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Vegetable protein intake is associated with lower gallbladder disease risk: findings from the Women's Health Initiative prospective cohort

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Abstract

Objective—This study aimed to measure associations between gallbladder disease and protein intake patterns, separated by quantity and type (vegetable vs. animal), among postmenopausal women.

Methods—Analyses were based on 130,859 postmenopausal women enrolled from 1993 to 1998 at 40 U.S. clinical centers in the Women's Health Initiative clinical trials and observational study. Women were excluded if they reported a history of gallbladder disease prior to baseline. Cox proportional hazards regression models, adjusted for gallbladder disease risk factors, were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for associations between energy-adjusted protein intake and gallbladder disease.

Results—In this study sample, 8.1% of postmenopausal women self-reported incident gallbladder disease. In multivariate analysis, women in the highest quintile of energy-adjusted vegetable protein intake (> 24.0 g/d) had a lower risk of gallbladder disease (HR, 0.87; 95% CI, 0.81–0.93) as compared to women in the lowest quintile (< 16.3 g/d) ($P_{trend} < 0.001$). Total protein intake was modestly protective against gallbladder disease ($P_{trend} < 0.021$). Animal protein intake was not associated with gallbladder disease risk. The protective effect of vegetable protein held

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stable only for women without history of diabetes (HR, 0.86; 95% CI, 0.80–0.92) and without recent weight loss (HR, 0.88; 95% CI, 0.80–0.97).

Conclusions—Vegetable protein intake is inversely associated with gallbladder disease risk in our sample of postmenopausal women. In addition to weight management, healthcare providers could emphasize vegetable protein as an additional dietary modality to promote lower risk for gallbladder disease.

Keywords

Protein; gallbladder; gallstone; diet; vegetable protein; animal protein; protein intake patterns; gallbladder disease

Introduction

Gastrointestinal disease affects 60 to 70 million people in the U.S. each year (Peery et al., 2012). One of the most common sites of gastrointestinal diseases is the gallbladder. The vast majority of gallbladder disease cases are associated with inflammation of the gallbladder caused by gallstones. Though up to 80 percent of cases are asymptomatic (Stinton and Shaffer, 2012), 20.5 million people have suffered from gallstones, resulting in over 750,000 cholecystectomies performed annually in the US (Stinton and Shaffer, 2012). It is estimated that gallstones cost the U.S. healthcare system an estimated $6.2 billion per year (Everhart and Ruhl, 2009a), and women account for 70% of cases (Shaffer, 2006). Though most cases are treatable, preventing gallbladder disease would result in a substantial reduction in public health burden.

One of the leading modifiable risk factors for gallbladder disease is diet (Jessri and Rashidkhani, 2015; Maclure et al., 1989; Stinton and Shaffer, 2012; Tseng et al., 1999). Specifically, excess energy intake resulting in obesity as well as greater dietary fat have been associated with elevated risk for gallbladder disease (Amaral and Thompson, 1985; Erlinger, 2000; Everhart, 1993; Tsai et al., 2004b). In an era of epidemic obesity, medical providers are promoting weight loss. Of note, however, is evidence that significant weight loss is a strong risk factor for gallbladder disease (Everhart, 1993; Liddle et al., 1989). Further, high-protein, low-fat diets that are often encouraged for weight loss (Noakes et al., 2005; Wycherley et al., 2012) may have limited efficacy (Schwingshackl and Hoffmann, 2013).

Dietary protein as a risk modulator for gallbladder disease has been explored with mixed results. Many animal studies have shown a reduction in gallstones, reduced biliary cholesterol and lithogenic index level (Kritchevsky and Klurfeld, 1979, 1983; Mahfouz-Cercone et al., 1984; Sullivan et al., 1985; Tomotake et al., 2006), and lower crystallization rates (Catala et al., 2000) with higher vegetable protein intake compared to animal protein-rich diets. Human feeding studies examining associations between type (vegetable vs. animal) and quantity of protein and gallbladder disease are sparse. Epidemiological studies have observed that people consuming vegetarian diets have a lower incidence of gallbladder disease (Kratzer et al., 1997; Pixley et al., 1985; Pradhan et al., 2009), but specific aspects of the vegetarian diet were not fully elucidated. The studies that probe for associated risks between protein intake and gallbladder disease are conflicting. In the prospective Nurses'

Health Study, women with increased vegetable protein consumption had reduced risk of developing symptomatic gallstones (Maclure et al., 1990) and lower risk for cholecystectomy (Tsai et al., 2004a), but two case-control studies (Misciagna et al., 1999; Pixley et al., 1985) and one other prospective cohort study (Attili et al., 1998) found no association between gallbladder disease and protein intake.

Considering that vegetable and animal protein consumption is a modifiable behavior, additional investigation into the effects of protein intake on gallbladder disease is warranted. The Women's Health Initiative (WHI), with a diverse, well-characterized sample of over 160,000 postmenopausal women (1998; Hays et al., 2003), provides ample opportunity to robustly analyze whether vegetable and/or animal protein intake impacts the risk of developing gallbladder disease. The relationships were evaluated under the hypothesis that vegetable protein is inversely associated with gallbladder disease, while total protein and animal protein are positively associated.

Subjects and Methods

Study design and sample

The WHI recruited a large, diverse sample of postmenopausal women (n = 161,808) between 1993 and 1998 at 40 U.S. clinical centers. Women participated in either the clinical trials (n = 61,132) or the Observational Study (n = 93,646) (1998). The three randomized, overlapping clinical trials comprised Hormone Therapy, Calcium/Vitamin D, and Dietary Modification interventions. Women were eligible if they were unlikely to relocate or die within three years, postmenopausal, between 50 and 79 years of age, and not participating in other clinical trials (1998). All women provided written informed consent, and study procedures were approved by the Institutional Review Boards of the 40 U.S. participating clinical centers. Detailed information about the study design and the reliability of baseline measures has been previously described (1998; Langer et al., 2003). Women were excluded from the current analysis if they were missing follow-up data (n = 923), reported extremely low or high total energy intake (< 600 or > 5,000 kcal/day, n = 4837), or had a history of gallbladder disease (n = 25,189). Thus, 130,859 women were included in the analysis.

Protein intake measurement

Daily protein intake was acquired from a food-frequency questionnaire (FFQ) administered at baseline, including 122 individual food/food group items, 19 adjustment items, and summary questions (Patterson et al., 1999). Across quintiles of intake, energy-adjusted dietary protein intake was evaluated as vegetable protein, animal protein, or total protein.

Gallbladder disease ascertainment

Women directly self-reported new diagnosis of gallbladder disease or gallstones on the mailed semiannual (clinical trial) or annual (observational study) medical history update form. Women were asked, "Since the date given on the front of this form, has a doctor told you for the first time that you have any of the following specific conditions… Gallbladder disease or gallstones?" Participants were censored at the date of self-reported gallbladder disease or date of last questionnaire returned during the initial WHI study period (i.e.

extension studies not included, because gallbladder disease outcomes were not collected beyond the initial WHI study period). The mean follow-up time of the entire sample was 7.5 ± 1.9 y, while the median follow-up was 7.9 y. Among women with incident gallbladder disease, the mean time-to-event was 4.4 ± 2.4 y.

Covariates

Demographic, clinical, and behavior variables were gathered at baseline using study-specific questionnaires. Neighborhood socioeconomic status (NSES) was derived through linkage of individual participant addresses to Federal Information Processing Standards codes from the 2000 U.S. census and tract-level socioeconomic data, with summary measures calculated according to previously described algorithms (Qi et al., 2012). Body mass index (BMI) (kg/m^2) was calculated from women's height and weight measured using study-specific protocols by trained research staff at baseline during local clinic visits; BMI was categorized in accordance with standard World Health Organization cutoffs (National Heart Lung and Blood Institute. and National Institute of Diabetes and Digestive and Kidney Diseases (U.S.), 1998).

Statistical analysis

Protein and other nutrients were adjusted for self-reported energy intake to reduce the risk of confounding (Brown et al., 1994; Willett et al., 1997). Energy-adjusted nutrients were calculated by finding the unstandardized energy residuals using linear regression, calculating the predicted value of each nutrient, and finding the mean of the predicted value of the nutrient. The mean of the predicted value of each nutrient was added to each of the nutrient residuals to obtain the final energy-adjusted value. Baseline characteristics of participants were compared across quintiles of energy-adjusted vegetable protein intake.

Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between energy-adjusted protein intake and gallbladder disease. Protein measures were categorized in quintiles and additionally assessed as continuous variables (estimates presented per 10 g/d increase in energy-adjusted protein intake). Tests for trend used the median of each quintile. In assessing confounding, none of the variables listed in Table 1 changed the association between vegetable protein and gallbladder disease by ≥ 10%. The following potential confounders were selected based on previously published literature and included in multivariate models: age (continuous), total energy (log-transformed; continuous), BMI (continuous), physical activity (MET-hr/wk; continuous), NSES (continuous), race/ethnicity (non-Hispanic white, black, Hispanic, Asian, Native American, other/unknown), oral contraceptive use (ever, never), hormone therapy use (never, past, current), history of liver disease (yes, no), statin use at baseline (yes, no), thiazide use at baseline (yes, no), alcohol use (< 1 drink/wk, 1 to < 7 drink/wk, ≥ 7 drink/wk), and clinical trial arms.

Associations between energy-adjusted vegetable protein intake and gallbladder disease were stratified by history of diabetes. An additional stratified analysis considered weight change between baseline and year 3 and categorized women according to weight loss > 10%. Women with weight change ≥ 25% were excluded from this analysis, because such dramatic

changes in weight are likely due to data errors. Outcomes from this stratified analysis were limited to those occurring ≥ 3 y after baseline for this stratified analysis. Potential interactions between vegetable protein and each stratification variable on gallbladder disease were tested using likelihood ratio tests. All statistical analyses were conducted using Stata 14.0 (StataCorp, College Station, TX).

Results

Baseline characteristics of study participants

The mean (non-energy-adjusted) vegetable and animal protein intakes in the study sample were 20.4 g/d and 47.8 g/d, respectively. Women who reported higher energy-adjusted vegetable protein intake were more commonly non-Hispanic whites or Asian with a lower BMI, higher reported physical activity, higher education (≥ college degree), and higher NSES (Table 1). Compared to women in the lowest quintile of vegetable protein intake, those reporting higher vegetable protein intake also reported lower intake of animal protein, total fat, saturated fat, cholesterol, and alcohol, and higher intake of fruit and vegetables.

Vegetable protein intake and gallbladder disease risk

A total of 8.1% of women (n = 10,615) self-reported gallbladder disease during 7.5 ± 1.9 y mean follow-up. Upon multivariate analysis, the highest quintile of energy-adjusted vegetable protein intake (> 24.0 g/d) was significantly associated with reduced gallbladder disease (HR, 0.87; 95% CI, 0.81–0.93; $P_{trend} < 0.001$), suggesting 13% lower risk than the lowest quintile (Table 2). A positive association was shown in the age- and energy-adjusted model between animal protein intake and gallbladder disease, suggesting 12% higher risk (quintile 5 versus 1 HR, 1.12; 95% CI, 1.06–1.19), but the association was attenuated in the multivariate analysis (quintile 5 versus 1 HR, 1.00; 95% CI, 0.94–1.07). Only the fourth quintile of total protein intake was associated with lower risk of gallbladder disease in the multivariate model (HR, 0.92; 95% CI, 0.86–0.99); however, the test for trend was statistically significant ($P_{trend} = 0.021$), as was the continuous model (HR, 0.98; 95% CI, 0.97–1.00; $P = 0.014$), indicating a modest protective association.

When the analytical sample was stratified by history of diabetes—a possible risk factor for gallbladder disease (Haffner et al., 1990; Jorgensen, 1989; Pacchioni et al., 2000)—no significant association between energy-adjusted vegetable protein intake and gallbladder disease was observed among women with diabetes ($P_{trend} = 0.535$; Table 3). Among women without diabetes, higher vegetable protein consumption showed a significant protective effect against gallbladder disease (HR, 0.86; 95% CI, 0.80–0.92 quintile 5 versus 1; $P_{trend} < 0.001$). Further, since weight loss is a well-documented risk factor for gallbladder disease (Everhart, 1993; Liddle et al., 1989; Stampfer et al., 1992; Yang et al., 1992), an independent assessment of the relationship between weight loss and gallbladder disease was conducted according to weight loss > or ≤ 10% of body weight between baseline and year 3. Among women with weight loss > 10% during the first 3 years of follow-up, 7.2% developed gallbladder disease versus 5.7% of women reporting lower percentage of body weight loss or weight gain (chi-square test, $P < 0.001$; crude HR, 1.31; 95% CI, 1.18–1.47). When stratified by weight loss (> or ≤ 10% body weight change between baseline visit and year 3),

a significant protective association between vegetable protein intake and gallbladder disease was observed in women with ≤ 10% weight loss and/or weight gain (HR, 0.88; 95% CI, 0.80–0.97 quintile 5 versus 1; P_{trend} = 0.009), and not in women with > 10% weight loss.

Discussion

To our knowledge, this is the largest prospective cohort study to investigate protein intake and gallbladder disease risk. Consistent with our hypothesis, multivariate analysis revealed decreasing risk of gallbladder disease across increasing levels of energy-adjusted vegetable protein intake, with 13% lower risk among postmenopausal women in the highest (> 24.0 g/d) versus lowest (< 16.3 g/d) quintile. Contrary to our hypothesis that animal protein would be associated with higher risk for gallbladder disease, no association between gallbladder disease and animal protein intake was observed. There was a modest association between energy-adjusted total protein and gallbladder disease, with an HR estimate in between those calculated for vegetable protein intake (protective) and animal protein (null). Though the suggested protective effect of vegetable protein on gallbladder disease is modest, gallstones were diagnosed during 1.8 million outpatient hospital visits in 2004, and age-adjusted rates were 162% for women compared to men (Everhart and Ruhl, 2009b). Incidence of gallbladder disease increases rapidly after age 40, and remains elevated in women even after menopause (Shaffer, 2005; Stinton and Shaffer, 2012). Considering that 8.1% (n = 10,615) of women in our sample reported gallbladder disease, efforts to prevent this disease burden are warranted.

Existing studies examining protein intake and gallbladder disease are conflicting. Maclure and colleagues conducted a similar analysis with a prospective cohort of 88,837 female registered nurses ages 34–59 y from 11 states in the Nurses' Health Study (Maclure et al., 1990). Their analysis was energy-adjusted, but established risk factors were not included as covariates. Symptomatic gallstones were noted less frequently (RR, 0.7) among normal weight women (BMI < 25 kg/m^2) in the highest quintile of vegetable protein intake (≥ 20.5 g/d). Upon inclusion of all women, no significant association was observed. Our study–with greater geographical diversity, only postmenopausal women (mean age 63 y), and longer average follow-up–found a lower risk of gallbladder disease with higher vegetable protein consumption in the entire sample. Both studies suggested there is no associated risk between animal protein or total protein consumption and gallbladder disease. Tsai et al. later analyzed the Nurses' Health Study for incidence of cholecystectomy (Tsai et al., 2004a). After multivariate analysis similar to the present study, they reported a lower risk of cholecystectomy (RR = 0.8) among the highest quintile of vegetable protein consumers and no associated risk between animal or total protein intake and cholecystectomy.

Other case-control studies (Misciagna et al., 1999; Pixley et al., 1985) and a prospective cohort study (Attili et al., 1998) have reported total protein intake and gallstone relationships, but not specific analyses for vegetable versus animal protein. Moreover, samples and study designs differ too greatly from the present study for proper comparison. Mathew and Ko conducted a case-control study with pregnant women in Washington wherein the findings suggested that no associations existed between vegetable or animal protein intake and biliary stones (Mathew and Ko, 2015). The study design and sample

differences may be too great for informative comparisons to be made. Replication of our results in other large prospective samples is needed to inform on plausibility.

These results must be considered in the larger context of several dietary and lifestyle risk factors for gallbladder disease that are highly correlated. For example, the findings may be related to exposures such as dietary saturated fat and cholesterol that are reflected in an animal- versus vegetable-rich diet. It is known that plant-based diets contain fewer dietary triglycerides and cholesterol than animal-based diets. Cholesterol stone formation, constituting approximately 80% of gallstone cases, occurs when cholesterol formation continually exceeds the solubilizing capacity of bile. Saturated fats are known to have the largest effect on cholesterol synthesis (Hu et al., 2001). Therefore, diets rich in cholesterol and saturated fats may be elevating cholesterol to levels beyond bile solubilization capacity, a known pathogenesis of cholesterol gallstones (Acalovschi, 2001). In fact, we note significantly lower cholesterol, saturated fat, and animal protein intake among women in the highest quintile of vegetable protein intake compared to lowest. Our results may also be attributed to the lifestyle characteristics of women in our sample that are collinear with vegetable protein intake. With regard to physical activity, women in the highest quintile of energy-adjusted vegetable protein intake reported exercise of 16.4 MET-hr/wk, compared to 9.9 MET-hr/wk in the lowest quintile. The average BMI of women in the highest quintile of vegetable protein intake was lower (26.5 kg/m^2) than for women in the lowest quintile (28.9 kg/m^2). When analysis was restricted to normal-weight women (BMI: 18.5–24.9 kg/m^2), there was no association between vegetable protein and gallbladder disease (data not shown). Increased physical activity and lower BMI have both been well associated with significantly lower risk of symptomatic gallstones (Banim et al., 2010; Erlinger, 2000; Everhart, 1993; Leitzmann et al., 1999; Misciagna et al., 1999).

Secondarily, upon assessing women with weight loss > 10% within the first 3 years after baseline, we noted 31% higher risk of gallbladder disease among women with > 10% body weight loss compared to women with ≤ 10% weight loss, reinforcing weight loss as a risk factor for gallbladder disease (Everhart, 1993; Liddle et al., 1989; Stampfer et al., 1992; Yang et al., 1992). Vegetable protein was not associated with gallbladder disease when restricted to women with weight loss > 10%, though the sample size in this subgroup was small. When stratified by history of diabetes, we observed lower gallbladder disease risk with increased vegetable protein intake only in those without a history of diabetes (P_{trend} < 0.001). Currently there is controversy as to whether diabetes mellitus affects gallbladder disease risk (Haffner et al., 1990; Jorgensen, 1989; Pacchioni et al., 2000).

Study limitations and strengths

Strengths of our study include our large sample with well-characterized demographic and lifestyle variables. The capacity to study variance in protein intake and the robust data for controlling for confounders in multivariate analysis in a sample of this size are rare. However, the results may not be generalizable to all postmenopausal women, as WHI participants tend to be healthier and receive more healthcare monitoring than the general population; these features could reduce disease rates or raise diagnosis rates, respectively. Though family history is a known risk factor for gallbladder disease, we did not collect

information regarding family history of gallbladder disease. The observed incidence of gallbladder disease events in our sample may be lower than the current rates given the continuing rise in obesity, a primary risk factor for gallbladder disease, over the past decade even among older women (Mokdad et al., 1999; Ogden et al., 2015). Also, there is known measurement error among FFQs in capturing energy intake. For those substituting vegetable protein for animal protein (e.g. vegetarians), it is possible that the FFQ may not accurately capture quantities of intake. Vegetable protein may be an indicator of healthier lifestyle for which statistical analysis cannot fully adjust. Lastly, participant self-reports of gallbladder disease were not physician adjudicated, though hospitalization records were collected. Thus, some error in outcome reporting may exist.

Conclusion

In summary, vegetable protein intake was associated with lower gallbladder disease risk among postmenopausal women, while there was no association between animal protein or total protein intake and gallbladder disease. The association between vegetable protein intake and gallbladder disease was attenuated when stratified by > 10% body weight change, a known risk factor for gallbladder disease. Vegetable protein contains less cholesterol and saturated fat than animal protein, two components that may increase the likelihood of gallstones. Lower risk of gallbladder disease may be related to healthier lifestyle choices (e.g. diet, exercise, sleep patterns) among women with higher vegetable protein intake. The well-characterized sample of postmenopausal women participating in the WHI program contributes to the small amount of conflicting literature on this subject, necessitating future analysis of large, diverse samples to strengthen existing findings. Vegetable and animal protein intake are modifiable risk factors for gallbladder disease, allowing for lifestyle intervention. Combined evidence of other epidemiological studies may inform the hypothesis on which a plant-based diet clinical intervention to prevent gallbladder disease may be tested. In addition to weight management, healthcare providers could recommend vegetable protein as an additional dietary modality to promote lower risk for gallbladder disease.

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Highlights

- We examined the association of protein intake with gallbladder disease risk.
- Analyses were based on postmenopausal women from the Women's Health Initiative.
- Vegetable and total protein intake were inversely associated with gallbladder disease risk.
- No association between animal protein and gallbladder disease risk was observed.

Table 1

Baseline characteristics across quintiles of energy-adjusted vegetable protein intake: mean ± SD or %[a]

Characteristic	Quintile of energy-adjusted vegetable protein intake				
	1 (< 16.3 g/d) *n* = 26,172	**2 (16.3–18.7 g/d) *n* = 26,172**	**3 (18.7–20.9 g/d) *n* = 26,172**	**4 (20.9–24.0 g/d) *n* = 26,172**	**5 (> 24.0 g/d) *n* = 26,171**
Age (y)	62.2 ± 7.2	63.1 ± 7.2	63.2 ± 7.2	63.4 ± 7.2	63.2 ± 7.3
BMI (kg/m^2)	28.9 ± 6.2	27.8 ± 5.7	27.4 ± 5.5	27.0 ± 5.4	26.5 ± 5.6
Physical activity (MET-hr/wk)	9.9 ± 12.4	11.4 ± 12.7	12.8 ± 13.6	13.9 ± 14.0	16.4 ± 15.6
NSES	74.7 ± 9.4	75.7 ± 8.8	76.2 ± 8.3	76.5 ± 8.1	76.4 ± 8.1
Race/ethnicity					
Non-Hispanic white	79.5	83.1	84.3	85.2	82.7
Black	13.9	10.2	8.38	6.59	6.40
Hispanic	3.85	3.30	3.23	3.28	3.56
Asian	1.01	1.60	2.35	3.17	5.54
Native American	0.44	0.39	0.38	0.34	0.34
Other/unknown	1.26	1.36	1.39	1.44	1.44
Income					
< $20k	18.3	15.8	14.6	13.9	14.6
$20k to < $35k	24.7	24.0	23.5	23.2	22.9
$35k to < $50k	20.4	20.8	21.1	20.5	20.2
$50k to < $75k	19.4	20.5	20.7	21.2	20.8
$75k to < $100k	8.81	8.89	9.59	9.98	10.3
≥ $100k	8.41	9.99	10.6	11.2	11.3
Education					
≤ High school	25.7	23.5	20.9	19.0	15.8
Some college	39.8	38.4	37.8	36.7	34.0
≥ College degree	34.6	38.0	41.3	44.4	50.2
Oral contraceptive use ever	44.1	41.8	41.9	41.3	41.3
Hormone therapy use					
Never	47.7	45.0	42.9	42.1	42.7
Former	15.8	15.4	15.8	15.8	15.3
Current	36.5	39.6	41.3	42.1	42.0

Characteristic	Quintile of energy-adjusted vegetable protein intake				
	1 (< 16.3 g/d) *n* = 26,172	**2 (16.3–18.7 g/d) *n* = 26,172**	**3 (18.7–20.9 g/d) *n* = 26,172**	**4 (20.9–24.0 g/d) *n* = 26,172**	**5 (> 24.0 g/d) *n* = 26,171**
History of diabetes	3.28	3.50	3.46	4.01	4.19
History of cancer	8.55	9.17	8.99	8.92	9.39
History of liver disease	2.11	2.14	2.04	2.14	2.25
Statin use (current)	5.75	7.20	7.65	8.17	7.70
Thiazide use (current)	5.73	5.56	5.65	5.10	4.42
Dietary intake					
Animal protein (g/d)[b]	52.8 ± 17.1	50.6 ± 12.3	48.8 ± 11.6	46.5 ± 11.8	40.2 ± 14.0
Total protein (g/d)[b]	66.6 ± 17.1	68.3 ± 12.3	68.6 ± 11.6	68.9 ± 11.7	68.8 ± 13.1
Total energy (kcal/d)	1860 ± 737	1490 ± 585	1478 ± 559	1554 ± 555	1802 ± 629
Sugar (g/d)[b]	98.9 ± 42.1	96.2 ± 27.6	97.3 ± 26.6	98.7 ± 27.0	99.9 ± 30.4
Total fat (g/d)[b]	69.5 ± 16.9	64.8 ± 12.0	61.2 ± 11.7	57.4 ± 12.2	50.2 ± 15.5
Saturated fat (g/d)[b]	24.5 ± 7.0	22.1 ± 4.7	20.5 ± 4.4	18.9 ± 4.5	15.5 ± 5.5
Fruit (serving/d)[b]	1.39 ± 1.1	1.70 ± 1.0	1.92 ± 1.1	2.13 ± 1.2	2.43 ± 1.4
Vegetable (serving/d)[b]	1.60 ± 0.9	1.89 ± 0.9	2.14 ± 1.0	2.46 ± 1.2	2.97 ± 1.5
Fiber (g/d)[b]	10.8 ± 3.2	13.9 ± 2.7	15.8 ± 3.0	18.0 ± 3.5	22.2 ± 5.3
Cholesterol (g/d)[b]	264.2 ± 122	236.4 ± 79.4	220.1 ± 71.7	202.9 ± 71.1	167.6 ± 81.5
Caffeine (g/d)[b]	164.7 ± 137	167.5 ± 126	169.5 ± 126	173.3 ± 130	166.0 ± 139
Calcium (g/d)[b]	783.6 ± 417	818.0 ± 312	831.1 ± 299	844.4 ± 298	851.5 ± 334
Alcohol (g/d)	8.8 ± 16.3	5.8 ± 10.1	4.9 ± 8.8	4.4 ± 8.1	3.8 ± 7.6
Alcohol use (drink/wk)					
< 1	55.1	57.7	60.1	62.2	66.3
1 to < 7	24.2	28.1	28.5	28.4	25.5
≥7	20.7	14.2	11.4	9.38	8.22

[a] NSES, Neighborhood socioeconomic status

[b] Energy-adjusted

Table 2

Association between sources of protein intake and gallbladder disease using Cox proportional hazards regression

Protein source	*n* events (%)	Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)
Vegetable protein[c]			
Quintile 1	2381 (9.1)	1.00	1.00
Quintile 2	2208 (8.4)	0.95 (0.90–1.01)	0.95 (0.89–1.02)
Quintile 3	2062 (7.9)	0.88 (0.83–0.94)	0.90 (0.84–0.96)
Quintile 4	2066 (7.9)	0.88 (0.83–0.93)	0.91 (0.85–0.97)
Quintile 5	1898 (7.3)	0.79 (0.74–0.84)	0.87 (0.81–0.93)
Test for trend		$P < 0.001$	$P < 0.001$
Continuous		0.86 (0.83–0.89)	0.91 (0.88–0.95)
Animal protein[c]			
Quintile 1	2047 (7.8)	1.00	1.00
Quintile 2	2081 (8.0)	1.05 (0.98–1.11)	1.00 (0.93–1.07)
Quintile 3	2096 (8.0)	1.06 (1.00–1.13)	1.00 (0.94–1.07)
Quintile 4	2117 (8.1)	1.06 (1.00–1.13)	0.96 (0.90–1.03)
Quintile 5	2274 (8.7)	1.12 (1.06–1.19)	1.00 (0.94–1.07)
Test for trend		$P < 0.001$	$P = 0.754$
Continuous		1.02 (1.01–1.04)	0.99 (0.98–1.01)
Total protein[c]			
Quintile 1	2142 (8.2)	1.00	1.00
Quintile 2	2125 (8.1)	1.03 (0.97–1.09)	1.00 (0.94–1.07)
Quintile 3	2100 (8.0)	1.01 (0.95–1.08)	0.96 (0.90–1.03)
Quintile 4	2061 (7.9)	0.99 (0.93–1.05)	0.92 (0.86–0.99)
Quintile 5	2187 (8.4)	1.03 (0.97–1.09)	0.95 (0.89–1.01)
Test for trend		$P = 0.718$	$P = 0.021$
Continuous		1.00 (0.99–1.01)	0.98 (0.97–1.00)

[a] Model 1 is adjusted for age and total energy (log-transformed)

[b] Model 2 is further adjusted for BMI, physical activity, neighborhood socioeconomic status, race/ethnicity, oral contraceptive use, hormone therapy use, history of liver disease, statin use, thiazide use, alcohol use, and clinical trial arm(s)

[c] Energy-adjusted

Table 3

Association between energy-adjusted vegetable protein intake and gallbladder disease, stratified by history of diabetes or recent weight loss

Subpopulation	*n* events (%)	Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)
History of diabetes = no			
Quintile 1	2298 (9.1)	1.00	1.00
Quintile 2	2085 (8.3)	0.93 (0.88–0.99)	0.94 (0.88–1.00)
Quintile 3	1974 (7.8)	0.88 (0.83–0.93)	0.90 (0.84–0.96)
Quintile 4	1948 (7.8)	0.86 (0.81–0.92)	0.90 (0.84–0.96)
Quintile 5	1793 (7.2)	0.78 (0.73–0.83)	0.86 (0.80–0.92)
Test for trend		$P < 0.001$	$P < 0.001$
Continuous		0.85 (0.82–0.88)	0.91 (0.87–0.95)
History of diabetes = yes			
Quintile 1	83 (9.7)	1.00	1.00
Quintile 2	122 (13.4)	1.49 (1.12–1.98)	1.39 (1.02–1.90)
Quintile 3	87 (9.6)	1.02 (0.75–1.39)	0.96 (0.68–1.35)
Quintile 4	117 (11.2)	1.20 (0.90–1.59)	1.08 (0.78–1.47)
Quintile 5	104 (9.5)	1.02 (0.76–1.36)	1.02 (0.74–1.40)
Test for trend		$P = 0.461$	$P = 0.535$
Continuous		0.96 (0.82–1.12)	0.97 (0.81–1.15)
Recent weight loss > 10%[c]			
Quintile 1	73 (6.5)	1.00	1.00
Quintile 2	82 (8.2)	1.28 (0.93–1.77)	1.34 (0.94–1.92)
Quintile 3	58 (6.8)	1.03 (0.73–1.46)	1.20 (0.82–1.76)
Quintile 4	69 (7.8)	1.20 (0.86–1.67)	1.23 (0.84–1.79)
Quintile 5	54 (6.6)	1.01 (0.71–1.44)	1.21 (0.82–1.79)
Test for trend		$P = 0.957$	$P = 0.449$
Continuous		1.05 (0.87–1.28)	1.15 (0.92–1.44)
Recent weight loss ≤ 10%/any gain[c]			
Quintile 1	1242 (6.4)	1.00	1.00
Quintile 2	1169 (5.8)	0.93 (0.86–1.01)	0.94 (0.86–1.03)
Quintile 3	1152 (5.6)	0.90 (0.83–0.98)	0.92 (0.84–1.01)
Quintile 4	1144 (5.5)	0.88 (0.81–0.96)	0.92 (0.84–1.01)
Quintile 5	1072 (5.1)	0.81 (0.75–0.88)	0.88 (0.80–0.97)
Test for trend		$P < 0.001$	$P = 0.009$
Continuous		0.86 (0.82–0.91)	0.91 (0.86–0.96)

[a] Model 1 is adjusted for age and total energy (log-transformed)

[b] Model 2 is further adjusted for BMI, physical activity, neighborhood socioeconomic status, race/ethnicity, oral contraceptive use, hormone therapy use, history of liver disease, statin use, thiazide use, alcohol use, and clinical trial arm(s)

[c] Weight loss categories are restricted to women within 25% of their baseline weight at year 3 (data outside that range are likely to be errors). Also, outcomes for this analysis are restricted to time points at year 3 or later.