Original Contribution

A Prospective Study of Dairy-Food Intake and Early Menopause

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Early natural menopause, the cessation of ovarian function prior to age 45 years, affects approximately 10% of women and increases risk of cardiovascular disease and other adverse conditions. Laboratory evidence suggests a potential role of dairy foods in the ovarian aging process; however, no prior epidemiologic studies have evaluated how dairy-food intake is associated with risk of early menopause. We therefore evaluated how intakes of total, low-fat, high-fat, and individual dairy foods were associated with early menopause in Nurses' Health Study II. Women who were premenopausal at the start of follow-up in 1991 were followed until 2011 for early menopause. Food frequency questionnaires were used to assess dietary intake. In Cox proportional hazards models adjusting for age, smoking, and other factors, total baseline dairy-food intake of ≥4 servings/day versus <4 servings/week was associated with 23% lower risk of early menopause (hazard ratio = 0.77, 95% confidence interval: 0.64, 0.93; *P* for trend = 0.08). Associations appeared to be limited to low-fat dairy foods (for ≥2 servings/day vs. <3 servings/month, hazard ratio = 0.83, 95% confidence interval: 0.68, 1.01; *P* for trend = 0.02), whereas high-fat dairy-food intake was not associated with early menopause. Low-fat dairy foods may represent a modifiable risk factor for reducing risk of early menopause among premenopausal women.

dairy food; menopause; menopause timing; milk; ovarian aging; ovarian function; yogurt

Early natural menopause is defined as the cessation of ovarian function prior to age 45 years, and it affects approximately 10% of women in Western populations (1). Early menopause is associated with increased risk of cardiovascular disease, osteoporosis, depression, and early cognitive decline (2–5). Furthermore, because female fertility declines substantially during the 10 years preceding the final menses, early menopause may interfere with family planning as women increasingly choose to delay childbearing. The inability of a couple to conceive as desired may have substantial psychological and financial implications (1, 6). Population-based studies indicate that genetic factors account for relatively little of the variation in menopausal timing, and recent prospective studies have identified several modifiable risk factors for early menopause, including diet (7–9). Bovine milk and dairy foods may be of particular interest, as they are comprised of a number of nutritive and nonnutritive components that may be physiologically related to ovarian aging and ovarian reserve (10).

Milk is an excellent source of vitamin D and calcium, as well as other macro- and micronutrients, including dairy fat, dairy protein, lactose, vitamin A, B vitamins, magnesium, phosphorus, potassium, and zinc (11). Many of these nutrients, particularly vitamin D, are hypothesized to be related to ovarian aging through potential effects on adiposity, inflammation, and anti-Müllerian hormone, a glycoprotein involved in follicle recruitment and a reliable proxy for ovarian reserve (12–14). In addition, milk also contains naturally occurring exogenous sex hormones, including estrogens and progesterone, and dairy-food consumption has been positively associated with plasma levels of total and free estradiol (15).

While few studies have considered the relationship between milk and dairy-food consumption and menopause timing, inverse associations of vitamin D and calcium from food sources, particularly dairy foods, with risk of early menopause were observed in Nurses' Health Study II (NHS2) (8). However, in a subsequent analysis, no associations between plasma 25-hydroxyvitamin D

level and risk of early menopause were observed, suggesting that observed associations with vitamin D intake are instead explained by other components of dairy food (16). To answer this specific question, we evaluated risk of early menopause with respect to intakes of total, low-fat, high-fat, and individual dairy foods among participants in NHS2.

METHODS

NHS2 is a prospective study of 116,429 female US registered nurses who were 25–42 years of age in 1989 when they responded to a mailed baseline questionnaire. Information regarding lifestyle behaviors and medical conditions is collected through biennial questionnaires, and the follow-up rate for each cycle has been at least 89%. The study protocol was approved by the Institutional Review Board at Brigham and Women's Hospital in Boston, Massachusetts.

Assessment of early menopause

On the 1989 baseline questionnaire, nurses were asked whether their menstrual periods had ceased permanently, with the following response options: 1) no—premenopausal; 2) yes—no menstrual periods; 3) yes—had menopause but now have periods induced by hormones; and 4) not sure (e.g., started using hormones prior to cessation of periods). Nurses who indicated that their periods had ceased were then asked the following questions: 1) At what age did your periods cease? (open response); and 2) For what reason did your periods cease? (response options were surgery; radiation or chemotherapy; and natural menopause). Women were also asked about their current and past use of hormone therapy. These questions were repeated on all questionnaires thereafter. Age at natural menopause was defined as age after 12 consecutive months of amenorrhea not due to radiation, chemotherapy, or surgery. A small number of women reported being postmenopausal on one questionnaire and then subsequently reported being premenopausal. For these women, we defined age at menopause as age after which periods had been absent for 12 months or more, and we then confirmed that this status persisted for at least 3 consecutive questionnaires.

We were interested in prospectively evaluating dairy-food intake and risk of early menopause; participants were thus eligible for inclusion in our study if they indicated being premenopausal and reported no age at menopause on the baseline 1989 questionnaire (*n* = 108,811). We then excluded women who did not respond to the 1991 food frequency questionnaire (FFQ) or reported implausible caloric intake (<500 kcal/day or ≥3,500 kcal/day) on the 1991 FFQ (*n* = 21,897), who were diagnosed with cancer before 1991 (*n* = 391), or whose date of menopause was before their date of return of the 1991 FFQ (*n* = 283). After baseline exclusions, 86,240 women comprised the analytical study sample (Figure 1).

Women were then followed prospectively until 2011 for menopause, as defined above, or first report of hysterectomy, bilateral or unilateral oophorectomy, cancer (not including nonmelanoma skin cancer), loss to follow-up, or death. Early menopause was defined as natural menopause occurring before the age of 45 years.
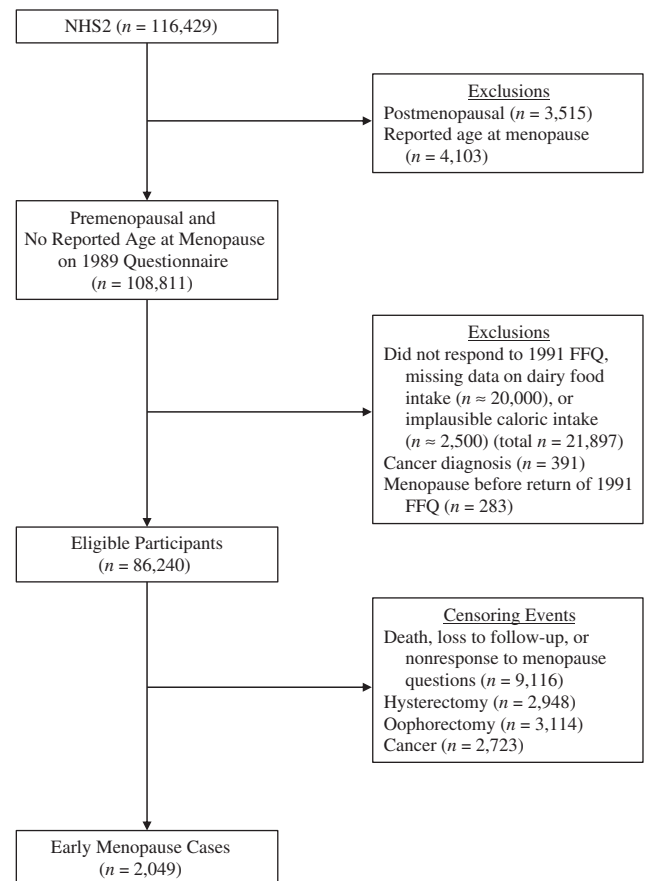
Figure 1. Selection of participants from Nurses' Health Study II (NHS2) for an analysis of dairy-food intake and early menopause, 1989–2011. FFQ, food frequency questionnaire.

Dietary assessment

Nurses were queried about their usual intake of 131 foods, beverages, and supplements over the preceding year via validated semiquantitative FFQs in 1991, 1995, 1999, 2003, 2007, and 2011 (17–19). Nine response categories were given for each item (i.e., <1 or 1–3 servings/month; 1, 2–4, or 5–6 servings/week; or 1, 2–3, 4–5, or ≥6 servings/day). We calculated low-fat dairy-food intake by summing intakes of skim and low-fat milk, frozen yogurt/sherbet, yogurt, cottage/ricotta cheese, and other low-fat cheese. High-fat dairy-food intake was equal to the sum of intakes of whole milk, cream, ice cream, cream cheese, other high-fat cheese, and butter. Total dairy-food intake was calculated by summing intakes of all dairy products. Deattenuated Pearson correlation coefficients for comparison of dairy-food intake measured via FFQ with that measured via four 1-week diet records among 173 Nurses' Health Study participants were moderate to strong (range, 0.54–0.77), with the exception of hard cheese (*r* = 0.33) (18). Women who responded to the 1991 FFQ were similar to those who did not with regard to age (34 years vs. 34 years), body mass index (BMI) (calculated as weight (kg)/height (m)^2; 24 vs. 24), and current smoking (13% vs. 16%).

In 1998, 45,947 nurses completed a retrospective 124-item FFQ assessing usual diet in high school. Dairy foods assessed on this questionnaire included chocolate milk, whole milk, low-fat milk, skim milk, yogurt, cottage or ricotta cheese, cheese, cream cheese, and butter. Correlations for comparison of 2 high school diet FFQs completed 4 years apart ($n = 333$) were high for total dairy foods ($r = 0.64$) and for milk specifically ($r = 0.76$) [20].

Assessment of covariates

Information regarding age, race, height, ethnicity, maternal and paternal education, physical activity during high school, body mass index at age 18 years, smoking during high school, and age at menarche was collected at baseline in 1989. Updated information on weight, parity, oral contraceptive use, breast-feeding, hormone therapy, and smoking was collected biennially throughout follow-up. Baseline height and updated weight were used to calculate updated BMI for each questionnaire cycle. Information on physical activity was collected in 1991, 1997, 2001, 2005, and 2009 using nurses' responses to questions regarding average time spent per week participating in specific activities (i.e., walking, running, biking, etc.), from which we calculated metabolic equivalent of task (MET)-hours per week [21].

Intakes of micro- and macronutrients were also assessed via FFQ every 4 years, including intakes of vegetable protein, dairy protein, lactose, dairy fat, dietary magnesium, phosphorus, potassium, zinc, vitamins B1, B2, B5, and B12, folate, calcium, vitamin D, vitamin A, and alcohol. For example, calcium intake from food sources was estimated by summing calcium content per serving of each food and beverage and multiplying it by the frequency of consumption. We calculated percentage of total energy derived from vegetable protein by multiplying vegetable protein intake (g/day) by 4 kcal/g and then dividing by total kilocalories.

Nurses were also asked to indicate their average use and dosage of multivitamins, calcium, and vitamin D supplements every 2 years on FFQs or biennial questionnaires, which we used to estimate intakes of each nutrient from supplemental sources. We calculated total vitamin D and calcium intakes by summing intakes from foods and supplements. We adjusted intakes of all nutrients for total energy intake using the residual method [22].

Statistical analysis

We assessed baseline characteristics of participants according to category of total dairy-food intake in 1991 using age-adjusted generalized linear models. We then used Cox proportional hazards regression to estimate age-adjusted and multivariable hazard ratios and 95% confidence intervals for early menopause according to category of adult intake of total, high-fat, low-fat, and individual dairy foods. We repeated these analyses for adolescent intakes of total dairy food and individual dairy foods. Tests for linear trend were conducted by modeling each exposure as a continuous variable (servings/day). Participants contributed person-time (in months) from the date of return of the 1991 questionnaire to menopause, first report of hysterectomy, bilateral or unilateral oophorectomy, cancer (not including nonmelanoma skin cancer), loss to follow-up, or death, whichever occurred first. Analyses were stratified by age (in months) and questionnaire cycle.

We separately modeled timing of intake using both baseline (1991) and cumulative average intakes for each exposure. Cumulative average values for each exposure were calculated as mean intakes estimated from all FFQs up to and including the cycle prior to menopause.

There was very little evidence of confounding in our analyses, and thus covariate selection for multivariable models (model 2) was based on factors identified a priori (i.e., age, race/ethnicity, parity, multivitamin use, age at menarche, and physical activity) and factors previously identified as risk factors for early menopause in our population (i.e., smoking, BMI, duration of breast-feeding, and intakes of alcohol and vegetable protein). To assess whether estimates for dairy-food exposures were confounded by intakes of vitamin D and calcium, we additionally adjusted for intakes of these nutrients in an additional model (model 3). We also further adjusted total dairy-food intake for intakes of dairy protein, lactose, dairy fat, dietary magnesium, phosphorus, potassium, zinc, vitamins B1, B2, B5, and B12, folate, and vitamin A to evaluate whether these nutrients explained the associations between dairy-food intake and risk of early menopause.

In multivariable models assessing adolescent dairy-food intake, we adjusted for a priori factors, including age, race/ethnicity, BMI at age 18 years, and physical activity and smoking during high school.

We considered potential effect modification by BMI (underweight/normal vs. overweight/obese) and oral contraceptive use (ever vs. never) using likelihood ratio tests comparing models with and without multiplicative interaction terms.

Finally, we conducted sensitivity analyses to evaluate the robustness of our estimates to potential residual confounding. Because some evidence suggests that polycystic ovary syndrome is related to both dairy-food intake and timing of menopause, we excluded women who reported diagnoses of polycystic ovary syndrome (n for analysis = 1,852 cases) [23, 24]. We also excluded women who reported diagnoses of autoimmune conditions, including rheumatoid arthritis, multiple sclerosis, lupus, Crohn's disease, and ulcerative colitis, as these conditions are associated with earlier menopause [25] and could be related to diet (n for analysis = 1,895 cases). Furthermore, in order to assess the adequacy of our control for confounding by smoking, we restricted an analysis to never smokers (n for analysis = 1,226 cases).

All statistical analyses were conducted with SAS software, version 9.4 (SAS Institute, Inc., Cary, North Carolina). We used 2-sided statistical tests with $\alpha = 0.05$.

RESULTS

Over 20 years of follow-up (1991–2011), 2,049 women in the sample experienced early menopause. At baseline, women reporting the highest dairy-food intake were on average younger, were more physically active, were less likely to smoke, and had higher BMI than those reporting the lowest intake (Table 1). Dairy-food intake was also positively associated with calcium, vitamin D, and alcohol intake and inversely associated with vegetable protein intake.

Table 1.   Age-Adjusted Characteristics of Premenopausal Women According to Category of Total Dairy-Food Intake at Baseline, Nurses' Health Study II, 1991[a,b]

Characteristic	Total Dairy-Food Intake, no. of servings					
	<2/Week (n = 2,091)	2–4/Week (n = 5,334)	5–6/Week (n = 6,062)	1/Day (n = 28,422)	2–3/Day (n = 32,432)	≥4/Day (n = 11,899)
Age, years[c]	36.8 (4.5)	36.5 (4.6)	36.3 (4.6)	36.0 (4.6)	35.6 (4.6)	35.2 (4.5)
Body mass index[d]	24.1 (0.16)	24.5 (0.07)	24.5 (0.07)	24.5 (0.03)	24.5 (0.03)	24.6 (0.05)
Calcium intake, mg/day	711 (8.5)	729 (5.3)	785 (5.0)	894 (2.3)	1,127 (2.2)	1,304 (3.6)
Vitamin D intake, IU/day	302 (5.6)	295 (3.5)	318 (3.3)	349 (1.5)	428 (1.4)	476 (2.3)
Age at menarche, years	12.4 (0.03)	12.4 (0.02)	12.4 (0.02)	12.4 (0.01)	12.4 (0.01)	12.5 (0.01)
Parity, no. of children	1.4 (0.03)	1.4 (0.02)	1.5 (0.02)	1.5 (0.01)	1.6 (0.01)	1.7 (0.01)
Physical activity, MET-hours/week	22.3 (1.4)	21.4 (0.9)	22.7 (0.8)	23.7 (0.4)	24.8 (0.4)	26.7 (0.6)
Vegetable protein intake, % of total kcal	5.6 (0.02)	5.3 (0.01)	5.2 (0.01)	5.1 (0.01)	4.9 (0.01)	4.6 (0.01)
Alcohol intake, g/day	2.6 (0.13)	3.0 (0.08)	3.2 (0.08)	3.2 (0.04)	3.1 (0.03)	3.3 (0.06)
Ever use of oral contraceptives, %	82	85	85	85	84	82
Current smoking, %	17	15	14	12	10	12

Abbreviations: BMI, body mass index; MET, metabolic equivalent of task.

[a] All characteristics were calculated with the use of generalized linear models adjusting for the age of participants in 1991.

[b] All values are presented as mean (standard error) unless otherwise indicated.

[c] Values are presented as mean (standard deviation).

[d] Weight (kg)/height (m)^2.

In age-adjusted analyses (model 1), women who consumed ≥4 servings of total dairy food per day versus <4 servings per week at baseline experienced a 26% lower risk (hazard ratio (HR = 0.74, 95% confidence interval (CI): 0.62, 0.88) of early menopause (Table 2). In particular, women who consumed the most low-fat dairy foods (≥2 servings/day) were 24% less likely (HR = 0.76, 95% CI: 0.64, 0.91) to experience early menopause than women with the lowest intake (<3 servings/month). In contrast, no association was observed for high-fat dairy-food intake (for ≥2 servings/day vs. <3 servings/month, HR = 1.03, 95% CI: 0.87, 1.23).

After adjustment for BMI, smoking, and other factors (model 2), estimates for total and low-fat dairy-food intake were very similar but slightly attenuated (Table 2). After further adjustment for total vitamin D and calcium intake (model 3), the hazard ratio comparing ≥4 servings of total dairy food per day with <4 servings of total dairy food per week was 0.77 (95% CI: 0.64, 0.93). Each 1-serving/day increment of total dairy-food intake was associated with a marginally significant 3% lower risk (HR = 0.97, 95% CI: 0.94, 1.00; P for trend = 0.08). Specifically, high (≥2 servings/day) versus low (<3 servings/month) intake of low-fat dairy foods was associated with 17% lower risk (HR = 0.83, 95% CI: 0.68, 1.01) of early menopause, and each 1-serving/day increment was associated with 5% lower risk (HR = 0.95, 95% CI: 0.91, 0.99; P = 0.02).

Intake of high-fat dairy food was not associated with risk of early menopause (for ≥2 servings/day vs. <3 servings/month, model 3 HR = 1.03, 95% CI: 0.87, 1.23). Estimates from models adjusting intakes of high- and low-fat dairy food for each other were substantively unchanged (data not shown). Further adjustment for dairy protein, lactose, dairy fat, dietary magnesium, phosphorus, potassium, zinc, vitamins B1, B2, B5, and B12,

folate, and vitamin A did not meaningfully change the estimates for dairy-food exposures (results not shown).

Our findings for total and low-fat dairy-food intake were consistent with those for individual low-fat dairy foods, including skim milk and yogurt. For example, in fully adjusted models (model 3), each 1-serving/day increment of skim milk intake was associated with 6% lower risk (HR = 0.94, 95% CI: 0.89, 0.99; P for trend = 0.02) of early menopause. Yogurt intake was not linearly associated with risk of early menopause (HR = 0.88, 95% CI: 0.72, 1.07; P for trend = 0.19); however, in categorical analyses, high (≥2 servings/day) versus low (almost never) intake of yogurt was associated with 14% lower risk (HR = 0.86, 95% CI: 0.75, 0.98) (see Web Table 1, available at https://academic.oup.com/aje). Other individual dairy foods were not significantly associated with risk of early menopause.

Estimates from models using cumulative averages of dairy-food intake were similar to those from models using baseline intake but were attenuated slightly. For example, 1-serving/day increments of total dairy-food and low-fat dairy-food intake were associated with 2% (HR = 0.98, 95% CI: 0.94, 1.01; P for trend = 0.15) and 5% (HR = 0.95, 95% CI: 0.90, 1.00; P for trend = 0.04) lower risks, respectively (complete data not shown).

Our findings for adolescent intake of dairy foods in the subset (n = 1,012 cases) of women who completed high school diet questionnaires are presented in Table 3. Dairy-food intake was substantially higher during adolescence (median, 10 servings/day) than adulthood (median, 2 servings/day), and therefore ranges were not comparable between time periods. Although statistical power for analyses of dairy-food intake during adolescence was low, estimates were similar to those for adult dairy-food intakes. For example, in multivariable analyses,

Table 2. Hazard Ratios for Early Menopause According to Category of Baseline (1991) Intake of Total, High-Fat, and Low-Fat Dairy Food and Individual Dairy Foods, Nurses' Health Study II, 1991–2011

Dairy Food and Intake Measure	No. of Cases	No. of Person-Years	Model 1[a]		Model 2[b]		Model 3[c]	
			HR	95% CI	HR	95% CI	HR	95% CI
Total dairy food, no. of servings								
≤4/week	214	90,448	1.00	Referent	1.00	Referent	1.00	Referent
5–6/week	160	76,591	0.92	0.75, 1.13	0.94	0.77, 1.16	0.94	0.76, 1.15
1/day	673	372,648	0.81	0.69, 0.94	0.84	0.72, 0.98	0.83	0.71, 0.98
2–3/day	736	441,958	0.75	0.65, 0.88	0.81	0.70, 0.95	0.80	0.68, 0.93
≥4/day	266	166,795	0.74	0.62, 0.88	0.79	0.66, 0.95	0.77	0.64, 0.93
Per 1-serving/day increment			0.96	0.94, 0.99	0.98	0.95, 1.00	0.97	0.94, 1.00
P for trend				0.01		0.10		0.08
High-fat dairy food, no. of servings								
<3/month	297	156,272	1.00	Referent	1.00	Referent	1.00	Referent
1/week	451	244,039	1.00	0.86, 1.15	1.03	0.89, 1.19	1.03	0.89, 1.19
2–4/week	441	262,545	0.92	0.79, 1.07	0.96	0.83, 1.12	0.96	0.83, 1.12
5–6/week	212	125,631	0.93	0.78, 1.11	0.98	0.82, 1.17	0.98	0.82, 1.17
1/day	413	236,695	0.96	0.83, 1.11	1.00	0.86, 1.17	1.00	0.86, 1.17
≥2/day	235	123,257	1.03	0.87, 1.23	1.03	0.87, 1.22	1.03	0.87, 1.23
Per 1-serving/day increment			1.01	0.97, 1.06	1.00	0.96, 1.04	1.00	0.96, 1.05
P for trend				0.51		0.85		0.83
Low-fat dairy food, no. of servings								
<3/month	158	70,531	1.00	Referent	1.00	Referent	1.00	Referent
1/week	242	115,308	0.97	0.79, 1.19	1.01	0.83, 1.24	1.01	0.83, 1.24
2–4/week	316	158,512	0.92	0.76, 1.12	0.99	0.81, 1.20	0.98	0.81, 1.19
5–6/week	167	95,026	0.81	0.65, 1.01	0.87	0.70, 1.09	0.86	0.69, 1.08
1/day	618	361,459	0.80	0.67, 0.95	0.88	0.74, 1.05	0.86	0.72, 1.03
≥2/day	548	347,603	0.76	0.64, 0.91	0.87	0.72, 1.04	0.83	0.68, 1.01
Per 1-serving/day increment			0.93	0.90, 0.97	0.96	0.93, 1.00	0.95	0.91, 0.99
P for trend				<0.01		0.05		0.02
Individual low-fat dairy foods, per 1-serving/day increment								
Skim milk			0.92	0.88, 0.97	0.96	0.91, 1.00	0.94	0.89, 0.99
P for trend				<0.01		0.04		0.02
Yogurt			0.86	0.71, 1.04	0.88	0.73, 1.07	0.88	0.72, 1.07
P for trend				0.13		0.19		0.19
Frozen yogurt/sherbet			0.91	0.74, 1.11	0.95	0.78, 1.15	0.95	0.78, 1.16
P for trend				0.35		0.61		0.61
Cottage/ricotta cheese			1.01	0.76, 1.36	1.08	0.81, 1.43	1.08	0.81, 1.43
P for trend				0.92		0.62		0.61
Low-fat other cheese			1.00	0.84, 1.20	1.03	0.86, 1.22	1.03	0.86, 1.23
P for trend				0.97		0.79		0.78
Individual high-fat dairy foods, per 1-serving/day increment								
Whole milk			1.09	0.96, 1.23	1.05	0.92, 1.19	1.05	0.92, 1.19
P for trend				0.19		0.46		0.46
Cream			1.04	0.97, 1.11	1.00	0.94, 1.07	1.00	0.94, 1.07
P for trend				0.26		0.97		0.96
Ice cream			0.81	0.62, 1.04	0.91	0.72, 1.17	0.92	0.72, 1.17
P for trend				0.09		0.47		0.47
Cream cheese			0.83	0.59, 1.18	0.88	0.63, 1.24	0.88	0.63, 1.24
P for trend				0.31		0.47		0.48

Table continues

Table 2. Continued

Dairy Food and Intake Measure	No. of Cases	No. of Person-Years	Model 1[a]		Model 2[b]		Model 3[c]	
			HR	95% CI	HR	95% CI	HR	95% CI
High-fat other cheese			1.00	0.90, 1.11	1.04	0.94, 1.16	1.04	0.94, 1.16
P for trend				0.99		0.46		0.44
Butter			1.01	0.91, 1.11	0.99	0.89, 1.09	0.99	0.89, 1.09
P for trend				0.92		0.78		0.78

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a] Model 1 adjusted for age (months; continuous).

[b] Model 2 adjusted for age (months; continuous), pack-years of smoking (0–10, 11–20, or ≥21), body mass index (weight (kg)/height (m)^2; <18.5, 18.5–24.9, 25.0–29.9, or ≥30), age at menarche (years; continuous), parity (nulliparous, 1–2 children, or ≥3 children), duration of breast-feeding (months; continuous), percentage of total kilocalories derived from vegetable protein (quintiles 1–3 or 4 + 5), alcohol intake (<10 g/day or ≥10 g/day), and current multivitamin use (yes or no).

[c] Model 3 adjusted for model 2 covariates plus total vitamin D intake (IU/day; continuous) and total calcium intake (mg/day; continuous).

the hazard ratio comparing total adolescent dairy-food intake of ≥6 servings/day with <4 servings/day was 0.86 (95% CI: 0.66, 1.12). When adolescent and adult total dairy-food intake were evaluated simultaneously, estimates for exposures in both time periods were similar but slightly stronger.

Analyses restricted to women without polycystic ovary syndrome or autoimmune conditions and women who had never smoked produced estimates similar to those seen in the full population. There was no evidence of multiplicative interaction by oral contraceptive use (P for interaction = 0.53) or BMI (P for

Table 3. Hazard Ratios for Early Menopause According to Category of Adolescent Intake of Total and Individual Dairy Foods, Nurses' Health Study II, 1991–2011

Dairy Food and Intake Measure	No. of Cases	No. of Person-Years	Model 1[a]		Model 2[b]	
			HR	95% CI	HR	95% CI
Total dairy food, no. of servings						
<4/day	60	27,571	1.00	Referent	1.00	Referent
4–5/day	112	56,833	0.92	0.67, 1.27	0.93	0.68, 1.28
≥6/day	840	462,393	0.86	0.66, 1.11	0.86	0.66, 1.12
Per 1-serving/day increment			1.00	0.99, 1.01	1.00	0.99, 1.01
P for trend				0.60		0.64
Skim milk, no. of servings						
Almost never	748	379,718	1.00	Referent	1.00	Referent
1/month–1/day	135	86,634	0.90	0.74, 1.08	0.92	0.76, 1.10
≥2/day	129	80,445	0.89	0.74, 1.08	0.92	0.76, 1.11
Per 1-serving/day increment			0.95	0.89, 1.02	0.96	0.90, 1.03
P for trend				0.17		0.29
Whole milk, no. of servings						
Almost never	414	241,591	1.00	Referent	1.00	Referent
1/month–6/week	207	100,797	1.10	0.92, 1.30	1.07	0.90, 1.27
1/day	118	64,149	0.99	0.81, 1.22	0.98	0.80, 1.20
≥2/day	273	140,260	1.02	0.87, 1.19	1.01	0.86, 1.18
Per 1-serving/day increment			1.00	0.95, 1.05	1.00	0.95, 1.05
P for trend				0.95		0.89

Abbreviations: CI, confidence interval; HR, hazard ratio; MET, metabolic equivalent of task.

[a] Model 1 adjusted for age (months; continuous).

[b] Model 2 adjusted for age (months; continuous), race (white or nonwhite), physical activity during high school (MET-hours/week; continuous), body mass index at age 18 years (weight (kg)/height (m)^2; continuous), and smoking during high school (pack-years; continuous).

interaction = 0.68) in the total dairy food–early menopause relationship.

DISCUSSION

In this prospective study, we found intake of low-fat dairy foods to be inversely associated with risk of early menopause. The relationship appeared to be strongly related to intakes of skim milk and yogurt. We also observed some suggestion of an inverse association with low-fat dairy-food intake in adolescence, though a lower sample size for these analyses limited our statistical power. In contrast, high-fat dairy-food intake at either time point was unrelated to risk.

To our knowledge, this was the first study to evaluate how dairy-food consumption is specifically related to risk of early menopause. However, our findings are largely consistent with those of a prospective analysis in the Nurses' Health Study evaluating dairy food consumption and overall timing of menopause. In that study, Carwile et al. (26) observed that intakes of low-fat dairy food and skim milk, but not total or high-fat dairy-food intake, were associated with earlier menopause only among participants under 51 years of age, suggesting that a similar relationship may have been observed specifically among women undergoing early menopause. Conversely, Nagel et al. (27) observed no association between total dairy-food intake and age at menopause among 5,110 participants in the European Prospective Investigation Into Cancer and Nutrition. Neither of these studies observed significant associations with total dairy food; this is perhaps explained by overall higher intakes of high-fat (vs. low-fat) dairy food in these populations.

A number of different mechanisms relating constituents of dairy food to ovarian aging have been proposed, including up-regulation of anti-Müllerian hormone by vitamin D and potential effects on vitamin D-mediated inflammatory pathways (14). In a recent analysis, inverse associations of vitamin D and calcium from food sources, specifically dairy foods, with risk of early menopause were observed among participants in NHS2 (8). Subsequent findings indicated that plasma 25-hydroxyvitamin D levels were not associated with risk of early menopause or anti-Müllerian hormone levels in the NHS2 population (16), suggesting that our findings are more likely to be explained by mechanisms involving other components of milk, rather than those involving vitamin D.

The observed relationships may be explained by associations of sex hormone levels with nonnutritive components of dairy food. Milk products contain varying concentrations of conjugated and unconjugated estrogen metabolites and progesterone (10, 28). Higher concentrations of lipophilic unconjugated estrogens and progesterone are present in high-fat dairy products, whereas hydrophilic conjugated estrogens are more concentrated in low-fat dairy products (10, 15). Hydrophilic conjugated estrogen metabolites, such as estrone sulfate, are considered more biologically active than their unconjugated counterparts because of their circumvention of hepatic metabolism (29). Differences in our findings for low-fat and high-fat dairy-food intake may thus be explained by the relative bioavailability and concentration of these hormones depending upon milk fat content.

Milk also contains androgens such as testosterone and androstenedione, which may be implicated in ovarian aging (30).

Epidemiologic evidence suggests that exogenous androgens are positively associated with circulating insulin-like growth factor 1 (IGF-1) in humans (31). Age is associated with a decrease in levels of circulating IGF-1, and in studies of rats, investigators have observed that low IGF-1 is associated with disruption of luteinizing hormone, which regulates ovulation (26, 32). Dairy-food consumption may therefore increase levels of IGF-1, potentially allowing for the continuation of normal menstrual cycles during the later reproductive years.

Further epidemiologic evidence demonstrates positive associations of dairy-food intake with plasma levels of total and free estradiol and IGF-1 (15, 33–35). Indeed, milk intake has been associated with other reproductive outcomes, including endometriosis (36) and premenstrual syndrome (37), as well as acne (38), suggesting that the levels of hormones present in dairy food are sufficient to alter circulating levels and, in turn, possibly influence risk of health outcomes. Given that associations for low-fat dairy-food intake persisted after we controlled for vitamin D and calcium intakes, as well as other nutrients in milk, the mechanisms involving hormones in milk appear to be the most likely physiological explanation for our findings.

Strengths of our study include its large sample size and high statistical power, prospective design, and high participant follow-up (>89%). Our study also had several limitations. First, some women may have misreported the timing of menopause, resulting in misclassification of case status. However, self-reporting of menopausal status has been shown to be a highly reproducible method of assessment (39). Among 6,591 Nurses' Health Study women who were premenopausal in 1976 and reported having undergone natural menopause on the 1978 questionnaire, 82% reported their age at menopause to within 1 year on the following 2 questionnaires (39), suggesting relatively limited misclassification. Importantly, misclassification of case status is unlikely to be related to dairy-food intake and would not explain our positive findings. Second, some degree of nondifferential misclassification due to error in self-reported dairy-food intake is expected. However, misclassification across extreme categories of dairy-food intake is improbable and would likely produce a bias towards the null. Finally, selection bias arising from missingness of 1991 FFQ data is unlikely, given the high degree of similarity between women completing the FFQ and those not completing the FFQ.

Although results evaluating adolescent dairy-food intake were similar to those for adult intakes, our ability to make direct comparisons across time periods was limited because of a lower sample size. Additionally, intake levels of dairy food during adolescence and adulthood were highly correlated in our population, making it difficult to assess each time point independently. Future large prospective studies may have better statistical power to evaluate how early-life dairy-food intake may be associated with risk of early menopause. NHS2 comprises a heterogeneous population with regard to many lifestyle and dietary variables; we therefore anticipate that our findings would apply to similar groups of premenopausal women. However, our findings may not be applicable to women who cannot consume dairy products because of milk allergies or lactose intolerance.

Findings of our study indicate that low-fat dairy products, including skim milk and yogurt, may represent modifiable risk factors women can alter in order to reduce their risk of early menopause.

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