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Vegetable Protein Intake and Early Menopause in the Nurses' Health Study II

Maegan Boutot
University of Massachusetts Amherst

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Vegetable Protein Intake and Early Menopause in the Nurses' Health Study II

A Thesis Presented

By

MAEGAN BOUTOT

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

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Epidemiology

Vegetable Protein Intake and Early Menopause in the Nurses' Health Study II

A Thesis Presented

By

MAEGAN BOUTOT

Approved as to style and content by:

Elizabeth Bertone-Johnson, Chair

Brian Whitcomb, Member

Paula Stamps, Graduate Program Director
Department of Biostatistics and Epidemiology

ABSTRACT

VEGETABLE PROTEIN INTAKE AND EARLY MENOPAUSE IN THE NURSES'

HEALTH STUDY II

MAY 2016

MAEGAN BOUTOT, B.A., THE COLLEGE OF NEW JERSEY

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Dr. Elizabeth Bertone-Johnson

Early menopause, the cessation of ovarian function prior to age 45, affects 5-10% of Western women and is associated with an increased risk of adverse health outcomes, including premature mortality and cardiovascular disease. Recent literature suggests that high vegetable protein intake may prolong female reproductive function, but no study has evaluated the association between this exposure and early menopause. Therefore, we evaluated the relationship between cumulative vegetable protein intake as a percentage of total calories and early menopause in the Nurses' Health Study II cohort. Women included in analyses were premenopausal at baseline (1991) and followed for up to 20 years. Cases (n=2,077) were defined as women experiencing natural menopause before age 45; women were excluded if early menopause was a result of hysterectomy, oophorectomy or radiation treatment. Non-cases were women whose age at menopause was 45 or greater or who were older than 45 and still premenopausal in 2011 (n=51,007). Intake of vegetable and animal protein was assessed every four years via food frequency questionnaires. In Cox proportional hazard models adjusting for age, smoking, diet, and behavioral factors, women in the highest quintile of cumulatively averaged vegetable protein intake (median=6.5%) had a significant 18% lower likelihood of experiencing early menopause as compared to women in the lowest

quintile (3.9%) (95% CI: 0.71-0.94; P-trend=0.004). In contrast, animal and total protein was unrelated to risk. Results were similar in analyses limited to never smokers and never oral contraceptive users. Our findings suggest vegetable protein intake may be inversely associated with early menopause.

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CHAPTER 1

INTRODUCTION

Early menopause, or the cessation of ovarian function prior to the age 45, affects 5-10% of Western women and is associated with increased risk of adverse health outcomes, including premature mortality and cardiovascular disease.¹⁻³ Additionally, women who develop early menopause may have reduced fertility as much as a decade prior to onset of menopause.^{1,3} The causes of natural early menopause are not well understood, and the majority of diagnoses are not attributable to genetic factors or autoimmune conditions.^{1,2}

Recent literature has suggested that diet composition may be associated with rate of ovarian aging, oocyte quality, and menopause timing.⁴⁻¹² In particular, vegetable protein and animal protein has been shown to have differential effects on ovarian function in animal models.⁶ Appt et al. found that cynomolgus macaques randomly assigned to receive a soy with isoflavins (i.e. vegetable protein) diet had more ovarian follicles than those randomized to receive a casein and lactalbumin (i.e. animal protein) diet (in secondary follicles, N=39, 95%CI: 29-50 as compared to N=25, 95%CI: 17-35, p=0.05).⁶ To our knowledge, the association between vegetable protein intake and early menopause has not been directly evaluated.

We have investigated the relationship between vegetable protein intake and natural early menopause among women enrolled in the Nurses' Health Study (NHS) II. We hypothesized that higher vegetable protein consumption would be inversely associated with incidence of early menopause.

CHAPTER 2

SUBJECT AND METHODS

The NHS II began in 1989 with the enrollment of 116,686 US female registered nurses, aged 25-42, through the use of a mailed baseline health questionnaire.¹³ The questionnaire was used to collect information on nurses' current and lifelong health, prescription medication use, and lifestyle factors.¹³ Participants have completed new questionnaires every two years since, and the follow-up for each questionnaire cycle has been at least 90%.¹³ The Institutional Review Board at Brigham and Women's Hospital (Boston, MA) approved of the NHS II study protocol.

A. Vegetable Protein Assessment

We assessed intake of total protein and protein from vegetable and animal sources in 1991 and every four years thereafter using the Harvard food frequency questionnaire (FFQ). The FFQ was added to the surveys starting in 1991. The FFQ measures participants' usual intake of 131 foods, beverages and dietary supplements over the previous year. Participants were asked to how often they consumed a single serving of an item using a 9 point scale, ranging from "Never or less than once a month" to "6+ per day".¹⁵

Vegetable protein intake was calculated by multiplying the vegetable protein content of a single serving of each food item by its frequency of consumption and then summing across all items. Foods explaining the greatest amount of variation in vegetable protein intake at baseline included pasta (7.85%), dark bread (6.33%), cold cereal (6.11%), and pizza (6.10%). Soy intake explained 0.55% of the variation. Intakes of total protein, animal protein, other macronutrients and micronutrients were derived similarly. We calculated vegetable and animal protein intake as percentages of total calories by multiplying intake of each protein

type in grams by four (the number of kilocalories in one gram of protein), dividing by the total number of calories consumed, and multiplying by 100. Intakes of micronutrients were adjusted for total energy intake using the residual method.²¹

We excluded participants from analysis if they reported less than 600 kcal or more than 3,500 kcal per day, were missing two or more sections of food categories, were missing the entire section on sweets and other miscellaneous foods, were missing an entire page of the FFQ, or did not provide information on more than 70 food items.¹⁴

The validity and reproducibility of the FFQ have been evaluated previously in a similar cohort (the Nurses' Health Study). In validation studies, the correlation coefficient for total protein assessed using the FFQ in the NHS vs. from 4 weeks of daily food records was 0.47.^{16,17} Reproducibility within the same cohort was found to be high.¹⁷

B. Outcome Assessment

The present analysis was limited to NHS II participants who were premenopausal in 1991 and had a reported age at menopause or who were followed through 2011 and remained premenopausal.¹⁴ Additionally, women younger than 45 who began using hormone replacement therapy (HRT), had a hysterectomy, or had an oophorectomy were excluded.

Menopausal status and timing have been assessed every two years since 1989. First, participants were asked if their menstrual periods had ceased, with response options of: "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)". Women who reported that their periods had ceased were asked to report the age at which their periods ceased (open response) and for what reason their periods ceased: "1) Surgery, 2) Radiation or chemotherapy, or 3) Natural". Women were subsequently asked

about their use of replacement sex hormones. Because menopause is a transitional process (perimenopause), it is not uncommon for women to be unsure of their menopausal status.^{19,20} In our analyses, age of menopause was assigned by examining the pattern of responses given on surveys from 1989 to 2011 to confirm correct menopausal status reports. Briefly, women were assigned an age at menopause by using the age reported on the first questionnaire on which they reported being postmenopausal, so long as they consistently did not report being premenopausal on any subsequent questionnaire. For censoring purposes, we used age at menopause in months assigned to the mid-year, which was derived by adding participants' birthday in months plus six months plus derived age at menopause in years times 12. Women who reported premenopausal on the 1991 survey, reported menopausal in the 1993 survey, and whose derived age at menopause in months was before the midpoint were excluded from analyses for not meeting baseline criteria of reporting premenopausal on the 1991 survey.

We classified women as having early menopause if their periods ceased naturally and their age at menopause was less than 45. Non-cases included women whose age at menopause was 45 or greater and women who were at least 45 years old and still premenopausal at the end of follow-up in 2011. We retained women in our analysis who reported surgical menopause, radiation/chemotherapy-induced menopause, and hormone therapy use prior to menopause if these events first occurred after age 45. Our method of assessing menopausal status in a prospective cohort has been validated in the both the NHS cohort and a subgroup of women who provided medical record confirmation of menopause age. Eighty-two percent of women experiencing natural menopause in the cohort repeatedly recalled the same age at menopause within one year on repeated health questionnaires, while

99% women whose age at menopause was confirmed via medical records accurately reported their age at menopause within one year.²⁰

C. Covariate Assessment

Additional dietary factors evaluated as confounders in our analysis were animal protein as a percentage of total calories, total calories, saturated fat, vitamin D without supplementation, B6 without supplementation, omega 3 intake, and calcium without supplementation.^{1,4,6-8,12} As with protein intake, these factors were assessed every four years starting in 1991 using the FFQ.

Lifestyle variables considered as possible confounders include physical activity in METs per week, body mass index (BMI), smoking history in pack years, alcohol consumption in grams/day, and ethnicity (*white non-Hispanic* or *other*).^{2,4,5,10} Physical activity was assessed every four years using a frequency questionnaire consisting of eight aerobic activities, including running and swimming. These responses were converted into MET hours.^{22,23} BMI is derived using participants' 1989 height and self-reported weight on each two year survey.

Smoking pack-years was assessed from nurses' self-reported smoking habits. Pack-years were defined as the number of cigarettes smoked per day, and this value is carried forward for non-respondents and for those who quit smoking. Alcohol consumption was assessed biannually with three questions regarding the frequency of one serving of wine, beer, and liquor. Ethnicity was self-reported in 1989.

Oral contraception, parity, breastfeeding, and age at menarche were considered variables relevant to lifetime ovulation and were also tested in the model. Current oral contraception use was assessed biannually from 1989 until 2011, and history and duration of

oral contraception was assessed in 1989. Parity, considered any pregnancy that lasted at least 6 months, was assessed biannually from 1989 until 2009. Months spent breastfeeding was self-reported biannually from 1989 until 2003. Age at menarche was reported on the 1989 survey.

Additionally, to confirm that any potential relationship between vegetable protein intake and early menopause was not related to total protein intake, cumulative total protein as a percentage of total calories was run in all final models, excluding vegetable and animal protein variables.

D. Statistical Analyses

We divided participants into quintiles of vegetable protein intake in 1991 based on the distribution within the cohort. We then compared the distribution of covariates measured at baseline with vegetable protein intake in age-adjusted general linear models.

We first evaluated the association between vegetable protein intake and early menopause using age-adjusted Cox proportional hazards models to calculate hazard ratios (HR) and 95% confidence intervals (CI). Cumulative average updating was used for dietary variables, including vegetable protein intake, and BMI. To test for linear trend across quintiles, we modeled the median value of each quintile as a continuous variable.

We included animal protein intake as a percentage of total calories and total calorie intake in our multivariable models *a priori* due to their strong relationship with the exposure variable and potential for confounding. All other potential covariates were evaluated for confounding through the individual addition of each into an age-adjusted model of vegetable protein intake as a percent of total calories, animal protein intake as a percent of total calories, and total calorie intake. Covariates whose addition resulted in a 10% or greater

change in the HRs for vegetable protein were to be retained; however, no covariates caused a 10% or greater change in the HR of any quintiles of vegetable protein intake. Therefore, the additional models were specified including risk factors from the literature. We decided upon two additional models: 1) one adjusting for lifestyle variables only and 2) one adjusting for lifestyle variables, variables related to cumulative number of lifetime ovulation, and dairy protein intake as a percentage of total calories.

Animal protein, total calories, and dairy protein were broken into quintiles based on the population distribution. BMI was categorized as <18.5 , 18.5 to <25 , 25 to <30 , and ≥ 30 . Smoking in pack-years was categorized as never, < 20 years, and ≥ 20 years. Oral contraceptive use was categorized into never, <2 years, and ≥ 2 years. Parity was categorized into nulliparous, 1-2 children, and ≥ 3 children. Breastfeeding was categorized as never, <2 years, and ≥ 2 years. Age at menarche was categorized as $12<$, 12 (referent), 13-15, and ≥ 16 .

To address residual confounding by smoking status, we repeated our analyses in never smokers, defined as participants who had zero pack-years of smoking at the end of follow-up in 2011. Similarly, we also repeated our analyses in women who never used oral contraceptives as of 2009 (oral contraceptive use was not reported in 2011).

Finally, we ascertained if timing of consumption was related to risk. To accomplish this, we identified each participant's protein intake at age 35 and at age 40 and then assessed how intake at each time period was associated with risk in logistic regression models. In order to preserve the prospective nature of these analyses, women whose age at menopause was less than or equal to 40 were excluded. Additionally, only women with exposures for age 35 and age 40 were included in the models, such that the comparisons between groups would be more interpretable. The same basic covariates used in the Cox models described

previously; however, they were age specific rather than cumulative or updated. For example, BMI associated with age 35 was used in the 35 model, similarly for age 40 models.

Statistical analyses were conducted with SAS v9.3 software (SAS Institute Inc, Cary, NC).

CHAPTER 3

RESULTS

After all exclusions, a total of 53,084 participants were included in our analyses and 2,077 women met criteria for early menopause.

Distributions of baseline covariates by quintile of vegetable protein consumption in 1991 are presented in Table 1. In general, women in the highest quintile of vegetable protein intake were older, consumed fewer calories from animal protein, consumed fewer calories overall, had a lower BMI, and were less likely to be parous. Despite being less parous, however, these women were more likely to breastfeed longer, given they were parous. Women in the fifth quintile were also more likely to be former smokers and less likely to be current smokers; however, former and current smokers in the fifth quintile smoked less than former or current smokers of the other quintiles. Women in the fifth quintile were also more likely to have never used oral contraceptives and were less likely to have been using oral contraceptives at baseline. In those who had used oral contraceptives (former or current), women in the fifth quintile used oral contraceptives for fewer months.

More broadly, comparing characteristics by quintiles of vegetable protein, decreasing linear trends from first to fifth quintile of vegetable protein were found for animal protein consumption, BMI, percentage of current smokers, pack-years smoking, percentage of current oral contraceptive users, and oral contraceptive use in months. For example, the average, age-adjusted BMI for quintile five in 1991 was 23.6 and decreased continuously towards quintile one. Similarly, increasing linear trends from first to fifth quintile were found for age, percentage of former smokers, percentage of never oral contraceptive users, and number of months breastfeeding in parous women (Table 1).

In the age-adjusted Cox model (Model 1), women in the highest quintile of vegetable protein intake (median = 6.5%) had a 21% lower likelihood of early menopause as compared to women in the lowest quintile (median = 3.9%) (95% CI: 0.69-0.91; *P*-trend: 0.0006) (Table 2). Addition of covariates only slightly attenuated results. When adjusting for all covariates (Model 4), women in the highest quintile of vegetable protein intake had a significant 18% lower likelihood of experiencing early menopause as compared to women in the lowest quintile (95% CI: 0.71-0.94; *P*-trend=0.004). In contrast, neither animal protein intake (*P*-trend Model 4 = 0.825) nor total protein intake (*P*-trend Model 4 = 0.791) was associated with risk of early menopause (Table 2).

Results limited to never smokers (N=34,602, 1,238 cases) and never oral contraceptive users (N=6,387, 228 events) were similar to results including the whole cohort (data not shown).

Results from logistic regression models comparing vegetable protein intake suggested that there may be differential effects by age (N=13,911 with 533 incidence early menopause cases) (Table 3). No significant associations were found between vegetable protein intake at 35 and incidence of early menopause; however, the odds ratios reported looked similar to those found for the age 40 analyses. In the age 40 analyses, women in the highest quintile of vegetable protein intake (median=6.8%) had a 33% lower odds of early menopause as compared to women in the lowest quintile (median=3.8%) when adjusting for age (95% CI: 0.51-0.871; *P*-trend=0.005). These results were attenuated but remained significant when adjusting for animal protein, total calories, smoking in pack-years, and BMI. Upon the addition of age specific Model 4 covariates, the results were no longer significant. Specifically, women in the highest quintile of vegetable protein consumption are at a non-

significant 24.9% lower likelihood of early menopause as compared to women in the first quintile (95% CI: 0.56-1.004, *P*-trend=0.11).

CHAPTER 4

DISCUSSION

In this prospective study, we found that high vegetable protein intake was significantly associated with lower likelihood of early menopause. Conversely, animal protein intake and total protein intake were not associated with early menopause.

Our results do not directly correspond to previous research done in the field, as our outcome was concerned primarily with the outcome of early menopause, in contrast to age at menopause. Disregarding that difference, there is only one study, to our knowledge, that has examined vegetable protein intake and age at menopause. Nagata et al. (2000) reported a non-linear association between vegetable protein intake, in grams, and age at menopause in 1,130 Japanese women. After adjusting for age BMI, smoking status, and age at which menses became regular, women in the second tertile of vegetable protein intake had a 51% higher risk of menopause than women in the first tertile. However, when adjusting for the same covariates, women in the third tertile of vegetable protein intake had no significant change in risk of menopause as compared to the first tertile (HR=0.99, 95%CI: 0.73-1.34).¹⁰ However, because these women only reported a single baseline dietary intake and were, on average, older than our cohort (range: 35-54 years of age, mean: 42.7, SD: 4.3)¹⁰, the association presented does not necessarily reflect a long-term cumulative effect of vegetable protein intake, nor does it correspond well to assessing the outcome of early menopause

Additionally, women in the Japanese cohort may be eating different sources of vegetable protein; women in the Japanese cohort reported median soy intake of 44 grams to 114 grams (approximately 1 to 4 ounces) per day, whereas, at baseline, the majority (89%) of the NHS II cohort included in our analyses reported “never or less than once per month” consumption of 3 to 4 ounces of soy and less than 1% reported one or more servings per day.

Rather, as mentioned previously, vegetable protein consumption in the NHS II cohort appears to be wheat based. This difference may be important as Nagata et al. (1998) found that higher consumers of soy had a lower age at menopause in a cross-sectional study²⁴; however, they did not find such an association in their more recent study, nor did other studies of European and Asian women.^{4,5,10}

Our results for total protein and animal protein intake are more in line with the literature, as neither of these factors have been found to be associated with age at menopause.^{4,5,10}

Given that controlling for confounding by animal protein intake had no effect on the effect estimates in the vegetable protein models and that total protein showed no association with early menopause, we believe that the risks shown are related to the specific effect of vegetable protein on outcome. The biological mechanism through vegetable protein acts on early menopause unknown. After finding that cynomolgus macaques randomly assigned to receive a soy-with-isoflavins diet had more ovarian follicles than those randomized to receive a casein-and-lactalbumin diet, Appt et al. (2010) suggested that soy isoflavones or their metabolites, possibly functioning as exogenous estrogens, may slow ovarian aging by decreasing inflammation or oxidative stress.⁶ It is unclear if this mechanism functions in this scenario however, as vegetable protein in the NHS II cohort is derived mostly from wheat products, making the percentage of daily calories from soy products much smaller than those consumed by the macaques. Relatedly, the results from Appt. et al contradict the epidemiological studies by Nagata et al.^{10, 23}

Results from our target age analyses suggest that vegetable protein consumption at age 40 may decrease the occurrence of early menopause, whereas results from age 35 suggest

a weaker, if any, association. It is important to note that these results only apply to women who undergo menopause between 40 and 45, as other women with early menopause were excluded to keep the data prospective. These results may be detecting a narrow window of exposure, in that exposure at age 35 is not strong enough to affect an outcome five to ten years in the future, whereas exposure at 40 has a much stronger impact for the upcoming one to five years. If an association between age 35 consumption and menopause between 40 and 45 does exist, it is possible that we were underpowered to detect an effect of age 35 exposure, as we lost many participants due to missing data. Nevertheless, these results suggest that women may be able to prolong menopause through vegetable protein consumption at age 40.

A main limitation of our study is that our exposure and outcome were self-reported. Cumulative vegetable protein intake was calculated using multiple self-reported FFQs. Although this technique is both common and validated, it is possible that women incorrectly reported their intake due to a misunderstanding of what constitutes a single serving of a food, causing an under or overestimation depending on the food, or due to a desire of having a better diet; however, adjusting for total calories and percent of total energy would reduce misclassification. Additionally, women would have to misreport for vegetable protein sources while still accurately reporting for animal protein and other dietary variables, which seems unlikely. Similarly, age at menopause was self-reported; however, reporting has been shown to be highly accurate within this population.²⁰ If some misclassification did occur, we would expect the error would be non-differential, and thus the HR would be, on average, biased towards the null.

In regards to generalizability, we would expect that our results would be generalizable to populations with similar vegetable protein consumption and main sources of vegetable protein. Considering that, it is possible that populations that, on average, eat much higher amounts of vegetable protein may experience a plateau effect, such that higher consumption of vegetable protein would not necessarily lead to decreased incidence of early menopause. Similarly, it is possible that in populations where the main sources of vegetable protein are different than those in NHS II cohort, the relationship may not hold. This latter finding would possibly shed light upon the mechanism by which our vegetable protein acts upon the reproductive system.

In conclusion, long term vegetable protein intake is associated with a reduced likelihood in the incidence of early menopause. Given the potential public health impact this may have and how unstudied early menopause is in the literature, more studies are warranted to discern which foods, if any, have the greatest impact, possible unaccounted confounders, and the biological mechanism that drives this relationship.

Tables

Table 1. Distribution of 1991 Age Standardized Covariates According to % Calories from Vegetable Protein in 1991; NHS II, 1991-2011.

Characteristic ¹	% Calories from Vegetable Protein				
	Q1 (n=9973) Median: 3.7%	Q2 (n=10470) Median: 4.4%	Q3 (n=10765) Median: 4.9%	Q4 (n=10930) Median: 5.4%	Q5 (n=10946) Median: 6.3%
Age (Years) ^{2,3}	36.3 (4.6)	36.5 (4.5)	36.8 (4.4)	37.0 (4.3)	37.2 (4.3)*
% of Calories from Animal Protein	15.9 (0.0)	15.1 (0.03)	14.5 (0.0)	13.9 (0.0)	12.1 (0.0)*
Total Caloric Intake	1796 (5.4)	1818 (5.3)	1810 (5.2)	1788 (5.2)	1736 (5.2)*
Body Mass Index (BMI)	25.0 (0.1)	24.6 (0.1)	24.5 (0.1)	24.2 (0.1)	23.6 (0.1)*
Smoking Status ⁴					
% Never ⁵	63.5	66.6	66.7	66.7	65.34*
% Former ⁵	22.2	23.4	24.8	25.6	28.1*
% Current ⁵	14.3	10.0	8.5	7.7	6.6*
Pack-Years (PY) Smoking ⁶	13.5 (0.14)	12.1 (0.14)	11.6 (0.14)	11.2 (0.14)	11.0 (0.13)*
Oral Contraceptive Use ⁴					
% Never ⁵	16.3	16.9	16.7	17	18.4*
% Former ⁵	72.5	72.6	72.8	73.6	72.9*
% Current ⁵	11.5	10.6	10.5	9.4	8.7*
Oral Contraceptive Use (Months) ⁶	50.9 (0.5)	48.3 (0.5)	47.1 (0.5)	45.7 (0.5)	42.9 (0.5)*
Parity (Pregnancies≥6 Months)	1.6 (0.01)	1.7 (0.01)	1.7 (0.01)	1.7 (0.01)	1.5 (0.01)*
Percent Parious ⁴	75.0	77.8	78.5	77.0	71.6*
Breastfeeding (Months) ⁷	10.5 (0.16)	12.9 (0.15)	13.4 (0.15)	14.2 (0.15)	15.6 (0.16)*
Age at Menarche (Years)	12.5 (0.01)	12.5 (0.01)	12.4 (0.01)	12.4 (0.01)	12.4 (0.01)*

¹Values are means and SEs or percentages standardized to the age distribution of the study population in 1991.

²Values are not age-adjusted.

³Standard deviation reported instead of standard error.

⁴Percentage

⁵P represents difference across all categories of use (never, former, current)

⁶Former and current users only

⁷Parious women only

*P<0.01

Table 2. Unadjusted and Adjusted Hazard Ratios and 95% Confidence Intervals for Protein Intake and Early Menopause; NHS II 1991-2011.

	Median	Cases	Early Menopause											
			Model 1			Model 2			Model 3			Model 4		
			HR	95% CI		HR	95% CI		HR	95% CI		HR	95% CI	
% Calories from Vegetable Protein														
1st Quintile	3.9%	456	1.0	Referent		1.0	Referent		1.0	Referent		1.0	Referent	
2nd Quintile	4.6%	410	0.84	0.73	0.96	0.84	0.73	0.96	0.87	0.76	0.99	0.88	0.76	1.00
3rd Quintile	5.1%	429	0.86	0.75	0.98	0.86	0.75	0.98	0.90	0.78	1.03	0.91	0.79	1.04
4th Quintile	5.6%	386	0.77	0.67	0.88	0.77	0.67	0.88	0.81	0.70	0.93	0.81	0.71	0.94
5th Quintile	6.5%	396	0.79	0.69	0.91	0.77	0.67	0.89	0.81	0.70	0.94	0.82	0.71	0.94
p-trend				0.0006			0.0002			0.003			0.004	
% Calories from Animal Protein*														
1st Quintile	10.0%	427	1.0	Referent		1.0	Referent		1.0	Referent		1.0	Referent	
2nd Quintile	12.2%	420	0.96	0.84	1.10	0.95	0.83	1.09	0.96	0.83	1.10	0.98	0.86	1.13
3rd Quintile	13.7%	410	0.95	0.83	1.09	0.92	0.80	1.06	0.94	0.81	1.08	0.97	0.85	1.12
4th Quintile	15.4%	416	1.00	0.87	1.14	0.95	0.83	1.10	0.97	0.84	1.11	1.01	0.88	1.17
5th Quintile	17.9%	404	1.04	0.90	1.19	0.94	0.81	1.08	0.96	0.83	1.12	1.01	0.86	1.17
p-trend				0.485			0.450			0.703			0.825	
% Calories from Total Protein ⁺														
1st Quintile	15.4%	423	1.0	Referent		1.0	Referent		1.0	Referent		1.0	Referent	
2nd Quintile	17.5%	436	1.01	0.88	1.16	1.01	0.88	1.16	1.03	0.90	1.18	1.06	0.93	1.22
3rd Quintile	18.9%	405	0.96	0.83	1.10	0.95	0.83	1.09	0.97	0.85	1.12	1.01	0.88	1.16
4th Quintile	20.4%	412	0.99	0.86	1.14	0.98	0.85	1.12	1.00	0.87	1.15	1.05	0.91	1.21
5th Quintile	22.8%	401	1.03	0.90	1.18	1.00	0.87	1.15	1.03	0.89	1.18	1.06	0.92	1.23
p-trend				0.756			0.867			0.845			0.791	

Model 1 Age-adjusted

Model 2 Adjusted for animal protein (quintiles) and total calories (quintiles)

Model 3 Adjusted for Model 2 covariates, BMI (categorical), and pack-years smoking (categorical)

Adjusted for Model 3 covariates, percent calories from dairy protein (quintiles), age at menarche (categorical), total months breastfeeding (categorical), OC use (categorical),

Model 4 parity (categorical)

* Adjusted for vegetable protein in place of animal protein in Models 1-4

+ Not adjusted for vegetable protein, animal protein, or dairy protein in Models 1-4

Table 3. Adjusted Odds Ratios and 95% Confidence Intervals for Vegetable Protein Intake at Age 40 and Age 35 and Early Menopause; NHS II 1991-2011.

	Median	Cases	Early Menopause											
			Model 1			Model 2			Model 3			Model 4		
			OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI	
% Calories from Vegetable Protein at Age 40														
1st Quintile	3.84%	111	1.00	Referent		1.00	Referent		1.00	Referent		1.00	Referent	
2nd Quintile	4.56%	91	0.77	0.58	1.02	0.80	0.60	1.06	0.82	0.62	1.10	0.85	0.63	1.13
3rd Quintile	5.10%	101	0.80	0.61	1.05	0.84	0.63	1.11	0.88	0.67	1.17	0.91	0.69	1.21
4th Quintile	5.69%	116	0.81	0.62	1.06	0.86	0.65	1.14	0.92	0.70	1.21	0.94	0.71	1.25
5th Quintile	6.77%	114	0.67	0.51	0.87	0.69	0.52	0.92	0.74	0.55	0.98	0.75	0.56	1.00
p-trend				0.01			0.03			0.09			0.11	
% Calories from Vegetable Protein at Age 35														
1st Quintile	3.74%	111	1.00	Referent		1.00	Referent		1.00	Referent		1.00	Referent	
2nd Quintile	4.43%	93	0.81	0.61	1.08	0.82	0.62	1.09	0.84	0.64	1.12	0.86	0.64	1.14
3rd Quintile	4.94%	114	0.96	0.74	1.25	0.97	0.74	1.26	0.99	0.76	1.30	1.01	0.77	1.32
4th Quintile	5.46%	108	0.93	0.71	1.22	0.91	0.69	1.20	0.95	0.72	1.25	0.95	0.72	1.26
5th Quintile	6.44%	107	0.89	0.67	1.16	0.80	0.60	1.07	0.83	0.62	1.11	0.82	0.61	1.09
p-trend				0.64			0.24			0.34			0.28	

Model 1 Age-adjusted

Model 2 Adjusted for animal protein (quintiles) and total calories (quintiles)

Model 3 Adjusted for Model 2 covariates, BMI (categorical), and pack-years smoking (categorical)

Model 4 Adjusted for Model 3 covariates, dairy protein (quintiles), age at menarche (categorical), total months breastfeeding (categorical), OC use (categorical), and parity (categorical)

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