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A prospective study of dairy foods intake and anovulatory infertility

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BACKGROUND: Dairy foods and lactose may impair fertility by affecting ovulatory function. Yet, few studies have been conducted in humans and their results are inconsistent. We evaluated whether intake of dairy foods was associated with anovulatory infertility and whether this association differed according to fat content. METHODS: We prospectively followed 18 555 married, premenopausal women without a history of infertility who attempted a pregnancy or became pregnant during an 8-year period. Diet was assessed twice during the study using food—frequency questionnaires RESULTS: During follow-up, 438 women reported infertility due to an ovulatory disorder. The multivariate-adjusted relative risks (RR) [95% confidence interval (CI); *P*, trend] of anovulatory infertility comparing women consuming ≥2 servings per day to women consuming ≤1 serving per week was 1.85 (1.24–2.77; 0.002) for low-fat dairy foods. The RR (95% CI; *P*, trend) comparing women consuming ≥1 serving per day of high-fat dairy foods to those consuming ≤1 serving per week was 0.73 (0.52–1.01; 0.01). There was an inverse association between dairy fat intake and anovulatory infertility (*P*, trend = 0.05). Intakes of lactose, calcium, phosphorus and vitamin D were unrelated to anovulatory infertility. CONCLUSIONS: High intake of low-fat dairy foods may increase the risk of anovulatory infertility whereas intake of high-fat dairy foods may decrease this risk. Further, lactose (the main carbohydrate in milk and dairy products) may not affect fertility within the usual range of intake levels in humans.

Key words: dairy/epidemiology/infertility/lactose/ovarian function

Introduction

Lactose, the main carbohydrate in milk and other dairy foods, is cleaved in the intestine into glucose and galactose, sugars which are subsequently absorbed and metabolized. Women with galactosemia, an inborn error of metabolism in which galactose cannot be adequately metabolized, often develop premature ovarian failure (POF) (Kaufman *et al.*, 1981; Waggoner *et al.*, 1990; Guerrero *et al.*, 2000). In animal experiments, rodents fed high amounts of galactose have decreased ovulatory rates and develop POF (Swarts and Mattison, 1988; Bandyopadhyay *et al.*, 2003). These observations have led to the hypothesis that high intake of milk and dairy products may increase the risk of infertility due to ovulatory dysfunction in otherwise healthy women (Cramer *et al.*, 1994). However, few studies have been conducted in humans (Cramer *et al.*, 1994; Greenlee *et al.*, 2003) and their results are not consistent. In addition, there are reasons to believe that dairy foods may be beneficial to ovarian function, and that not all dairy foods have the same relation to fertility. Dairy food intake has been associated with a lower risk of insulin resistance syndrome and type 2 diabetes (Pereira *et al.*, 2002; Choi *et al.*, 2005) which have been associated with ovulatory dysfunction (Hjollund *et al.*, 1999; Azziz *et al.*, 2001; Brettenthaler *et al.*, 2004), suggesting that dairy foods may actually improve ovarian function. Also, intake of low-fat dairy foods, but not high-fat dairy foods, has been associated with clinical manifestations of androgen excess (Adebamowo *et al.*, 2005), a component of the polycystic ovary syndrome (PCOS) (The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004) which may also lead to anovulatory infertility (Ehrmann, 2005). To address these issues, we prospectively evaluated whether the intakes of low-fat dairy foods, high-fat dairy foods, lactose and other nutrients concentrated in dairy foods were associated with anovulatory infertility in a large cohort of healthy women.

Materials and methods

Study population

The Nurses' Health Study II (NHS II) is a prospective cohort started in 1989 when more than 116 000 female registered nurses aged 24–42 completed and returned a mailed baseline questionnaire. Participants have been followed every 2 years since then with mailed questionnaires, including information on exposures and disease outcomes. Here we present a prospective analysis of incident anovulatory infertility among participants of this cohort. The association between dietary factors and infertility overall or due to other causes is not addressed in this report. 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, and concluded in 1999. On biennial questionnaires, participants were asked if they had tried to become pregnant for more than 1 year without success since the previous questionnaire administration, and to indicate whether their inability to conceive was caused by tubal blockage, ovulatory disorder, endometriosis, cervical mucous factor and spousal factor was not found, was not investigated or was due to another reason. Women were also asked if they became pregnant during the preceding 2-year period, including pregnancies resulting in spontaneous abortions or induced abortions. Using this information, we identified a cohort of women most likely attempting to become pregnant. Only married women, 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-year period in which they reported a pregnancy or a failed pregnancy attempt, and 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. Ten diabetic women met these criteria. Insulin resistance and hyperinsulinemia, hallmark characteristics of type 2 diabetes, may affect ovulatory function (Hjollund *et al.*, 1999; Cataldo *et al.*, 2006) and have been associated with intake of dairy products (Pereira *et al.*, 2002; Choi *et al.*, 2005). Since the small number of diabetics would preclude meaningful statistical adjustment or exploration of modification of the associations by diabetes, diabetic women were excluded from the analysis. After exclusions, we identified 18 555 women without a history of infertility who tried to become pregnant or became pregnant during the 8-year follow-up period.

Women who met the selection criteria for the study and reported infertility due to ovulatory disorder, including those reporting multiple diagnoses for infertility, were considered cases. If a pregnancy and infertility were reported in the same 2-year period it was assumed that infertility preceded the pregnancy. In a validation substudy, self-reported diagnosis of anovulatory infertility was confirmed by review of medical records in 95% of the cases (Rich-Edwards *et al.*, 1994).

Dietary assessment

Dietary information was collected in 1991 and 1995 using a food-frequency questionnaire (FFQ) with more than 130 food items and 11 individual dairy foods. Participants were asked to report how often, on average, they consumed each of the foods and beverages included in the FFQ during the previous year. The questionnaire offered nine options for frequency of intake, ranging from never or less than once per month to six or more times per day. Nutrient intakes were estimated by summing the nutrient contribution of all food items in the questionnaire, taking into consideration the use of dietary supplements. The nutrient content of each food and specified portion size was obtained from a nutrient database derived from the US Department of Agriculture (2001) and additional information obtained from food manufacturers. To reduce extraneous variation in nutrient intakes, nutrient intakes were adjusted for total energy intake using the nutrient residual method (Willett and Stampfer, 1986).

The FFQ has been previously found to provide valid estimates of intake of dairy foods and nutrients concentrated in them (Salvini *et al.*, 1989; Willett and Lenart, 1998). The de-attenuated correlation coefficients between estimated intake from the average of four 1-week diet records and from the FFQ were 0.81 for skim/low-fat milk, 0.62 for whole milk, 0.94 for yogurt, 0.73 for ice cream, 0.80 for cottage cheese and 0.57 for other (hard) cheeses (Salvini *et al.*, 1989). The corresponding de-attenuated correlation coefficient for calcium intake was 0.75 (Willett and Lenart, 1998).

Analyses were performed using the most recent intakes, whereby the 1991 diet was assigned to events reported in 1993 and 1995 and the 1995 diet was assigned to events reported in 1997 and 1999, and cumulative averaged intakes, whereby the 1991 diet was assigned to events reported in 1993 and the average of the 1991 and 1995 diets was assigned to the remainder of the follow-up period. The results obtained using both methods were similar. Since cumulative averaged intakes reduce measurement error due to within-person random variation over time (Hu *et al.*, 1999), only results using this method are presented.

Statistical analyses

Intakes of individual dairy foods were added to obtain intakes of low-fat dairy foods (skim/low-fat milk, sherbet, yogurt and cottage cheese), high-fat dairy foods (whole milk, cream, ice cream, cream cheese and other cheese) and total dairy foods. Spearman correlation coefficients were used to estimate the association between intakes of low-fat dairy foods, high-fat dairy foods and dairy fat. Relative risks (RR's) relating intakes of dairy foods to incidence of anovulatory infertility were estimated using logistic regression. The generalized estimating equation approach (Fitzmaurice *et al.*, 2004), with an exchangeable working correlation structure, was used to account for the within-person correlation in outcomes at different time periods. We also performed secondary analyses using only the first event reported in the study for each person to avoid any within-person correlation in outcomes. We first divided women into groups according to their intake frequency of total, low-fat, high-fat and specific dairy foods. Women were also divided into five groups according to quintiles of intake of dietary components of dairy foods intake (dairy fat, lactose, calcium, phosphorus and vitamin D). Tests for linear trend were conducted using the median values of intake in each category as a continuous variable. The RR associated with a 1 serving per day increase in dairy food consumption was estimated using the intake of dairy foods as a continuous variable.

All models were adjusted for total energy intake, age and calendar time at the beginning of each questionnaire cycle. Multivariate models included additional terms for body mass index [wt (kg) ht^{-2} (m)] (BMI), parity, smoking history, physical activity (measured in metabolic equivalents), history of contraceptive use and dietary factors found to be related to infertility in preliminary analyses (use of multivitamins and intakes of alcohol, coffee and iron). Values of the dietary and non-dietary variables were updated as new data became available from the follow-up questionnaires.

Lastly, we examined whether the association between dairy food intake and anovulatory infertility was modified by subject characteristics (age, parity and BMI), or the presence of long menstrual cycles (>40 days), by introducing cross-product terms between dairy foods intake and the variable of interest. Using the same methodology, we also evaluated whether the association between high-fat dairy food intake and anovulatory infertility was modified by the intake level of low-fat dairy foods and vice versa. All *P*-values were two sided. Analyses were performed using SAS version 9.1.

Results

Between 1991 and 1999, 26 971 eligible pregnancies and pregnancy attempts were accrued among 18 555 women. Of these events, 3 430 were incident reports of infertility from any cause, of which 2 165 were of women who underwent medical investigation for infertility and 438 were incident reports of anovulatory infertility. At baseline, women who consumed more low-fat dairy foods were less likely to smoke or drink more than two cups of coffee per day, and had a higher average level of physical activity (Table I). Women who consumed more high-fat dairy foods were more likely to consume alcohol and less likely to be nulliparous and to exercise. In addition, women consuming more dairy foods, regardless of fat content, were also more likely to use multivitamins and less likely to use oral contraceptives. Intakes of low-fat and high-fat dairy foods were positively related ($r = 0.07$). Low-fat dairy intake was inversely related to whole milk intake ($r = -0.12$) and positively related to skim/low-fat milk intake ($r = 0.93$). Dairy fat intake was positively related to the intakes of low-fat ($r = 0.26$) and high-fat dairy foods ($r = 0.64$).

Intake of total dairy foods was not associated with the risk of anovulatory infertility (Table II). When low-fat and high-fat dairy foods were considered separately, we found a positive association between low-fat dairy food intake above 5 servings per week and risk of anovulatory infertility and an inverse association between high-fat dairy food intake and risk of developing this condition. Adjustment for known and suspected risk factors for infertility, especially adjustment for parity, changed these associations but they remained statistically significant. After adjustment, an increase in low-fat dairy foods of 1 serving per day, while holding calories constant, was associated with an 11% greater risk of anovulatory infertility [95% confidence interval (CI) = 2–21%]. The corresponding RR (95% CI) associated with an increase in intake of high-fat dairy foods by 1 serving per day was 0.78 (0.64–0.95). Simultaneously, including low-fat and high-fat dairy food intake in the same model did not substantially change the results. In this model, the multivariate-adjusted RRs (95% CIs) associated with increasing intake by 1 serving per day, keeping energy intake constant, were 1.09 (1.01–1.19) for low-fat dairy foods and 0.80 (0.65–0.97) for high-fat dairy foods.

When only the first event for each woman was considered in the analysis, the associations between dairy foods intake and anovulatory infertility persisted. For low-fat dairy foods, the multivariate-adjusted RRs (95% CIs) for ovulatory infertility were 1.32 (0.83–2.11) for women consuming 2–4 servings per week, 1.77 (1.06–2.94) for women consuming 5–6 servings per week, 1.64 (1.06–2.52) for women consuming 1 serving per day and 1.67 (1.07–2.62) for women consuming 2 or more servings per day, when compared with women consuming 1 serving per week or less (*P*, trend = 0.05). For high-fat dairy foods, the corresponding RRs (95% CIs) were 0.91 (0.67–1.24) for women consuming 2–4 servings per week, 0.77 (0.52–1.15) for women consuming 5-6 servings per week and 0.68 (0.46–0.98) for women consuming 1 or more serving per day (*P*, trend = 0.02).

The results for intakes of specific dairy foods generally followed the aggregated results for low-fat and high-fat dairy products (Table III). Among the low-fat dairy foods, adding one serving per day of yogurt or sherbet/frozen yogurt without changing total energy intake was associated with a greater risk of anovulatory infertility in multivariate-adjusted models. Although there was not a linear relationship between skim/low-fat milk intake and anovulatory infertility, women consuming one or more servings per week of skim/low-fat milk had a significantly higher risk of anovulatory infertility when compared with women consuming less than one serving per week [RR (95% CI) = 1.40 (1.04–1.88)], after adjusting for potential confounders. Among the high-fat dairy foods, adding a daily serving of whole milk without increasing

Table I. Baseline[a] characteristics of the study population by levels of low-fat and high-fat dairy foods intake

	Low-fat dairy[b]		High-fat dairy[c]	
	≤1 serving per week	≥2 per day	≤1 per week	≥1 per day
Age, years	32.7	32.3	33.4	32.2
Alcohol intake, g day^{-1}	2.9	2.3	2.5	3.1
Coffee intake ≥2 cups day^{-1}, %	27	19	24	24
Multivitamin use, %	46	65	53	59
Current smoker, %	13	5	8	7
Body mass index (BMI), kg m^{-2}	23.3	24.2	23.7	23.9
Physical activity, METs week^{-1}[d]	17.6	22.6	23.2	21.0
Cycles ≥40 days, %	3	3	3	3
Hyperandrogenism, %	0.3	0.3	0.3	0.3
Nulliparous, %	23	17	32	18
Oral contraceptive use at the beginning of the mailing cycle, %	19	13	17	15
Low-fat dairy, servings per day	—	—	1.5	1.7
High-fat dairy, servings per day	1.0	0.9	—	—

[a]Baseline refers to the year of entry into the study for each individual. Values are presented as age-standardized means and proportions with the exception of values for age.
[b]Includes skim/low-fat milk, sherbet, yogurt and cottage cheese.
[c]Includes whole milk, cream, ice cream, cream cheese and other cheese.
[d]METs (metabolic equivalents) = metabolic rate consuming 1 kcal kg^{-1} body weight h^{-1}.

Table II. Relative risks (RRs) (95% CIs) for anovulatory infertility by cumulative averaged frequency of intake of total, low fat and high fat dairy products

Food						*P*, trend[a]
Total dairy foods (servings)	<1 day^{-1}	1 day^{-1}	2–3 day^{-1}	≥4 day^{-1}		
Cases/non-cases	50/3102	172/8284	175/11 219	41/3928		
Age and energy-adjusted[b]	1.00 (referent)	1.36 (0.99–1.88)	1.13 (0.81–1.56)	0.88 (0.55–1.40)		0.09
Multivariate-adjusted[c]	1.00 (referent)	1.36 (0.98–1.88)	1.30 (0.93–1.82)	1.12 (0.69–1.82)		0.94
Low-fat dairy foods[d]	≤1 week^{-1}	2–4 week^{-1}	5–6 week^{-1}	1 day^{-1}	≥2 / day	
Cases/non-cases	34/3014	64/4085	51/2183	147/7693	142/ 9558	
Age and energy-adjusted[b]	1.00 (referent)	1.37 (0.90–2.08)	2.06 (1.33–3.18)	1.76 (1.21–2.56)	1.58 (1.07–2.32)	0.13
Multivariate-adjusted[c]	1.00 (referent)	1.24 (0.81–1.91)	1.86 (1.19–2.91)	1.71 (1.16–2.52)	1.85 (1.24–2.77)	0.002
High-fat dairy foods[e]	≤1 week^{-1}	2-4 week^{-1}	5–6 week^{-1}	≥1 day^{-1}		
Cases/non-cases	80/3679	199/9855	61/4398	98/8601		
Age and energy-adjusted[b]	1.00 (referent)	0.97 (0.75–1.27)	0.69 (0.49–0.98)	0.61 (0.45–0.85)		<0.001
Multivariate-adjusted[c]	1.00 (referent)	1.05 (0.80–1.37)	0.76 (0.53–1.07)	0.73 (0.52–1.01)		0.01

[a]Calculated with median intake in each category as a continuous variable.
[b]Adjusted for age (continuous), total energy intake (continuous) and calendar time (four 2-year intervals).
[c]Age and energy-adjusted model further adjusted for BMI (<20, 20–24.9, 25–29.9, ≥30 kg m^{-2} and missing), parity (0, 1, ≥2 and missing), smoking history (never, past 1–4 cigarette per day, past 5–14 cigaretter per day, past 15–24 cigarette per day, past ≥25 cigarette per day or unknown amount, current 1–4 cigarette per day, current 5–14 cigarette per day, current 15–24 cigarette per day and current ≥25 cigarette per day or unknown amount), physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, ≥ 42 MET-h week^{-1} and missing), contraceptive use (current user, never user, past user 0–23 months ago, past user 24–47 months ago, past user 48–71 months ago, past user 72–95 months ago, past user 96–119 months ago, past user ≥120 months ago and missing), use of multivitamin supplements (yes/no), intake of alcohol (no intake, <2, 2–4.9 and ≥5 g day^{-1}), coffee (<1 cup per month, 1 cup per month, 2–6 cups per week, 1 cup per day, 2–3 cups per day, ≥4 cups per day) and quintiles of iron intake.
[d]Includes skim/low-fat milk, sherbet, yogurt and cottage cheese.
[e]Includes whole milk, cream, ice cream, cream cheese and other cheese.

Table III. RR (95% CIs) of anovulatory infertility associated with increasing the intake of specific dairy foods by 1 serving per day

Food	Age and energy-adjusted[a]		Multivariate-adjusted[b]	
	RR (95% CI)	*P*	RR (95% CI)	*P*
All milk	0.90 (0.82–0.99)	0.04	1.02 (0.92–1.12)	0.74
Skim or low fat milk[c]	0.95 (0.87–1.04)	0.29	1.06 (0.96–1.17)	0.26
Whole milk	0.37 (0.19–0.70)	0.002	0.46 (0.25–0.84)	0.01
Sherbet or frozen yogurt	2.16 (1.60–2.91)	<0.001	1.86 (1.35–2.57)	<0.001
Ice cream	0.46 (0.12–1.72)	0.25	0.70 (0.22–2.27)	0.55
Yogurt[d]	1.67 (1.32–2.11)	<0.001	1.34 (1.03–1.74)	0.03
Cream[e]	1.25 (0.65–2.40)	0.50	1.31 (0.64–2.69)	0.46
Cottage or ricotta cheese	1.43 (0.93–2.21)	0.11	1.40 (0.90–2.17)	0.14
Cream cheese	0.79 (0.35–1.75)	0.56	0.66 (0.28–1.57)	0.35
Other cheese	0.82 (0.63–1.05)	0.12	0.86 (0.67–1.09)	0.21

[a]Adjusted for age (continuous), total energy intake (continuous) and calendar time (four 2-year intervals).
[b]Age and energy-adjusted model further adjusted for BMI (<20, 20–24.9, 25–29.9, ≥30 kg m^{-2} and missing), parity (0, 1, ≥2 and missing), smoking history (never, past 1–4 cigarette per day, past 5–14 cigarette per day, past 15–24 cigarette per day, past ≥ 25 cigarette per day or unknown amount, current 1–4 cigarette per day, current 5–14 cigarette per day, current 15–24 cigarette per day and current ≥ 25 cigarette per day or unknown amount), physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, ≥42 MET-h week^{-1} and missing), contraceptive use (current user, never user, past user 0–23 months ago, past user 24–47 months ago, past user 48–71 months ago, past user 72–95 months ago, past user 96–119 months ago, past user ≥ 120 months ago and missing), use of multivitamin supplements (yes/no), intake of alcohol (no intake, <2, 2–4.9, ≥5 g day^{-1}), coffee (<1 serving per month, 1 serving per month, 2–6 servings per week, 1 serving per day, 2–3 servings per day, ≥4 servings per day) and quintiles of iron intake.
[c]Includes skim, 1% and 2% milk.
[d]Includes flavoured and plain yogurt.
[e]Includes whipped, heavy and sour cream.

energy intake was associated with a reduction in the risk of anovulatory infertility of more than 50% after accounting for potential confounders. Likewise, the multivariate-adjusted RRs (95% CIs) of anovulatory infertility for women in successively higher intakes of ice cream were 0.85 (0.64, 1.12) for women consuming ice cream once a week and 0.62 (0.43, 0.89) for women consuming ice cream twice or more each week, when compared with women consuming ice cream less than once weekly (*P*, trend = 0.01).

We then evaluated whether the intake of dairy fat, lactose, calcium, phosphorus and vitamin D was associated with anovulatory infertility (Table IV). In age and energy-adjusted analyses, there were inverse associations between the intakes of dairy fat, calcium, vitamin D and the risk of developing anovulatory infertility. After adjusting for other variables that could explain these associations, only dairy fat intake was associated with a lower risk of anovulatory infertility. The intakes of lactose and phosphorus were not associated with anovulatory infertility in any of the models.

Next, we examined the possibility that the association between intake of high-fat dairy foods and anovulatory infertility was mediated through the intake of dairy fat by including in

Table IV. RR (95% CIs) for anovulatory infertility by intake quintiles of dairy fat, lactose, calcium, phosphorus and vitamin D

Nutrient	Quintile of Intake					*P*, trend[a]
	1	2	3	4	5	
Dairy fat						
Median intake (g day^{-1})	8.6	11.8	14.5	17.3	22.7	
Cases/non-cases	108/5285	96/5290	97/5315	65/5317	72/5326	
Age and energy-adjusted[b]	1.00 (referent)	0.91 (0.69–1.20)	0.91 (0.69–1.21)	0.62 (0.45–0.84)	0.67 (0.49–0.91)	0.002
Multivariate-adjusted[c]	1.00 (referent)	0.95 (0.71–1.26)	1.00 (0.75–1.33)	0.70 (0.51–0.97)	0.79 (0.58–1.08)	0.05
Lactose						
Median intake (g day^{-1})	5.8	12.2	18.4	26.3	38.4	
Cases/non-cases	85/5306	93/5308	98/5292	89/5306	73/5321	
Age and energy-adjusted[b]	1.00 (referent)	1.11 (0.83–1.50)	1.16 (0.86–1.55)	1.10 (0.81–1.49)	0.85 (0.62–1.16)	0.21
Multivariate-adjusted[c]	1.00 (referent)	1.21 (0.89–1.63)	1.28 (0.95–1.74)	1.36 (0.99–1.86)	1.11 (0.80–1.55)	0.47
Calcium						
Median intake (mg day^{-1})	634	844	1049	1270	1621	
Cases/non-cases	95/5289	98/5308	114/5275	64/5331	67/5330	
Age and energy-adjusted[b]	1.00 (referent)	1.03 (0.77–1.37)	1.25 (0.94–1.64)	0.71 (0.51–0.98)	0.71 (0.52–0.98)	0.003
Multivariate-adjusted[c]	1.00 (referent)	1.10 (0.82–1.48)	1.51 (1.13–2.03)	0.93 (0.66–1.32)	1.02 (0.71–1.45)	0.81
Phosphorus						
Median intake (mg day^{-1})	1127	1288	1412	1548	1743	
Cases/non-cases	92/5293	80/5319	91/5316	82/5296	93/5309	
Age and energy-adjusted[b]	1.00 (referent)	0.86 (0.63–1.16)	1.00 (0.74–1.34)	0.92 (0.68–1.24)	1.01 (0.76–1.36)	0.77
Multivariate-adjusted[c]	1.00 (referent)	0.94 (0.69–1.28)	1.07 (0.79–1.45)	1.09 (0.80–1.49)	1.22 (0.90–1.66)	0.13
Vitamin D						
Median intake (IU day^{-1})	162	285	419	578	783	
Cases/non-cases	105/5272	106/5299	91/5318	66/5310	70/5334	
Age and energy-adjusted[b]	1.00 (referent)	1.04 (0.79–1.36)	0.91 (0.68–1.22)	0.67 (0.49–0.92)	0.64 (0.47–0.87)	<0.001
Multivariate-adjusted[c]	1.00 (referent)	1.12 (0.84–1.48)	1.12 (0.80–1.56)	0.98 (0.66–1.45)	1.01 (0.65–1.57)	0.91

[a]Calculated with median intake in each category as a continuous variable.
[b]Adjusted for age (continuous), total energy intake (continuous) and calendar time (four 2-year intervals).
[c]Age and energy-adjusted model further adjusted for BMI (<20, 20–24.9, 25–29.9, ≥30 kg m^{-2} and missing), parity (0, 1, ≥2 and missing), smoking history (never, past 1–4 cigarette per day, past 5–14 cigarette per day, past 15–24 cigarette per day, past ≥ 25 cigarette per day or unknown amount, current 1–4 cigarette per day, current 5–14 cigarette per day, current 15–24 cigarette per day and current ≥25 cigarette per day or unknown amount), physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, ≥42 MET-h week^{-1} and missing), contraceptive use (current user, never user, past user 0–23 months ago, past user 24–47 months ago, past user 48–71 months ago, past user 72–95 months ago, past user 96–119 months ago, past user ≥ 120 months ago and missing), use of multivitamin supplements (yes/no), intake of alcohol (no intake, <2, 2–4.9, ≥5 g day^{-1}), coffee (<1 serving per month, 1 serving per month, 2–6 servings per week, 1 serving per day, 2–3 servings per day, ≥4 servings per day) and quintiles of iron intake.

the same multivariate-adjusted model terms for intake of high-fat dairy foods and dairy fat. Adjustment for dairy fat intake attenuated the association between intake of high-fat dairy foods and anovulatory infertility, although a suggestion of a linear trend was still present. The RRs (95% CI) for women in successively higher intakes of high-fat dairy were 1.06 (0.79, 1.42) for women consuming 2 to 4 servings weekly, 0.78 (0.53, 1.15) for women consuming 5 to 6 servings per week and 0.77 (0.51, 1.17) for women consuming at least one serving per day, when compared with women consuming one or less servings of high-fat dairy each week (*P*, trend = 0.07). Dairy fat intake was unrelated to anovulatory infertility in this model.

Lastly, we assessed the possibility that the associations between intake of dairy foods and anovulatory infertility could be modified by personal characteristics, including age, menstrual cycle length, BMI and parity. The positive association between of low-fat dairy foods intake and anovulatory infertility appeared to be stronger among women above the median age (32 years), eumenorrhoeic women, women with a BMI below 25 kg m^{-2} and parous women (Table V). The association between intake of high-fat dairy foods and anovulatory infertility appeared to be stronger among women above 32 years, eumenorrhoeic women and overweight or obese women. However, none of the tests for effect modification reached statistical significance. There were no appreciable differences in the association between low-fat dairy food intake and anovulatory infertility by levels of high-fat dairy food intake or in the association between high-fat dairy food intake and ovulatory infertility by levels of low-fat dairy food intake.

Discussion

In this cohort of healthy women, we found that intake of low-fat dairy foods was associated with a greater risk of anovulatory infertility, whereas intake of high-fat dairy foods was associated with a lower risk of this condition. We found no evidence of an association between lactose and anovulatory infertility within the intake range of consumption in this population. In addition, we did not observe any association between important constituents of dairy foods and anovulatory infertility, with the exception of an inverse association for intake of dairy fat.

Only two previous studies have examined whether intake of milk or dairy foods could affect fertility in women. Cramer *et al.* (1994) found a positive correlation between per capita milk consumption and age-related decrease in fertility rates in 31 countries; milk consumption statistically explained 36% of the variation in the fertility decline associated with

Table V. RR and 95% CI[a] of anovulatory infertility associated with increasing dairy foods intake by 1 serving/day in subgroups of the study population

Subgroup	Cases (*n*)	Low fat dairy[b]		High-fat dairy[c]	
		RR (95% CI)	*P*, interaction	RR (95% CI)	*P*, interaction
Age ≤ 32 years	214	1.08 (0.97–1.20)	0.35	0.86 (0.69–1.09)	0.08
Age > 32 years	224	1.14 (1.03–1.26)		0.67 (0.52–0.87)	
Cycles ≥ 40 days	52	0.94 (0.73–1.21)	0.16	1.09 (0.66–1.79)	0.16
Cycles < 40 days	386	1.14 (1.04–1.24)		0.75 (0.61–0.92)	
BMI < 25	248	1.17 (1.05–1.29)	0.11	0.85 (0.67–1.08)	0.23
BMI ≥ 25	190	1.03 (0.91–1.17)		0.67 (0.50–0.91)	
Nulliparous	208	1.07 (0.95–1.21)	0.45	0.77 (0.55–1.07)	0.93
Parous	230	1.14 (1.02–1.26)		0.78 (0.62–0.99)	
High fat dairy <5/week	279	1.11 (1.01–1.23)	0.66	—	—
High fat dairy ≥5/week	159	1.07 (0.95–1.22)		—	
Low fat dairy <1/day	149	—	—	0.81 (0.62–1.06)	0.82
Low fat dairy ≥1/day	289	—		0.78 (0.59–1.02)	

[a]Adjusted for age (continuous), total energy intake (continuous), calendar time (four 2-year intervals), BMI (<20, 20–24.9, 25–29.9, ≥30 kg m^{-2} and missing), parity (0, 1, ≥2 and missing), smoking history (never, past 1–4 cigarette per day, past 5–14 cigarette per day, past 15–24 cigarette per day, past ≥ 25 cigarette per day or unknown amount, current 1–4 cigarette per day, current 5–14 cigarette per day, current 15–24 cigarette per day and current ≥25 cigarette per day or unknown amount), physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, ≥42 MET-h $week^{-1}$ and missing), contraceptive use (current user, never user, past user 0–23 months ago, past user 24–47 months ago, past user 48–71 months ago, past user 72–95 months ago, past user 96–119 months ago, past user ≥120 months ago and missing), use of multivitamin supplements (yes/no), intake of alcohol (no intake, <2, 2–4.9, ≥ 5 g day^{-1}), coffee (<1 serving per month, 1 serving per month, 2–6 servings per week, 1 serving per day, 2–3 servings per day, ≥4 servings per day) and quintiles of iron intake.
[b]Skim/low-fat milk, sherbet, yogurt and cottage cheese.
[c]Whole milk, cream, ice cream, cream cheese and other cheese.

age. Following-up on these findings, Greenlee *et al.* (2003) questioned participants of a case–control study of agricultural occupations and female infertility about their usual milk consumption. In this study, women who consumed three or more glasses of milk daily had a 70% lower risk of infertility when compared with women who did not consume milk (Greenlee *et al.*, 2003).

Our study has overcome many of the limitations of the previous studies. We were able to account for differences in several personal, behavioural and dietary characteristics that could be alternative explanations for these associations. The prospective nature of the study, in which dietary information was collected 2–4 years before events were reported, makes it unlikely that our results are affected by disease status at the time dietary information was collected: an issue of concern in case–control studies. The use of previously validated questionnaires for dietary and outcome assessments also adds to the strength of our results. A limitation of our study is that it was not a cohort of women known to be planning a pregnancy. Cases were clearly trying to conceive, whereas some pregnancy non-cases may have conceived accidentally. However, we simulated a cohort of pregnancy planners by restricting the study to married women and by considering as non-cases women who were diagnosed with infertility from other causes, making it less likely for pregnancy intention to affect our results. Another potential limitation of our study is that we did not collect information on exposures of the participants' partners that might influence female fertility, such as smoking. This situation, however, is unlikely to have major impact on our results since the potential bias would be limited by the association between the partner risk factor for anovulatory infertility, which is likely to be non-existent or small in the case of smoking status of the partner (Hull *et al.*, 2000; Greenlee *et al.*, 2003), and could be accounted for in part by adjusting for the same factor in women, as we did for smoking and other lifestyle characteristics, since spouses often share environmental exposures and lifestyle practices (Knuiman *et al.*, 2005; Jurj *et al.*, 2006).

We found that eating low-fat dairy foods was associated with an increased risk of anovulatory infertility. It is possible that this association is spurious. If women who have been diagnosed with or suspect they have PCOS made health-conscious changes to their diet, such as consuming low-fat dairy instead of high-fat dairy, a positive association between any of these dietary changes and anovulatory infertility would be expected. If this situation arose, one would also expect that in analyses stratified by PCOS or PCOS symptoms, the association between low-fat dairy foods and anovulatory infertility remained only among women with PCOS or symptoms of this condition. This is not, however, the case in our data. Although the tests for effect modification did not reach statistical significance, our results suggest that the association between low-fat (and high-fat) dairy foods intake and infertility due to anovulation is stronger among women without some clinical manifestations of PCOS than among women with them.

There are other potential explanations for our results regarding low-fat dairy foods intake. Assuming that dietary factors associated with an increased frequency of phenotypic features of PCOS would increase the risk of infertility due to ovulatory disorders, we had anticipated the observed association for low-fat dairy foods. Adebamowo *et al.* (2005) found intakes of skim milk, low-fat milk, sherbet and cottage cheese to be associated with an increased frequency of physician-diagnosed acne. They hypothesized that the association between skim milk consumption and acne could be explained by milk's content of androgen precursors (Hartmann *et al.*, 1998), the ability of milk to increase circulating IGF-I levels (Heaney *et al.*, 1999; Holmes *et al.*, 2002; Giovannucci *et al.*, 2003). Also, changes in milk composition during the fat extraction process, such as the addition of some whey proteins including α-lactalbumin, have been found to have androgenic effects in animals (Bouthegourd *et al.*, 2002). Several of these mechanisms may be particularly relevant for anovulatory infertility, in particular, the increased IGF-I levels resulting from increased dairy intake as this association may be driven by the intake of low-fat dairy foods (E. Giovannucci, personal communication). Some have proposed that IGF-I may be involved in the pathogenesis of PCOS (Giudice, 1992), and in human ovarian cells, IGF-I can induce thecal cell function changes observed in PCOS (Duleba *et al.*, 1998). Also, exogenous IGF-I administration to women with specific endocrinopathies has resulted in clinical manifestations of PCOS (Klinger *et al.*, 1998). Whether changes in circulating IGF-I levels induced by diet can result in subclinical PCOS-like disorders is unknown, but this would be consistent with our results and should be addressed by future studies.

In contrast with the results for low-fat dairy, greater intake of high-fat dairy foods was associated with a lower risk of anovulatory infertility. Intake of dairy fat was also inversely associated with infertility due to anovulatory disorders and appeared to be partially responsible for the high-fat dairy food association. Although particular fatty acids in dairy products could potentially have a beneficial effect on ovulatory function, a more likely explanation is that a fat-soluble substance present in dairy products may exert this action. Whole milk and other high-fat dairy products have a higher estrogen concentration than their low-fat counterparts (Wolford and Argoudelsi, 1979). Since estrogens decrease circulating IGF-I levels (Jorgensen *et al.*, 2004; Veldhuis *et al.*, 2005), it is possible that their presence in high-fat dairy foods could explain the observed association. Alternatively, increased insulin sensitivity among high-fat dairy consumers may have improved ovulatory function. High-fat, but not reduced-fat, dairy foods were found to decrease the risk of developing abnormal glucose homeostasis in young adults (Pereira *et al.*, 2002), although this finding conflicts with another study suggesting the opposite (Choi *et al.*, 2005).

We did not observe an association between lactose intake and risk of infertility due to ovulatory problems. Our findings contrast with previous clinical and experimental evidence (Kaufman *et al.*, 1981; Swarts and Mattison 1988; Waggoner *et al.*, 1990; Guerrero *et al.*, 2000; Bandyopadhyay *et al.*, 2003) but are not unexpected. Although it has been reported that carriers of gene variants affecting galactose metabolism have an increased risk of infertility (Cramer *et al.*, 1989), these results have not been replicated (Herrington *et al.*, 1996; Lukac Bajalo *et al.*, 2005). In addition, rodents exposed to diets containing 42% lactose, rather than galactose, do not develop the alterations of ovarian morphology and function observed in rodents exposed to high galactose diets (Lui *et al.*, 2005). Also, the exposure to galactose in galactosemia and the rodent models where ovarian consequences have been observed may be well beyond the range of usual dietary exposure in humans. The diets in rodent models that have induced ovarian dysfunction on a high galactose diet usually provide between 35% and 50% of the animal's total energy intake as galactose (Swarts and Mattison, 1988; Bandyopadhyay *et al.*, 2003). For humans, this would require feeding exclusively or almost exclusively on dairy foods which would be, at the very least, challenging. Our findings for associations in opposite directions for low-fat and high-fat dairy foods and the fact that the strength of the associations did not seem to depend on the lactose content of the food (e.g. strong associations for sherbet/frozen yogurt which has a low lactose content) also suggest that intake of lactose at the usual levels of human intake is not deleterious to fertility. We also did not find associations between intake of calcium, phosphorus or vitamin D and anovulatory infertility. These nutrients have been important determinants of fertility in animal models under certain conditions (Johnson and De Luca, 2001, 2002). Our data cannot exclude the possibility, however, that intakes of lactose, calcium, phosphorus or vitamin D could have some effect on ovulatory function and fertility among women carrying gene variants affecting galactose or vitamin D metabolism.

In summary, we observed a positive association between intake of low-fat dairy foods (especially yogurt and sherbet/frozen yogurt) and anovulatory infertility and an inverse association between intake of high-fat dairy foods (especially whole milk and ice cream) and this disease. The intake of dairy fat, or a fat-soluble substance present in dairy foods, may partly explain the association between high-fat dairy and anovulatory infertility. Further, our data do not support the hypothesis that, within the observed range of intake, lactose significantly impairs ovulatory function to the point of affecting fertility. Given the scarcity of studies in this area, it is important that our findings are confirmed or refuted. It is also important to investigate whether dairy foods have an impact on fertility beyond their association with anovulatory infertility. Clarifying the role of dairy foods intake on fertility is particularly important since the current Dietary Guidelines for Americans recommend that adults consume three or more daily servings low-fat milk or equivalent dairy products (United States Department of Health and Human Services and United States Department of Agriculture, 2005); a strategy that may be deleterious for women planning to become pregnant.

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References

Adebamowo CA, Spiegelman D, Danby FW, Frazier AL, Willett WC and Holmes MD (2005) High school dietary dairy intake and teenage acne. J Am Acad Dermatol 52,207–214.

Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, O'Keefe M and Ghazzi MN (2001) Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. J Clin Endocrinol Metab 86,1626–1632.

Bandyopadhyay S, Chakrabarti J, Banerjee S, Pal AK, Goswami SK, Chakravarty BN and Kabir SN (2003) Galactose toxicity in the rat as a model for premature ovarian failure: an experimental approach readdressed. Hum Reprod 18,2031–2038.

Bouthegourd J-CJ, Roseau SM, Makarios-Lahham L, Leruyet PM, Tome DG and Even PC (2002) A preexercise α-lactalbumin-enriched whey protein meal preserves lipid oxidation and decreases adiposity in rats. Am J Physiol Endocrinol Metab 283,E565–E572.

Brettenthaler N, De Geyter C, Huber PR and Keller U (2004) Effect of the insulin sensitizer pioglitazone on insulin resistance, hyperandrogenism, and ovulatory dysfunction in women with polycystic ovary syndrome. J Clin Endocrinol Metab 89,3835–3840.

Cataldo NA, Abbasi F, McLaughlin TL, Basina M, Fechner PY, Giudice LC and Reaven GM (2006) Metabolic and ovarian effects of rosiglitazone treatment for 12 weeks in insulin-resistant women with polycystic ovary syndrome. Hum Reprod. 21,109–120.

Choi H, Willett WC, Stampfer MJ, Rimm EB and Hu FB (2005) Dairy consumption and risk of type 2 diabetes mellitus in men: a prospective study. Arch Intern Med 165,997–1003.

Cramer DW, Harlow BL, Barbieri RL and Ng WG (1989) Galactose-1-phsphate uridyl transferase activity associated with age at menopause and reproductive history. Fertil Steril 51,609–615.

Cramer DW, Xu H and Sahi T (1994) Adult hypolactasia, milk consumption and age-specific fertility. Am J Epidemiol 139,282–289.

Duleba AJ, Spaczynski RZ and Olive DL (1998) Insulin and insulin-like growth factor i stimulate the proliferation of human ovarian theca-interstitial cells. Fertil Steril 69,335–340.

Ehrmann DA (2005) Polycystic ovary syndrome. N Engl J Med 352,1223–1236.

Fitzmaurice GM, Laird NM and Ware JH (2004) Applied longitudinal analysis. Wiley & Sons, Hoboken, NJ.

Giovannucci E, Pollak M, Liu Y, Platz EA, Majeed N, Rimm EB and Willett WC (2003) Nutritional predictors of insulin-like growth factor i and their relationships to cancer in men. Cancer Epidemiol Biomarkers Prev 12,84–89.

Giudice L (1992) Insulin-like growth factors and ovarian follicular development. Endocr Rev 13,641–669.

Greenlee AR, Arbuckle TE and Chyou PH (2003) Risk factors for female infertility in an agricultural region. Epidemiology 14,429–436.

Guerrero NV, Singh RH, Manatunga A, Berry GT, Steiner RD and Elsas LJ II (2000) Risk factors for premature ovarian failure in females with galactosemia. J Pediatr 137833–841.

Hartmann S, Lacorn M and Steinhart H (1998) Natural occurrence of steroid hormones in food. Food Chem 62,7–20.

Heaney RP, McCarron DA, Dawson-Hughes B, Oparil S, Berga SL, Stern JS, Barr SI and Rosen CJ (1999) Dietary changes favorably affect bone remodeling in older adults. J Am Diet Assocn 99,1228–1233.

Herrington LJ, Lemaitre RN, Beresford SAA, Stanford JL, Wolfla DM, Feng Z, Scott CR and Weiss NS (1996) Lactose metabolism and time to pregnancy. Fertil Steril 66,384–388.

Hjollund NHI, Jensen TK, Bonde JPE, Henriksen NE, Andersson AM and Skakkebaek NE (1999) Is glycosilated haemoglobin a marker of fertility? A follow-up study of first-pregnancy planners. Hum Reprod 14,1478–1482.

Holmes MD, Pollak MN, Willett WC and Hankinson SE (2002) Dietary correlates of plasma insulin-like growth factor i and insulin-like growth factor binding protein 3 concentrations. Cancer Epidemiol, Biomarkers Prev 11,852–861.

Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D and Willett WC (1999) Dietary fat and coronary heart disease: a comparison of approaches for adjusting total energy intake and modeling repeated dietary measurements. Am J Epidemiol 149,531–540.

Hull MGR, North K, Taylor H, Farrow A and Ford WCL (2000) Delayed conception and active and passive smoking. Fertil Steril 74,725–733.

Johnson LE and DeLuca HF (2001) Vitamin d receptor null mutant mice fed high levels of calcium are fertile. J Nutr 131,1787–1791.

Johnson LE and DeLuca HF (2002) Reproductive defects are corrected in vitamin d-deficient female rats fed a high calcium, phosphorus and lactose diet. J Nutr 132,2270–2273.

Jorgensen JO, Christensen JJ, Krag M, Fisker S, Ovesen P and Christiansen JS (2004) Serum insulin-like growth factor-i levels in growth hormone deficient adults: influence of sex steroids. Horm Res 62,73–76.

Jurj AL, Wen W, Li H-L, Zheng W, Yang G, Xiang Y-B, Gao Y-T and Shu X-O (2006) Spousal correlations for lifestyle factors and selected diseases in chinese couples. Ann Epidemiol 16,285–291.

Kaufman F, Kogut M, Donnell G, Goebelsmann U, March C and Koch R (1981) Hypergonadotropic hypogonadism in female patients with galactosemia. N Engl J Med 304,994–998.

Klinger B, Anin S, Silbergeld A, Eshet R and Laron Z (1998) Development of hyperandrogenism during treatment with insulin-like growth factor-i (igf-i) in female patients with laron syndrome. Clin Endocrinol 48,81–87.

Knuiman MW, Divitini ML and Bartholomew HC (2005) Spouse selection and environmental effects on spouse correlation in lung function measures. Ann Epidemiol 15,39–43.

Lui G, Shi F, Blas-Machado U, Dong Q, Davis VL, Foster WG and Hughes CL (2005) Ovarian effects of a high lactose diet in the female rat. Reprod Nutr Dev 45,185–192.

Lukac Bajalo J, Mencej S, Karas N, Mlinar B, Prodan Zitnik I and Gersak K (2005) Q188r, k285n, and n314d mutation-associated alleles in the galactose-1-phosphate uridyltransferase gene and female infertility. Fertil Steril 83,776–778.

Pereira MA, Jacobs DR Jr, Van Horn L, Slattery ML, Kartashov AI and Ludwig DS (2002) Dairy consumption, obesity and the insulin resistance syndrome in young adults: the cardia study. JAMA 287,2081–2089.

Rich-Edwards JW, Goldman MB, Willett WC, Hunter DJ, Stampfer MJ, Colditz GA and Manson JE (1994) Adolescent body mass index and ovulatory infertility. Am J Obstet Gynecol 171,171–177.

Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B and Willett WC (1989) Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. Int J Epidemiol 18,858–867.

Swarts WJ and Mattison DR (1988) Galactose inhibition of ovulation in mice. Fertil Steril 49,522–526.

The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (pcos). Hum Reprod 19,41–47.

United States Department of Agriculture (2001) USDA Nutrient Database for Standard Reference Release 14: US Department of Agriculture ARS.

United States Department of Health and Human Services and United States Department of Agriculture (2005) *Dietary Guidelines for Americans, 2005*, 6th ed. U.S. Government Printing Office, Washington, DC.

Veldhuis JD, Frystyk J, Iranmanesh A and Orskov H (2005) Testosterone and estradiol regulate free insulin-like growth factor i (igf-i), igf binding protein 1 (igfbp-1), and dimeric igf-i/igfbp-1 concentrations. J Clin Endocrinol Metab 90,2941–2947.

Waggoner DD, Buist NRM and Donnell GN (1990) Long-term prognosis in galactosemia: results of a survey of 350 cases. J Inherit Metab Dis 13,802–818.

Willett WC and Lenart E (1998) Chapter 6: Reproducibility and validity of food frequency questionnaires. In Willett WC (ed.) *Nutritional Epidemiology*, 2nd edn. Oxford University Press, New York.

Willett WC and Stampfer MJ (1986) Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 124,17–27.

Wolford ST and Argoudelsi CJ (1979) Measurement of estrogens in cow's milk, human milk and dairy products. J Dairy Sci 62,1458–1463.

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