A Prospective Study of Dairy Food Intake and Early Menopause

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ABSTRACT

Early natural menopause, the cessation of ovarian function prior to age 45, affects ~10% of women and increases risk of cardiovascular disease and other conditions. Laboratory evidence suggests a potential role of dairy foods in the ovarian aging process; however, no prior epidemiologic studies have evaluated how dairy intake is associated with risk of early menopause. We therefore evaluated how intakes of total, low-fat, high-fat and individual dairy foods are associated with early menopause in the 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 intake of ≥4 servings/day versus <4 servings/week was associated with 23% lower risk of early menopause (95% confidence interval (CI): 0.64, 0.93; *P*-trend = 0.08). Associations appeared to be limited to low-fat dairy foods (≥2 servings/day versus <3 servings/month HR: 0.83; 95% CI: 0.68, 1.01; *P*-trend = 0.02), whereas high-fat dairy intake was not associated with early menopause. Low-fat dairy foods may represent a modifiable risk factor to reduce risk of early menopause among premenopausal women.

Keywords:

Dairy; Milk; Yogurt; Ovarian aging; Menopause timing; Ovarian function

Abbreviations:

BMI, body mass index; CI, confidence interval; FFQ, food frequency questionnaire; HR, hazard ratio; IGF-1, insulin-like growth factor-1; MET, metabolic equivalent task; NHS2, Nurses' Health Study 2; PCOS, polycystic ovary syndrome.

Early natural menopause is defined as the cessation of ovarian function prior to age 45 and 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 non-nutritive 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 consumption has been positively associated with plasma levels of total and free estradiol. (15)

While few studies have considered milk and dairy consumption and menopause timing, inverse associations of vitamin D and calcium intakes from food sources, particularly from dairy foods, and risk of early menopause were observed in the Nurses' Health Study II (NHS2). (8)

However, in a subsequent analysis, no associations of plasma 25-hydroxyvitamin D levels and risk of early menopause were observed, suggesting that observed associations with vitamin D intake are instead explained by other components of dairy. (16) To answer this specific question, the present study evaluates risk of early menopause with respect to intakes of total, low-fat, high-fat, and individual dairy foods among participants of the NHS2.

METHODS

The NHS2 is a prospective study of 116,429 female U.S. registered nurses who were 25-42 years old in 1989 when they responded to a mailed baseline questionnaire. Information regarding lifestyle behaviors and medical conditions are collected through biennial questionnaires, for which 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, MA.

Assessment of early menopause

On the 1989 baseline questionnaire, nurses were asked if their 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 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). 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 were absent for 12 months or more, and then confirmed that this status persisted for at least 3 consecutive questionnaires.

We were interested in prospectively evaluating dairy 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 = 108,812). We then excluded women who did not respond to or who reported implausible caloric intake (<500 or ≥3,500 kcal/d) on the 1991 food-frequency questionnaire (FFQ) (n = 22,847), were diagnosed with cancer before 1991 (n = 391), or whose date of menopause was before their return date of the 1991 FFQ (n = 283). After baseline exclusions, 86,240 women comprised the analytic study sample (Figure 1).

Women were then followed prospectively until 2011 for menopause, as defined above, or first report of hysterectomy, bi-lateral or unilateral oophorectomy, cancer (not including non-melanoma skin cancer), loss to follow-up, or death. Early menopause was defined as natural menopause occurring before the age of 45.

Dietary assessment

Nurses were queried about their usual intake of 131 foods, beverages, and supplements over the preceding year via validated semi-quantitative FFQs in 1991, 1995, 1999, 2003, 2007, and 2011. (17–19) Nine response categories were given for each item (i.e., <1, 1-3 servings/month, 1, 2-4, 5-6 servings/wk, and 1, 2-3, 4-5, and ≥6 servings/d). We calculated low-fat dairy 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 intake was equal to the sum of whole milk, cream, ice cream, cream cheese, other high-fat cheese, and butter intakes. Total dairy intake was calculated by summing intakes of all dairy products. De-attenuated Pearson correlation coefficients comparing dairy food intake measured via FFQ to 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 vs. 34 years), BMI (24 vs. 24 kg/m^2) and current smoking (13% vs. 16%).

In 1998, 45,947 nurses completed a retrospective 124-item food frequency questionnaire 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 comparing two high school diet FFQ completed four years apart (n=333) were high for 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, smoking during high school, and age at menarche was collected at baseline in 1989. Updated information on weight, parity, oral contraceptive use, breastfeeding, hormone therapy use, and smoking were collected biennially throughout follow-up. Baseline height and updated weight were used to calculate updated body mass index (BMI) as weight (kg)/ height (m)^2 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 task (MET)-hours per week. (21)

Intakes of micro and macronutrients were also assessed via FFQ every four years, including vegetable protein, dairy protein, lactose, dairy fat, dietary magnesium, phosphorus, potassium, zinc, vitamins B_1, B_2, B_5 and B_{12}, folate, calcium, vitamin D, vitamin A and alcohol. For example, calcium intake from food sources was estimated by summing calcium content per 1 serving of each food and beverage and multiplying it by the frequency of consumption. We calculated % of total energy from vegetable protein by multiplying grams/day of vegetable protein by 4 kcal/g and then dividing by total kcal.

Nurses were also asked to indicate their average use and dosage of multivitamins, calcium and vitamin D supplements every two years on FFQs or biennial questionnaires, which we used to estimate intakes of each nutrient from supplement sources. Total vitamin D and calcium intakes were calculated by summing intakes from foods and supplements. We adjusted intakes of all nutrients for total energy 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 (HR) and confidence intervals (CI) 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 and individual dairy foods. Tests for linear trend were conducted by modeling each exposure as a continuous servings/day variable. Participants contributed person-time (in months) beginning on the date of return of the 1991 questionnaire until menopause, first report of hysterectomy, bi-lateral or unilateral oophorectomy, cancer (not including non-melanoma skin cancer), loss to follow-up, or death, whichever occurred first. Analyses were stratified on 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 breastfeeding, and intakes of alcohol and vegetable protein). To assess whether estimates for dairy 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 intake for dairy protein, lactose, dairy fat, dietary magnesium, phosphorus, potassium, zinc, vitamins B_1, B_2, B_5 and B_{12}, folate, and vitamin A to evaluate whether these nutrients explained the associations for dairy intake and risk of early menopause.

In multivariable models assessing adolescent dairy intake, we adjusted for *a priori* factors including age, race/ethnicity, BMI at age 18, 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 interactions 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 (PCOS) is related to both dairy intake and menopausal timing, **we** excluded women self-reporting diagnoses of PCOS (n for analysis = 1,852 cases). (23,24) We also excluded women self-reporting diagnoses of autoimmune conditions including rheumatoid arthritis, multiple sclerosis, lupus, Crohn’s disease, and ulcerative colitis, as these conditions are associated with earlier age at 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 v9.4 software (SAS Institute Inc., Cary, NC). We used two-sided statistical tests with alpha = 0.05.

RESULTS

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

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

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

Intake of high-fat dairy was not associated with risk of early menopause (<3 servings/month versus ≥2 servings/day, model 3 HR: 1.03; 95% CI: 0.87, 1.23). Estimates from models adjusting high and low fat dairy for each other were substantively unchanged (data not shown). Further adjustment for dairy protein, lactose, dairy fat, dietary magnesium, phosphorus, potassium, zinc, vitamins B_1, B_2, B_5 and B_{12}, folate, and vitamin A did not meaningfully change the estimates for dairy exposures (results not shown).

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

Estimates from models using cumulative averages of dairy intake were similar to models using baseline intake, but attenuated slightly. For example, each 1 serving/day increase in total dairy and low-fat dairy intake was associated with 2% (95% CI: 0.94, 1.01; *P*-trend = 0.15) and 5% (95% CI: 0.90, 1.00; *P*-trend = 0.04) lower risk, respectively (complete data not shown).

Our findings for adolescent intake of dairy foods among the subset (n=1,012 cases) of women who completed high school diet questionnaires are presented in Table 3. Dairy intake was substantially higher during adolescence (median = 10 servings/d) than adulthood (median = 2 servings/d) and therefore ranges were not comparable between time periods. Although power for analyses of dairy intake during adolescence was low, estimates were similar to those for adult dairy intakes. For example, in multivariable analyses the HR comparing total adolescent dairy intake of ≥6 versus <4 servings/d was 0.86 (95% CI: 0.66, 1.12). When adolescent and adult total dairy intake were evaluated simultaneously, estimates for exposures at both time periods were similar but slightly stronger.

Analyses restricted to women without PCOS or autoimmune conditions and women who never smoked produced estimates similar to those among the full population. There was no evidence of multiplicative interaction by oral contraceptive use (*P*-interaction = 0.53) or BMI (*P*-interaction = 0.68) on the total dairy-early menopause relation.

DISCUSSION

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

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

A number of different mechanisms relating constituents of dairy to ovarian aging have been proposed, including upregulation 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 intakes from food sources, specifically from dairy foods, and risk of early menopause were observed among participants of the NHS2. (8) Subsequent findings indicate that plasma 25-hydroxyvitamin D levels are not associated with risk of early menopause or anti-Müllerian levels in the NHS2 population, suggesting that our findings are more likely to be explained by mechanisms involving other components of milk, rather than those involving vitamin D. (16)

The observed associations may be explained via associations of sex hormone levels with non-nutritive components of dairy. 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 to be more biologically active than their unconjugated counterparts due to their circumvention of hepatic metabolism. (29) Differences in our findings for low-fat and high-fat dairy 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 circulating IGF-1, and studies in rats have observed that low IGF-1 is associated with disruption of luteinizing hormone, which regulates ovulation. (26,32) Dairy 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 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 are sufficient to alter circulating levels and in turn, possibly influence risk of health outcomes. Given that associations for low-fat dairy 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 physiologic explanation for our findings.

Strengths of our study include large sample size and high statistical power, prospective design, and high participant follow-up (>89%). Our study also has several limitations to note. First, some women may have misreported the timing of menopause, resulting in misclassification of case status. However, self-reported menopausal status has been shown to be a highly reproducible method of assessment. (39) Among 6,591 NHS women who were premenopausal in 1976 and reported having natural menopause on the 1978 questionnaire, 82% reported their age at menopause to within 1 year on the following two questionnaires, suggesting relatively limited misclassification. Importantly, misclassification of case status is unlikely to be related to dairy intake and would not explain our positive findings. Second, some degree of non-differential misclassification due to error in self-reported dairy food intake is expected. However, misclassification across extreme categories of dairy 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 in women completing versus not completing the FFQ.

Although results evaluating adolescent dairy intake were similar to those of adult intakes, our ability to make direct comparisons across time periods was limited due to lower sample size. Also, intake levels of dairy during adolescence and adulthood were highly correlated in our population, making it difficult assess each time point independently. Future large prospective studies may be more adequately powered to evaluate how early life dairy intake may be associated with risk of early menopause. The NHS2 is 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 due to 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 for women to reduce risk of early menopause.

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REFERENCES

1. Shuster LT, Rhodes DJ, Gostout BS, et al. Premature menopause or early menopause: long-term health consequences. *Maturitas*. 2010;65(2):161–6.

2. Wellons M, Ouyang P, Schreiner PJ, et al. Early menopause predicts future coronary heart disease and stroke: the Multi-Ethnic Study of Atherosclerosis. *Menopause*. 2012;19(10):1081–7.

3. van Der Voort D, van Der Weijer P, Barentsen R. Early menopause: increased fracture risk at older age. *Osteoporos Int*. 2003;14(6):525–30.

4. Fauser B. Female health implications of premature ovarian insufficiency [abstract]. International Federation of Fertility Societies 21st World Congress on Fertility and Sterility and the 69th Annual Meeting of the American Society for Reproductive Medicine, Boston, MA, 2013.

5. Bleil ME, Adler NE, Gregorich SE, et al. Does accelerated reproductive aging underlie premenopausal risk for cardiovascular disease? *Fertil Steril*. 2012;98(3):S43–S44.

6. Broekmans F, Soules M, Fauser B. Ovarian aging: mechanisms and clinical consequences. *Endocr Rev*. 2009;30(5):465–93.

7. Simpson J. Genetics of premature ovarian failure [abstract]. International Federation of Fertility Societies 21st World Congress on Fertility and Sterility and the 69th Annual Meeting of the American Society for Reproductive Medicine, Boston, MA, 2013.

8. Purdue-Smithe AC, Whitcomb BW, Szegda KL, et al. Vitamin D and calcium intake and risk of early menopause. *Am J Clin Nutr*. 2017;105(6):1493-1501.

9. Boutot ME, Purdue-Smithe A, Whitcomb BW, et al. Dietary protein intake and early menopause in the Nurses' Health Study II. Am J Epidemiol. 2018;187(2):270-277.

10. Hartmann S, Lacorn M, Steinhart H. Natural occurrence of steroid hormones in food. *Food Chem*. 1998;62(1):7–20.

11. Rice BH, Cifelli CJ, Pikosky MA, et al. Dairy components and risk factors for cardiometabolic syndrome: recent evidence and opportunities for future research. *Adv. Nutr*. 2011;2(5):396–407.

12. Szegda KL, Brian W. Whitcomb, Purdue-Smithe AC, et al. Adult adiposity and risk of early menopause. *Hum Reprod.* 2017;32(12):2522-2531.

13. Bertone-Johnson ER, Manson JE, Purdue-Smithe A, et al. Anti-Mullerian hormone levels and incidence of early natural menopause in a prospective study. *Hum Reprod*. 2018;33(6):1175-1182.

14. Purdue-Smithe AC, Bertone-Johnson ER. Dairy consumption, ovarian aging and reproductive lifespan: a narrative review. In: Berton-Johnson ER, ed. *Cursed? Biologic and Cultural Aspects of the Menstrual Cycle and Menstruation*. Hauppauge, NY: Nova Science Publishers; 2018:143–182.

15. Brinkman MT, Baglietto L, Krishnan K, et al. Consumption of animal products, their nutrient components and postmenopausal circulating steroid hormone concentrations. *Eur J Clin Nutr*. 2009;64(2):176–183.

16. Purdue-Smithe AC, Whitcomb BW, Manson JE, et al. Vitamin D status is not associated with risk of early menopause. *J Nutr*. 2018;148(9):1445-1452.

17. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol.* 1985;122(1):51–65.

18. Salvini S, Hunter DJ, Sampson L, et al. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. *Int J Epidemiol.* 1989;18(4):858–867.

19. Rimm EB, Giovannucci EL, Stampfer MJ, et al. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol.* 1992;135(10):1114-1126.

20. Maruti SS, Feskanich D, Colditz GA, et al. Adult recall of adolescent diet: reproducibility and comparison with maternal reporting. *Am J Epidemiol.* 2005;161(1):89–97.

21. Wolf AM, Hunter DJ, Colditz GA, et al. Reproducibility and validity of a self-administered physical activity questionnaire. *Int J Epidemiol.* 1994;23(5):991–999.

22. Willett WC. Nutritional Epidemiology. 2nd ed. New York, NY: Oxford University Press; 1998.

23. Schmidt J, Brännström M, Landin-Wilhelmsen K, et al. Reproductive hormone levels and anthropometry in postmenopausal women with polycystic ovary syndrome (PCOS): a 21-year follow-up study of women diagnosed with PCOS around 50 years ago and their age-matched controls. *J Clin Endocrinol Metab.* 2011;96(7):2178–2185.

24. Rajaeieh G, Marasi M, Shahshahan Z, et al. The relationship between intake of dairy products and polycystic ovary syndrome in women who referred to Isfahan University of Medical Science Clinics in 2013. *Int J Prev Med.* 2014;5(6):687–694.

25. Ebrahimi M, Akbari Asbagh F. The role of autoimmunity in premature ovarian failure. *Iran J Reprod Med*. 2015;13(8):461–472.

26. Carwile JL, Willett WC, Michels KB. Consumption of low-fat dairy products may delay natural menopause. *J Nutr*. 2013;143(10):1642–50.

27. Nagel G, Altenburg H-P, Nieters A, et al. Reproductive and dietary determinants of the age at menopause in EPIC-Heidelberg. *Maturitas*. 2005;52(3–4):337–347.

28. Farlow DW, Xu X, Veenstra TD. Quantitative measurement of endogenous estrogen metabolites, risk-factors for development of breast cancer, in commercial milk products by LC–MS/MS. *J Chromatogr B*. 2009;877(13):1327–1334.

29. Remesar X, Tang V, Ferrer E, et al. Estrone in food: a factor influencing the development of obesity? *Eur J Nutr.* 1999;38(5):247–253.

30. Gaiani R, Chiesa F, Mattioli M, et al. Androstenedione and testosterone concentrations in plasma and milk of the cow throughout pregnancy. *J Reprod Fertil.* 1984;70(1):55–59.

31. Klinger, Anin, Silbergeld, et al. Development of hyperandrogenism during treatment with insulin-like growth factor-I (IGF-I) in female patients with Laron syndrome. *Clin Endocrinol. (Oxf.)*. 1998;48(1):81–87.

32. Todd BJ, Merhi ZO, Shu J, et al. Hypothalamic insulin-like growth factor-I receptors are necessary for hormone-dependent luteinizing hormone surges: implications for female reproductive aging. *Endocrinology*. 2010;151(3):1356–1366.

33. Holmes MD, Pollak MN, Willett WC, et al. Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiol. Biomark Prev*. 2002;11(9):852–861.

34. Ma J, Giovannucci E, Pollak M, et al. Milk intake, circulating levels of insulin-like growth factor-I, and risk of colorectal cancer in men. *J Natl Cancer Inst.* 2001;93(17):1330–1336.

35. Giovannucci E, Pollak M, Liu Y, et al. Nutritional predictors of insulin-like growth factor I and their relationships to cancer in men. *Cancer Epidemiol Prev Biomark.* 2003;12(2):84–89.

36. Harris HR, Chavarro JE, Malspeis S, et al. Dairy-food, calcium, magnesium, and vitamin D intake and endometriosis: a prospective cohort study. *Am J Epidemiol*. 2013;177(5):420–30.

37. Bertone-Johnson ER, Hankinson SE, Bendich A, et al. Calcium and vitamin D intake and risk of incident premenstrual syndrome. *Arch Intern Med.* 2005;165(11):1246–1252.

38. Adebamowo CA, Spiegelman D, Danby FW, et al. High school dietary dairy intake and teenage acne. *J Am Acad Dermatol.* 2005;52(2):207–214.

39. Colditz GA, Stampfer MJ, Willett WC, et al. Reproducibility and validity of self-reported menopausal status in a prospective cohort study. *Am J Epidemiol.* 1987;126(2):319–325.

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

Characteristic	Total Dairy Intake (servings)					
	<2/wk (n=2091)	**2-4/wk** (n=5334)	**5-6/wk** (n=6062)	**1/d** (n=28422)	**2-3/d** (n=32432)	**≥4/d** (n=11899)
Age, y[b]	36.8 (4.5)	36.5 (4.6)	36.3 (4.6)	36.0 (4.6)	35.6 (4.6)	35.2 (4.5)
BMI, kg/m^2[c]	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/d	711 (8.5)	729 (5.3)	785 (5.0)	894 (2.3)	1127 (2.2)	1304 (3.6)
Vitamin D intake, IU/d	302 (5.6)	295 (3.5)	318 (3.3)	349 (1.5)	428 (1.4)	476 (2.3)
Age at menarche, y	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, *n*	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-h/wk	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/d	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 used oral contraceptives[d]	82	85	85	85	84	82
Current smoker[d]	17	15	14	12	10	12

Abbreviations: BMI, body mass index; IU, International units; MET-h, metabolic equivalent task hours.

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

[b]Values are means (SD).

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

[d]Values are expressed as percentages.

Table 2. Hazard Ratios and 95% Confidence Intervals for Early Menopause by Category of Baseline (1991) Intake of Total, High-fat and Low-fat Dairy, and Individual Dairy Foods: Nurses' Health Study II (1991-2011)

Dairy Food	Cases	Person-years	Model 1[a] HR	Model 1[a] 95% CI	Model 2[b] HR	Model 2[b] 95% CI	Model 3[c] HR	Model 3[c] 95% CI
Total dairy								
≤4/wk	214	90,448	1		1		1	
5-6/wk	160	76,591	0.92	0.75, 1.13	0.94	0.77, 1.16	0.94	0.76, 1.15
1/d	673	372,648	0.81	0.69, 0.94	0.84	0.72, 0.98	0.83	0.71, 0.98
2-3/d	736	441,958	0.75	0.65, 0.88	0.81	0.70, 0.95	0.8	0.68, 0.93
≥4/d	266	166,795	0.74	0.62, 0.88	0.79	0.66, 0.95	0.77	0.64, 0.93
per 1 serving/d			0.96	0.94, 0.99	0.98	0.95, 1.00	0.97	0.94, 1.00
P-trend				0.01		0.1		0.08
High-fat dairy								
<3/m	297	156,272	1		1		1	
1/wk	451	244,039	1.00	0.86, 1.15	1.03	0.89, 1.19	1.03	0.89, 1.19
2-4/wk	441	262,545	0.92	0.79, 1.07	0.96	0.83, 1.12	0.96	0.83, 1.12
5-6/wk	212	125,631	0.93	0.78, 1.11	0.98	0.82, 1.17	0.98	0.82, 1.17
1/d	413	236,695	0.96	0.83, 1.11	1.00	0.86, 1.17	1.00	0.86, 1.17
≥2/d	235	123,257	1.03	0.87, 1.23	1.03	0.87, 1.22	1.03	0.87, 1.23
per 1 serving/d			1.01	0.97, 1.06	1.00	0.96, 1.04	1.00	0.96, 1.05
P-trend				0.51		0.85		0.83
Low-fat dairy								
<3/m	158	70,531	1		1		1	
1/wk	242	115,308	0.97	0.79, 1.19	1.01	0.83, 1.24	1.01	0.83, 1.24
2-4/wk	316	158,512	0.92	0.76, 1.12	0.99	0.81, 1.20	0.98	0.81, 1.19
5-6/wk	167	95,026	0.81	0.65, 1.01	0.87	0.70, 1.09	0.86	0.69, 1.08
1/d	618	361,459	0.8	0.67, 0.95	0.88	0.74, 1.05	0.86	0.72, 1.03
≥2/d	548	347,603	0.76	0.64, 0.91	0.87	0.72, 1.04	0.83	0.68, 1.01
per 1 serving/d			0.93	0.90, 0.97	0.96	0.93, 1.00	0.95	0.91, 0.99
P-trend				<0.01		0.05		0.02
Low-fat dairy foods								
Skim milk								
per 1 serving/d			0.92	0.88, 0.97	0.96	0.91, 1.00	0.94	0.89, 0.99
P-trend				<0.01		0.04		0.02
Yogurt								
per 1 serving/d			0.86	0.71, 1.04	0.88	0.73, 1.07	0.88	0.72, 1.07
P-trend				0.13		0.19		0.19
Frozen yogurt/sherbet								
per 1 serving/d			0.91	0.74, 1.11	0.95	0.78, 1.15	0.95	0.78, 1.16
P-trend				0.35		0.61		0.61
Cottage/ricotta cheese								

per 1 serving/d	1.01	0.76, 1.36	1.08	0.81, 1.43	1.08	0.81, 1.43
P-trend		0.92		0.62		0.61
Low-fat other cheese						
per 1 serving/d	1.00	0.84, 1.20	1.03	0.86, 1.22	1.03	0.86, 1.23
P-trend		0.97		0.79		0.78
High-fat dairy foods						
Whole milk						
per 1 serving/d	1.09	0.96, 1.23	1.05	0.92, 1.19	1.05	0.92, 1.19
P-trend		0.19		0.46		0.46
Cream						
per 1 serving/d	1.04	0.97, 1.11	1.00	0.94, 1.07	1.00	0.94, 1.07
P-trend		0.26		0.97		0.96
Ice cream						
per 1 serving/d	0.81	0.62, 1.04	0.91	0.72, 1.17	0.92	0.72, 1.17
P-trend		0.09		0.47		0.47
Cream cheese						
per 1 serving/d	0.83	0.59, 1.18	0.88	0.63, 1.24	0.88	0.63, 1.24
P-trend		0.31		0.47		0.48
High-fat other cheese						
per 1 serving/d	1.00	0.90, 1.11	1.04	0.94, 1.16	1.04	0.94, 1.16
P-trend		0.99		0.46		0.44
Butter						
per 1 serving/d	1.01	0.91, 1.11	0.99	0.89, 1.09	0.99	0.89, 1.09
P-trend		0.92		0.78		0.78

Abbreviations: CI, confidence interval; BMI, body mass index; HR, hazard ratio; MET, metabolic equivalent task hours.

[a]Model 1 adjusted for age.

[b]Model 2 adjusted for age (months; continuous), pack-years of smoking (0-10, 11-20, ≥21), BMI (in kg/m2; <18.5, 18.5-24.9, 25.0-29.9, ≥30), age at menarche (continuous), parity (nulliparous, 1-2, ≥3), breastfeeding duration (continuous), % of total kcal from vegetable protein (quintiles 1-3, 4+5), alcohol intake (<10 or ≥10 g/d), and current multivitamin use (yes or no).

[c]Model 3 adjusted for model 2 covariates + total vitamin D intake (continuous) and total calcium intake (continuous).

Table 3. Hazard Ratios and 95% Confidence Intervals for Early Menopause by Category of Adolescent Intake of Total and Individual Dairy Foods: Nurses' Health Study II (1991-2011)

Dairy Food	Cases	Person-years	Model 1[a]		Model 2[b]	
			HR	95% CI	HR	95% CI
Total dairy						
<4/d	60	27,571	1		1	
4-5/d	112	56,833	0.92	0.67, 1.27	0.93	0.68, 1.28
6+/d	840	462,393	0.86	0.66, 1.11	0.86	0.66, 1.12
per 1 serving/d			1.00	0.99, 1.01	1.00	0.99, 1.01
P-trend			0.60		0.64	
Skim milk						
Almost never	748	379,718	1		1	
1/m-1/d	135	86,634	0.90	0.74, 1.08	0.92	0.76, 1.10
2+/d	129	80,445	0.89	0.74, 1.08	0.92	0.76, 1.11
per 1 serving/d			0.95	0.89, 1.02	0.96	0.90, 1.03
P-trend			0.17		0.29	
Whole milk						
Almost never	414	241,591	1		1	
1/m-6/wk	207	100,797	1.10	0.92, 1.30	1.07	0.90, 1.27
1/d	118	4,149	0.99	0.81, 1.22	0.98	0.80, 1.20
2+/d	273	140,260	1.02	0.87, 1.19	1.01	0.86, 1.18
per 1 serving/d			1.00	0.95, 1.05	1.00	0.95, 1.05
P-trend			0.95		0.89	

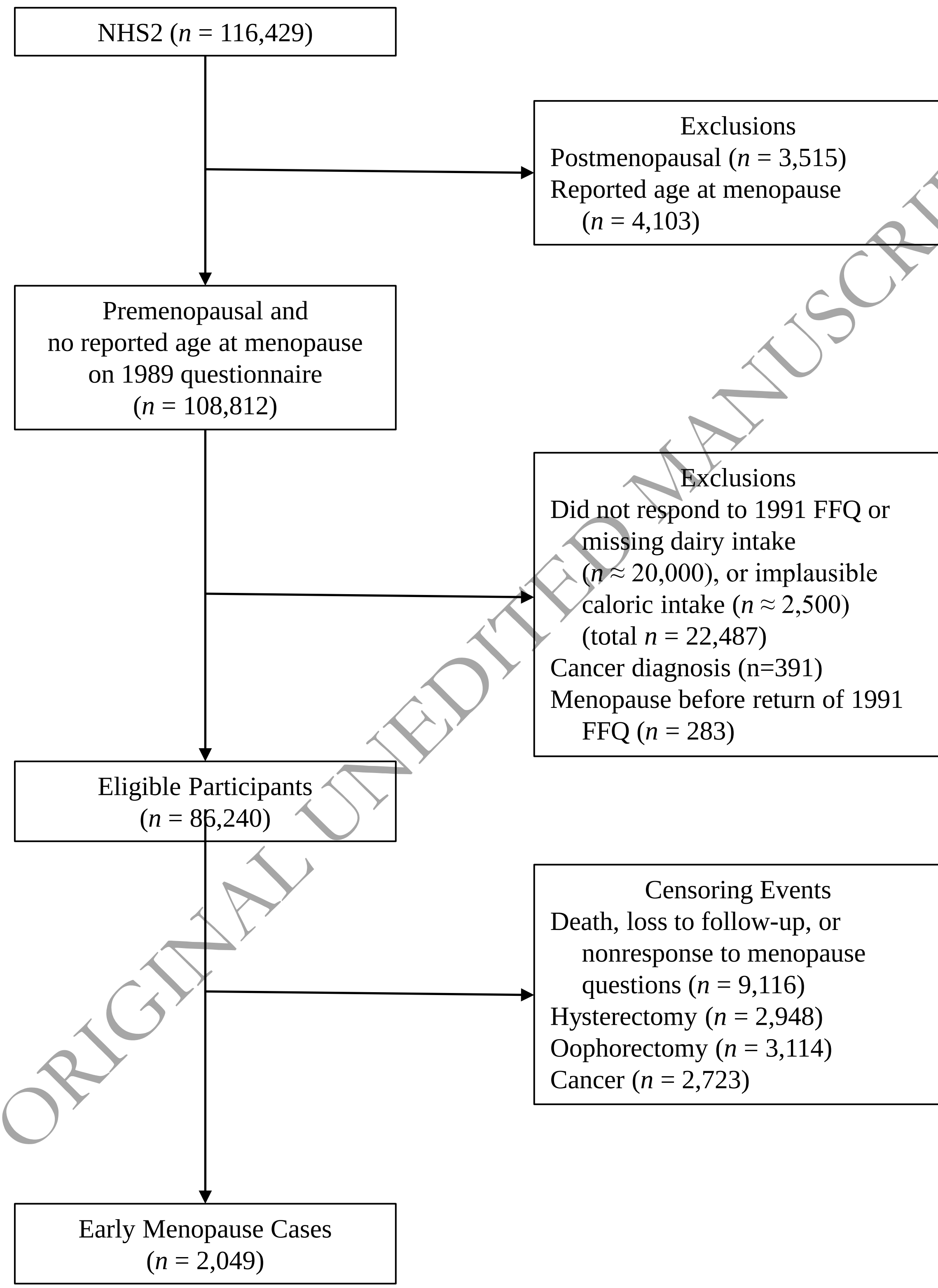
Abbreviations: CI, confidence interval; BMI, body mass index; HR, hazard ratio; MET, metabolic equivalent task hours.

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

[b]Model 2 adjusted for age (continuous months), race (white or non-white), physical activity during high school (continuous MET-h/wk), BMI at age 18 (continuous kg/m2), and smoking during high school (continuous pack-years).

Figure 1. Participant Flowchart: Nurses' Health Study II (1989-2011)

Figure 1 Legend: FFQ, food frequency questionnaire; NHS2, Nurses' Health Study II.


NHS2 (*n* = 116,429)
Exclusions
Postmenopausal (*n* = 3,515)
Reported age at menopause (*n* = 4,103)
Premenopausal and no reported age at menopause on 1989 questionnaire (*n* = 108,812)
Exclusions
Did not respond to 1991 FFQ or missing dairy intake (*n* ≈ 20,000), or implausible caloric intake (*n* ≈ 2,500) (total *n* = 22,487)
Cancer diagnosis (n=391)
Menopause before return of 1991 FFQ (*n* = 283)
Eligible Participants (*n* = 86,240)
Censoring Events
Death, loss to follow-up, or nonresponse to menopause questions (*n* = 9,116)
Hysterectomy (*n* = 2,948)
Oophorectomy (*n* = 3,114)
Cancer (*n* = 2,723)
Early Menopause Cases (*n* = 2,049)