	
Age- and energy-adjusted[3]	1.00 (referent)	0.87 (0.66, 1.15)	0.73 (0.55, 0.98)	0.63 (0.46, 0.86)	0.80 (0.60, 1.06)	0.02
Multivariate-adjusted 1[4]	1.00 (referent)	0.97 (0.74, 1.29)	0.87 (0.65, 1.18)	0.72 (0.53, 1.00)	0.90 (0.66, 1.21)	0.18
Cholesterol						
Median intake (mg)	162	202	230	262	314	
Cases/noncases (*n*)	103/5330	82/5188	81/5459	81/5204	91/5352	
Age- and energy-adjusted[3]	1.00 (referent)	0.84 (0.63, 1.12)	0.80 (0.60, 1.07)	0.86 (0.64, 1.15)	0.89 (0.67, 1.18)	0.53
Multivariate-adjusted 1[4]	1.00 (referent)	0.89 (0.66, 1.20)	0.86 (0.64, 1.16)	0.92 (0.68, 1.24)	0.94 (0.70, 1.26)	0.77
Saturated fat						
Median intake (% of calories)	8.0	9.8	11.0	12.2	14.1	
Case/noncases (*n*)	11/5282	102/5294	81/5313	69/5325	75/5319	
Age- and energy-adjusted[3]	1.00 (referent)	0.96 (0.73, 1.25)	0.76 (0.57, 1.01)	0.64 (0.47, 0.88)	0.69 (0.52, 0.94)	< 0.01
Multivariate-adjusted 1[4]	1.00 (referent)	1.11 (0.84, 1.47)	0.91 (0.67, 1.23)	0.76 (0.56, 1.05)	0.82 (0.59, 1.13)	0.06
Multivariate-adjusted 2[5]	1.00 (referent)	1.14 (0.84, 1.55)	0.95 (0.66, 1.37)	0.77 (0.51, 1.16)	0.76 (0.48, 1.19)	0.11
Monounsaturated fat						
Median intake (% of calories)	8.6	10.4	11.6	12.8	14.5	
Case/noncases (*n*)	112/5282	95/5299	73/5321	68/5327	90/5304	
Age- and energy-adjusted[3]	1.00 (referent)	0.87 (0.66, 1.15)	0.68 (0.50, 0.91)	0.64 (0.47, 0.87)	0.82 (0.62, 1.08)	0.03
Multivariate-adjusted 1[4]	1.00 (referent)	0.95 (0.72, 1.26)	0.79 (0.59, 1.08)	0.74 (0.54, 1.01)	0.90 (0.66, 1.21)	0.23
Multivariate-adjusted 2[5]	1.00 (referent)	0.93 (0.67, 1.29)	0.80 (0.54, 1.20)	0.77 (0.48, 1.20)	0.94 (0.57, 1.56)	0.71
Polyunsaturated fat						
Median intake (% of calories)	3.8	4.5	5.1	5.8	6.9	
Case/noncases (*n*)	94/5299	90/5306	84/5310	78/5317	92/5301	
Age- and energy-adjusted[3]	1.00 (referent)	0.99 (0.74, 1.32)	0.93 (0.69, 1.25)	0.87 (0.64, 1.18)	1.01 (0.75, 1.35)	0.87
Multivariate-adjusted 1[4]	1.00 (referent)	1.03 (0.77, 1.38)	0.96 (0.71, 1.30)	0.87 (0.63, 1.19)	0.99 (0.63, 1.19)	0.70
Multivariate-adjusted 2[5]	1.00 (referent)	1.05 (0.78, 1.42)	0.99 (0.71, 1.36)	0.89 (0.63, 1.26)	1.03 (0.72, 1.47)	0.90
trans Unsaturated fat						
Median intake (% of calories)	0.9	1.2	1.4	1.7	2.3	
Cases/noncases (*n*)	108/5286	75/5320	80/5314	84/5310	91/5303	
Age- and energy-adjusted[3]	1.00 (referent)	0.69 (0.51, 1.93)	0.76 (0.56, 1.01)	0.81 (0.60, 1.08)	0.86 (0.64, 1.14)	0.74
Multivariate-adjusted 1[4]	1.00 (referent)	0.79 (0.59, 1.07)	0.92 (0.68, 1.24)	0.94 (0.70, 1.27)	0.90 (0.72, 1.34)	0.74
Multivariate-adjusted 2[5]	1.00 (referent)	0.87 (0.64, 1.20)	1.11 (0.79, 1.55)	1.21 (0.85, 1.73)	1.31 (0.88, 1.95)	0.09

[1] $n = 26\,971$.

[2] Calculated with median intake of fat in each quintile as a continuous variable.

[3] Model stratified by age (1-y intervals) and calendar time (four 2-y intervals) and adjusted for total energy intake (continuous).

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

[5] Multivariate-adjusted model 1 plus quintiles of intake for the remaining types of fat (saturated, monounsaturated, polyunsaturated, and *trans* fat) and quintiles of protein intake.

inversely related to the risk of infertility in age- and energy-adjusted models but unrelated to infertility in multivariate-adjusted models. When the intakes of protein and all major types of fat were simultaneously included in the models (to estimate the effect of the isocaloric substitution of fat for carbohydrates), intake of TFAs was positively associated with risk of ovulatory infertility. A 2% increase in energy intake from TFAs was associated with a 94% greater risk of ovulatory infertility (95% CI: 22%, 208%) in age- and energy-adjusted analyses. This association remained significant after adjustment for potential confounders, although the estimated risk increase was somewhat lower (Table 3). Adjustment for BMI, parity, use of oral contraceptives, and intakes of alcohol and iron produced the largest changes in the association between TFAs and ovulatory infertility. Intakes of SFAs, MUFAs, total PUFAs, n−3 PUFAs, and n−6 PUFAS were not associated with ovulatory infertility.

TABLE 3
Relative risks (RRs), 95% CIs, and significance of ovulatory infertility associated with the specified isocaloric substitution of major types of fat[1]

	Age- and energy-adjusted		Multivariate-adjusted[2]	
	RR (95% CI)	*P*	RR (95% CI)	*P*
Substitution for the average mixture of other energy sources[3]				
Saturated fat (5% of energy)	0.74 (0.61, 0.91)	< 0.01	0.84 (0.68, 1.04)	0.11
Monounsaturated fat (5% of energy)	0.78 (0.64, 0.95)	0.01	0.86 (0.70, 1.05)	0.14
Polyunsaturated fat (5% of energy)	1.01 (0.69, 1.47)	0.96	0.94 (0.65, 1.37)	0.76
trans Unsaturated fat (2% of energy)	0.99 (0.71, 1.39)	0.97	1.09 (0.77, 1.54)	0.61
Total fat intake (5% of energy)	0.90 (0.83, 0.98)	0.02	0.94 (0.86, 1.03)	0.17
Substitution for carbohydrates[4]				
Saturated fat (5% of energy)	0.77 (0.55, 1.07)	0.12	0.86 (0.61, 1.20)	0.38
Monounsaturated fat (5% of energy)	0.68 (0.45, 1.03)	0.07	0.75 (0.49, 1.13)	0.17
Polyunsaturated fat (5% of energy)	1.31 (0.83, 2.08)	0.25	1.09 (0.69, 1.73)	0.70
trans Unsaturated fat (2% of energy)	1.94 (1.22, 3.08)	< 0.01	1.73 (1.09, 2.73)	0.02
Total fat intake (5% of energy)[5]	0.89 (0.82, 0.97)	0.01	0.93 (0.85, 1.02)	0.12

[1] $n = 26\,971$.

[2] Models are stratified by age (1-y intervals) and calendar time (four 2-y intervals) and adjusted for total energy intake (continuous), BMI (<20, 20–24.9, 25–29.9, ≥30, or missing), parity (0, 1, ≥2, or missing), smoking history (never; previously 1–4, 5–14, 15–24, or ≥25 cigarettes/d or unknown amount; current 1–4, 5–14, 15–24, or ≥25 cigarettes/d or unknown amount), physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, or ≥42 MET-h/wk or missing), contraceptive use (current user; never user; past user 0–23, 24–47, 48–71, 72–95, 96–119, or ≥120 mo ago or missing), use of multivitamins (yes or no), intake of alcohol (no intake or <2, 2–4.9, or ≥5 g/d), coffee (<1 serving/mo, 1 serving/mo, 2–6 servings/wk, 1 serving/d, 2–3 servings/d, or ≥4 servings/d), and quintiles of retinol, iron, and α-carotene intakes.

[3] From separate models including linear terms for each type of fat and total energy intake as predictors.

[4] From a single model including linear terms for all types of fat (saturated, monounsaturated, polyunsaturated, and *trans* unsaturated), protein intake, and total energy intake as predictors.

[5] Total fat was entered into a different model not including the specific types of fat.

We used the regression coefficients from this multivariate model to estimate the effect of the isocaloric substitution of one type of fat for another and found that eating TFAs instead of MUFAs was significantly related to the risk of ovulatory infertility ($P = 0.028$). The replacement of 2% of energy from MUFAs with 2% of energy from TFAs was associated with a more than doubled risk of ovulatory infertility (RR = 2.31; 95% CI: 1.09, 4.87). Similarly, the consumption of 2% of energy from *trans* fats rather than from n−6 PUFAs was associated with a significantly greater risk of ovulatory infertility (RR = 1.79; 95% CI: 1.11, 2.89; $P = 0.02$).

Because the results of the models simulating nutrient substitutions rely on an assumption of a linear relation between fat intakes and ovulatory infertility, we evaluated that assumption. No evidence was found for a nonlinear relation between the intake of SFAs ($P = 0.32$), MUFAs ($P = 0.83$), PUFAs ($P = 0.21$), or TFAs ($P = 0.35$) and ovulatory infertility. Similarly, there was no evidence of differences in the associations between intake of fatty acids and ovulatory infertility by levels of age, BMI, menstrual cycle length, hyperandrogenism, parity, oral contraception use, smoking or multivitamin use (P for interaction > 0.05 in all cases). However, the association between the intake of PUFAs and ovulatory infertility was modified by the level of iron intake. Obtaining energy from PUFAs rather than from carbohydrates was associated with a lower risk of ovulatory infertility in women in the highest quintile of iron intake but not in women who consumed less iron (P for interaction = 0.03). The RRs (95% CIs) for a 5% increase in energy intake from PUFAs in the first, third, and fifth (highest) quintiles of iron intake were 1.88 (0.95, 3.73), 0.78 (0.33, 1.84) and 0.22 (0.06, 0.79), respectively.

The analyses exploring the association between cumulative averaged fat intakes and ovulatory infertility showed similar results, albeit slightly attenuated. The multivariate-adjusted RRs for the estimated isocaloric substitution of fat for carbohydrates were 0.84 for SFAs (5% of energy; 95% CI: 0.59, 1.20), 0.77 for MUFAs (5% of energy; 95% CI: 0.49, 1.22), 1.11 for total PUFAs (5% of energy; 95% CI: 0.69, 1.79), 1.40 for n−3 PUFAs (1% of energy; 95% CI: 0.64, 3.05), 0.99 for n−6 PUFAs (1% of energy; 95% CI: 0.87, 1.12) and 1.67 for TFAs (2% of energy; 95% CI: 1.04, 2.69).

DISCUSSION

We examined the association between the intakes of different types of fat and ovulatory infertility and found that consuming TFAs instead of carbohydrates, MUFAs, or n−6 PUFAs was associated with a greater risk of this disease. The results did not differ according to a woman's age, parity, past use of oral contraception, smoking, BMI, or menstrual cycle length or the presence of clinical manifestations of excess androgens.

Although the association between fat intake and the risk of infertility has not, to our knowledge, previously been examined in humans, studies of women with PCOS suggested that this association would resemble that between fat intake and insulin resistance. A randomized trial in which 782 women with PCOS were assigned to a daily intake of 150, 300, or 600 mg/d of troglitazone or placebo for a total of 44 wk documented dose-dependent improvements in signs of ovulatory dysfunction, such as ovulation rate and pregnancy rates, as well as in clinical and biochemical signs of hyperandrogenemia (13). Similar results

have been observed in trials involving other pharmacologic activators of PPAR-γ (14–16). These results support our finding regarding the associations between the intake of *trans* fats and ovulatory infertility because, at levels of usual human consumption, TFAs have been found to down-regulate PPAR-γ expression in vivo by ≈40% (33). The intake of TFAs has also been associated with greater insulin resistance (25), risk of type 2 diabetes (22), and concentrations of inflammatory markers (21, 24), which may adversely affect ovulatory function (34). These mechanisms could explain the observed association between the intake of TFAs and the risk of ovulatory infertility, although alternative mechanisms cannot be ruled out.

Intake of PUFAs was not protective of ovulatory infertility in the entire group of women. However, a strong inverse association was noted in women with high iron intake, and mechanisms that could explain this interaction have been described. The activity of Δ-6 desaturase (an enzyme that participates in the conversion of linoleic acid into arachidonic acid and of α-linolenic acid into eicosapentaenoic acid and docohexaenoic acid) is significantly impaired in persons with low serum iron concentrations (35), and iron is an important functional component of this enzyme (36). Because arachidonic acid and eicosapentaenoic acid bind PPAR-γ more efficiently than do PUFAs with shorter chain lengths (37), the observed interaction would be expected if women with low iron intakes had impairments in this metabolic pathway, whereas women with a high iron intake could endogenously produce long-chain PUFAs more efficiently through this pathway. In addition, iron is a known oxidant, and oxidated metabolites of PUFAs are more potent ligands of PPAR-γ than are PUFAs themselves (38). It is tempting to conclude that the mechanism described above explains the observed effect modification, especially after adjustment for the high prevalence of depleted iron stores observed among young women in national surveys (21%) (39). Nevertheless, this interaction should be interpreted with caution, given that the intake of heme iron has been associated with a greater risk of outcomes related with insulin resistance (40) and that multiple tests for effect modification were conducted, which makes it possible that this finding was due to chance.

We observed inverse associations between the estimated isocaloric substitutions of total fat and saturated fat for the average mixture of other energy sources and risk of ovulatory infertility in age- and energy-adjusted models but not in multivariate-adjusted models. Disturbances of menstrual cycles that could be causal intermediates for ovulatory infertility, such as secondary amenorrhea, increased menstrual cycle length, and increased follicular phase length, were previously associated in smaller studies (41–45) with low intakes of total fat or saturated fat. However, some of these studies did not consider differences in total energy intake or other subject characteristics as alternative explanations for their findings (43, 44), and feeding studies made simultaneous changes in intakes of protein (42) and the ratios of saturated to monounsaturated to polyunsaturated fats (41, 45), which limited the ability of the investigators to draw conclusions regarding intakes of specific types of fat.

Strengths of the current study include its prospective nature: diet was collected 2–4 y before events were reported, which made it unlikely that results were affected by a subject's fertility status at the time of dietary report. The use of previously validated questionnaires of dietary intake and outcome assessment is also a strength of the current study. The most important limitation is that the subjects are not a cohort of women known to be planning to become pregnant. Cases, who were clearly attempting to conceive, may have been more health-conscious than the pregnancy noncases, who may have conceived accidentally. However, TFA intake is inversely associated with markers of health consciousness, and thus increased health consciousness of cases is more likely to have caused an inverse association, rather than the positive association between TFAs and ovulatory infertility we observed. Moreover, we simulated a cohort of pregnancy planners in our study by including only married women and by including in the noncase group women who were diagnosed with infertility from other causes. These steps made it less likely that pregnancy intention would affect our results. Another limitation was that selection bias might have been introduced by including only clinically recognizable outcomes of a pregnancy attempt in the study. However, pregnancy attempts with clinically nonrecognizable outcomes, such as early pregnancy losses, are likely to have been identified as infertility of unknown etiology or to be due to other causes, thus minimizing any potential selection bias. In addition, any bias present is unlikely to be any more influential than that introduced by design into traditional case-control studies of infertility or retrospective time-to-pregnancy studies, both of which have been useful in identifying risk factors for infertility. Because the current study was observational, we cannot completely rule out the possibility that our findings may be due in part to unmeasured confounders of the associations. Nevertheless, our results were statistically adjusted for numerous recognized risk factors for infertility and several other factors associated with ovulatory infertility in this population. Finally, because the study included only 438 ovulatory infertility cases, our statistical power to detect a significant association in categorical analyses based on quintiles of intake was limited (≈40% in the *trans* fatty acids analysis). However, we complemented those analyses with more powerful analyses using fat intakes as continuous variables, and those analyses showed that some of the hypothesized relations were significant.

In conclusion, our data suggest that dietary *trans* fatty acids increase the risk of ovulatory infertility when they replace carbohydrates or the unsaturated fats that are commonly found in vegetable oils. Given that these associations have not previously been reported, our findings should be reproduced, preferably in large prospective studies and randomized trials involving couples known to be planning a pregnancy. Because replacing *trans* fats with nonhydrogenated vegetable oils is likely to reduce the risk of coronary heart disease (46) and type 2 diabetes (22), women planning to become pregnant should consider this strategy; it could reduce their risk of infertility as well.

All authors were responsible for the study concept and design; WCW obtained funding and collected data; JEC analyzed the data and drafted the manuscript; BAR provided statistical support; and all authors critically reviewed and revised the manuscript. None of the authors had a personal or financial conflict of interest.

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