

Original Research

Strawberry Intake, Lipids, C-Reactive Protein, and the Risk of Cardiovascular Disease in Women

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Objective: There is indirect evidence suggesting that strawberries, containing several key nutrients, may be associated with the risk of cardiovascular disease (CVD). In the Women's Health Study, we examined strawberry intake for both its prospective association with CVD risk in 38,176 women and its cross-sectional association with lipids and C-reactive protein (CRP) in a subset of 26,966 women.

Methods: Strawberry intake was assessed from a baseline semiquantitative food frequency questionnaire, along with other self-reported lifestyle, clinical and dietary factors. Participants returned baseline bloods which were assayed for lipids and CRP. We computed the relative risks (RRs) for total CVD (1,004 cases) (including confirmed myocardial infarction, stroke, revascularization, and cardiovascular death) occurring during 10.9 years of follow-up.

Results: At baseline, 25.6%, 41.9%, 24.8%, and 7.7% of women reported corresponding strawberry intake of none, 1–3 servings/month, 1 serving/week, and ≥ 2 servings/week. For total CVD, the multivariate RRs (95% confidence intervals) for increasing categories of strawberry intake were 1.00 (ref), 1.01 (0.85–1.19), 0.95 (0.77–1.17), and 1.27 (0.94–1.72) (P, trend = 0.06). We found a similar lack of an association for individual cardiovascular endpoints and comparing mean levels of lipids and CRP by category of strawberry intake. However, women consuming ≥ 2 servings/week versus none had a borderline significant, multivariate 14% lower likelihood of an elevated CRP of ≥ 3 mg/L.

Conclusions: Strawberry intake was unassociated with the risk of incident CVD, lipids, or CRP in middle-aged and older women, though higher strawberry intake may slightly reduce the likelihood of having elevated CRP levels. Additional epidemiologic data are needed to clarify any role of strawberries in CVD prevention.

INTRODUCTION

Higher levels of fruit and vegetable intake have consistently been associated with a reduced risk of cardiovascular disease (CVD) [1,2]. Strawberries are a dense source of several key

nutrients that may have a role in cardiovascular disease (CVD) prevention, including fiber, folate, potassium, vitamin C, and various flavonoids. However, it remains unclear whether consuming specific foods such as strawberries uniquely contributes to either improvements in coronary risk factors or the risk of

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Abbreviations: BMI = body mass index, CI = confidence interval, CVD = cardiovascular disease, HDL = high-density lipoprotein, LDL = low-density lipoprotein, MI = myocardial infarction, RR = relative risk, SFFQ = semiquantitative food frequency questionnaire, TC = total cholesterol, US = United States, WHS = Women's Health Study.

developing CVD. In one study, 240 g strawberry consumption in healthy older women markedly increased the antioxidant capacity of their serum up to 2 hours after consumption [3]. Using data from a large cohort of middle-aged and older women as part of the Women's Health Study, we therefore examined strawberry intake for its association with both the future risk of CVD and existing cross-sectional baseline measurements of lipids and C-reactive protein.

MATERIALS AND METHODS

Study Population

The Women's Health Study (WHS) [4,5] is a completed, randomized, double-blind, placebo-controlled 2×2 factorial trial of low-dose aspirin (100 mg every other day) [6] and vitamin E (600 IU every other day) [7] in the primary prevention of CVD and cancer. A total of 39,876 female United States (US) health professionals were enrolled and randomized into the study so long as they were aged ≥45 years in 1992, post-menopausal or not intending to become pregnant, and free from prior myocardial infarction (MI), stroke, transient ischemic attack, and cancer (except non-melanoma skin cancer).

A 131-item validated semiquantitative food frequency questionnaire (SFFQ) was completed at baseline by 39,310 women [8], of whom 829 women were excluded due to insufficient completion of food items on the SFFQ, or energy intake of either <2,510 or ≥14,644 kJ/day (<600 or ≥3,500 kcal/day). Among these 38,481 women with sufficient and reasonable dietary data, subjects were also excluded if they had self-reported pre-randomization angina, coronary artery bypass graft surgery, or percutaneous transluminal coronary artery angioplasty. Finally, participants must have reported strawberry intake from the SFFQ. These exclusions resulted in a study population of 38,176 women for the present analyses.

Assessment of Strawberry Intake and Other Foods or Nutrients

For each individual food and beverage included on the SFFQ, a common unit or portion size was specified, with participants selected from 9 responses from "Never or less than once per month" up to "6+ per day." Strawberry intake was represented as "strawberries, fresh, frozen, or canned (1/2 cup)." The measurement of various nutrients from the SFFQ was based upon food tables maintained by the Department of Nutrition at the Harvard School of Public Health, Boston, MA. Nutrient values were all energy-adjusted by using the residual method [9]. Nutrients considered for these analyses included fruit and vegetable intake (servings/day) and saturated fat intake (g/day), as well as some of the primary constituents of strawberries, including total fiber intake (g/day), folate intake (μg/day), dietary vitamin C intake (mg/day), potassium intake (mg/day), and total flavonoid intake (mg/day).

Other Covariates

On the WHS baseline questionnaire, women provided self-reported information on coronary risk factors such as age (in years), weight and height (converted to body mass index (BMI), in kg/m²), smoking status (categorized as never, former, current), alcohol use (categorized as rarely/never, 1 to 3 drinks/month, 1 to 6 drinks/week, and ≥1 drink/day), frequency of exercise (categorized as rarely/never, <1 times/week, 1 to 3 times/week, and ≥4 times/week), parental history of MI at <60 years (no, yes), history of hypertension (no, yes), history of hypercholesterolemia (no, yes), history of diabetes (no, yes), and post-menopausal hormone use (categorized as never, former, or current).

Baseline Bloods

Prior to randomization, baseline blood samples were collected from 28,345 participants and stored in liquid nitrogen until analysis. Of the samples received, 27,939 were evaluated and transferred to a core laboratory facility and assayed for lipids - including total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol - using a direct-measurement assay (Roche Diagnostics). We also calculated non-HDL cholesterol (TC minus HDL cholesterol) and the TC/HDL cholesterol ratio for each participant. In addition, C-reactive protein was assessed with a validated, high-sensitivity assay (Denka Seiken) [10]. Of the 38,445 women comprising the baseline population for analyses of strawberry intake and CVD, 26,965 provided baseline bloods and had lipids and C-reactive protein assayed.

Outcome Ascertainment

During a mean follow-up of 10.1 years (maximum follow-up, 10.8 years) in the WHS, participants completed annual questionnaires updating information on compliance, adverse effects to the study agents, health outcomes, and risk factors. In addition, midway through each year-long follow-up period, participants were sent a return postcard on which to report any significant problems affecting compliance or recently developed illnesses. An Endpoints Committee consisting of physicians requested medical records for all self-reported endpoints for confirmation. Our definition of total CVD included confirmed MI, stroke, coronary artery bypass graft surgery, percutaneous transluminal coronary angioplasty, and cardiovascular death. The diagnosis of MI was confirmed using World Health Organization criteria [11]. Revascularization procedures were confirmed by hospital records. A stroke was defined as a typical neurological deficit, sudden or rapid in onset, lasting >24 hours. CVD death was documented by convincing evidence of a cardiovascular mechanism from death certificates and medical records. All analyses are based on the time to the first confirmed CVD event.

Table 1. Baseline Characteristics of 38,176 Women according to Categories of Strawberry Intake

	None (n=9,780)	1–3 servings/month (n=16,010)	1 serving/week (n=9,451)	≥2 servings/week (n=2,935)	P value*
Age (years)	54.2 ± 7.0†	54.2 ± 6.9	54.8 ± 7.2	54.8 ± 7.3	<0.0001
Body mass index (kg/m ²)	26.0 ± 5.1	25.9 ± 5.0	26.0 ± 5.0	26.4 ± 5.2	0.0002
History of hypertension (%)	26.8	24.7	26.0	27.4	0.0003
History of hypercholesterolemia (%)	30.8	28.7	29.5	28.8	0.003
History of diabetes mellitus (%)	2.6	2.3	2.7	3.4	0.005
Parental history of MI <60 years (%)	14.9	14.7	14.1	16.0	0.09
Exercise (%)					<0.0001
Rarely/never	46.0	37.6	33.7	29.9	
<1 times/week	18.8	20.5	20.3	18.9	
1 to 3 times/week	26.5	31.5	34.2	36.7	
≥4 times/week	8.8	10.5	11.7	14.6	
Smoking status (%)					<0.0001
Never	45.7	52.0	53.3	55.7	
Former	36.1	36.0	35.7	36.1	
Current	18.2	12.0	11.0	8.2	
Alcohol consumption (%)					<0.0001
Rarely/never	47.4	43.2	44.2	45.9	
1 to 3 drinks/month	12.3	13.5	13.4	13.8	
1 to 6 drinks/week	28.3	32.9	33.2	32.7	
≥1 drink/day	12.0	10.4	9.3	7.6	
Post-menopausal hormone use (%)					<0.0001
Never	49.2	48.5	46.1	46.6	
Former	10.7	10.0	10.1	10.6	
Current	40.1	41.6	43.9	42.8	
Fruit/vegetable intake (servings/day)	5.7 ± 2.9	7.7 ± 2.8	9.8 ± 3.1	12.8 ± 4.2	<0.0001
Total energy intake (kcal/day)	1567 ± 514	1722 ± 515	1828 ± 528	1955 ± 569	<0.0001
Total fiber intake (g/day)‡	17.3 ± 6.0	18.7 ± 5.5	20.1 ± 5.7	22.7 ± 6.6	<0.0001
Folate intake (μg/day)‡	417 ± 247	422 ± 218	440 ± 213	467 ± 213	<0.0001
Saturated fat intake (g/day)‡	20.6 ± 5.3	19.8 ± 4.7	19.1 ± 4.5	17.9 ± 4.5	<0.0001
Total flavonoid intake (mg/day)‡	24.0 ± 20.8	23.9 ± 17.6	25.4 ± 17.6	27.8 ± 17.4	<0.0001
Potassium intake (mg/day)‡	3063 ± 625	3178 ± 554	3311 ± 557	3517 ± 610	<0.0001
Dietary Vitamin C intake (mg/day)‡	128 ± 65	143 ± 58	159 ± 59	193 ± 70	<0.0001

* We used a global ANOVA test for continuous variables and chi-square tests (3 degrees of freedom) for categorical variables.

† Mean ± standard deviation.

‡ Energy-adjusted.

Data Analyses

Based upon the distribution of strawberry intake in this population of middle-aged and older women, we categorized strawberry intake as rarely/never, 1–3 servings/month, 1 serving/week, and ≥2 servings/week. We first compared mean values (ANOVA) or proportions (chi-square tests) of behavioral, clinical, and dietary risk factors according to categories of strawberry intake. In addition, we compared age-adjusted mean levels of plasma lipids and C-reactive protein in each category of strawberry intake.

Cox proportional hazards modeled the relative risk (RR) and 95% confidence interval (CI) of CVD for increasing levels of strawberry intake, with the lowest category (rarely/never) serving as the reference group. The proportional hazards assumption was satisfied for analyses of strawberry intake ($p > 0.05$). Models first adjusted for age, total energy intake, and randomized treatment assignments; then further adjusting for BMI, smoking status, frequency of exercise, alcohol intake,

parental history of MI <60 years, and post-menopausal hormone use. The third model also adjusted for clinical risk factors including hypertension, hypercholesterolemia, and diabetes. The fourth and final model then added dietary components related to strawberry intake, including fiber, folate, and saturated fat intake, along with fruit and vegetable intake. Linear trend tests across categories of strawberry intake were assessed using the median for each category as an ordinal variable. We also considered alternative CVD endpoints, including MI, stroke, and CVD death. We also compared the overall RRs with those excluding women with baseline diabetes, hypertension, and high cholesterol out of concern that participants may have recently altered the composition of their diet.

A final set of cross-sectional analyses considered the association between increasing categories of strawberry intake and the likelihood of being classified at adverse National Cholesterol Education Program [12] clinical cutpoints for lipids (TC, ≥240 vs <240 mg/dL; LDL cholesterol, ≥160 vs <160 mg/

Table 2. Age-Adjusted Mean Levels of Baseline Lipids and C-Reactive Protein, and the Percentage of Women with Elevated Lipid [12] and C-Reactive Protein [13] Levels, among 26,966 Women according to Categories of Strawberry Intake

	None (n = 6,743)	1–3 servings/month (n = 11,498)	1 serving/week (n = 6,693)	≥2 servings/week (n = 2,032)	P value*
Total Cholesterol (mg/dL)	213.0 ± 41.2	211.6 ± 41.7	211.5 ± 42.3	211.1 ± 42.5	0.08
≥240 mg/dL (%)	22.5	21.7	22.2	22.1	0.63
LDL Cholesterol (mg/dL)	125.5 ± 34.4	123.7 ± 33.9	123.8 ± 34.4	123.5 ± 34.8	0.003
≥160 mg/dL (%)	14.6	13.3	14.2	13.9	0.06
HDL Cholesterol (mg/dL)	53.4 ± 14.9	54.2 ± 15.3	53.7 ± 14.9	53.1 ± 14.7	0.0004
<60 mg/dL (%)	71.0	69.2	70.5	71.6	0.024
Non-HDL Cholesterol (mg/dL)	159.6 ± 41.0	157.4 ± 41.1	157.8 ± 41.6	158.0 ± 41.4	0.005
≥190 mg/dL (%)	20.7	19.1	19.7	19.4	0.08
TC/HDL Ratio (units)	4.26 ± 1.34	4.17 ± 1.33	4.20 ± 1.32	4.23 ± 1.33	<0.0001
≥6 units (%)	10.9	9.2	9.6	10.3	0.002
C-reactive protein (mg/L)	3.63 ± 5.26	3.65 ± 5.77	3.56 ± 5.41	3.68 ± 5.74	0.70
≥3 mg/L (%)	37.2	36.6	37.6	36.7	0.57

* We used a global ANOVA test for continuous variables and chi-square tests (3 degrees of freedom) for categorical variables.

† Mean ± standard deviation.

dL; HDL cholesterol, <60 vs ≥60 mg/dL; non-HDL cholesterol, ≥190 vs <190 mg/dL; and TC/HDL cholesterol ratio, ≥6 vs <6 units) and the American Heart Association/Centers for Disease Control cutpoint [13] for C-reactive protein (≥3 vs <3 mg/L). Logistic regression models were used to generate RRs of having an adverse lipid level with a modeling strategy paralleling that for analyses of CVD.

RESULTS

Strawberry intake was modest in this population of 38,176 women with a mean age of 54.4 years in 1992, as 25.6% reported no strawberry intake at baseline, along with 41.9%, 24.8%, and 7.7% of women reporting intake of 1–3 servings/month, 1 serving/week, and ≥2 servings/week, respectively. Only 204 (0.6%) women reported strawberry intake at or exceeding 1 serving/day, of whom 9 women developed CVD during follow-up. As indicated in Table 1, strawberry intake was strongly and significantly associated with various baseline characteristics. Women consuming greater amounts of strawberries tended to be older, exercise more, smoke less, and currently use post-menopausal hormones (all $p < 0.0001$). Further, the average intake of key dietary factors reflecting a heart-healthy dietary pattern and constituents of strawberries were all strongly associated with baseline strawberry intake (all $p < 0.0001$). In contrast, women consuming greater amounts of strawberries tended to have higher BMIs ($p = 0.0002$) and were more likely to have hypertension ($p = 0.0003$) and diabetes ($p = 0.005$). Spearman correlations were strongly significant for strawberry intake with either fruit and vegetable intake ($r^2 = 0.57$, $p < 0.0001$), fruit intake ($r^2 = 0.74$, $p < 0.0001$) or total flavonoid intake ($r^2 = 0.12$, $p < 0.0001$). Individual flavone and flavanones had similar values of Spearman correlations as with strawberry intake and total flavonoid intake.

We then compared, in a subset of 26,966 women who provided baseline bloods, levels of various lipid parameters and C-reactive protein across categories of strawberry intake in Table 2. Total and LDL cholesterol levels were both modestly lower with higher levels of strawberry intake. Significant ($p < 0.05$) global differences for HDL cholesterol, non-HDL cholesterol, and the TC/HDL cholesterol ratio were not supported with possible dose-response effects across categories of strawberry intake. C-reactive protein was not associated with baseline strawberry intake ($p = 0.70$), a finding that extended to when we also considered a log transformation of C-reactive protein levels ($p = 0.54$). We also considered the proportion of women with adverse levels of each lipid parameter or C-reactive protein according to established clinical guidelines, but revealed no obvious associations with strawberry intake.

During a mean follow-up of 8.49 years (maximum follow-up, 10.9 years), a total of 1,004 women had CVD, including in part 289 cases of MI, 339 cases of stroke, and 204 cases of CVD death. As indicated in Table 3, for models adjusted for age and randomized WHS treatment assignment we found no association between higher levels of strawberry intake and the risk of total CVD (P , trend = 0.28) as well as more specific cardiovascular endpoints (all P , trend > 0.05). Additional adjustment for lifestyle factors strongly confounded the RRs, whereas adjustment for clinical factors did not. Controlling for a variety of dietary factors did not greatly impact the RRs except for the highest category (≥2 servings/week) of strawberry intake, for which there was a possible increased risk of MI and stroke. On the other hand, there was a suggestion of a lower risk of CVD death for strawberry intake at or above 1 serving/week that was limited by the relatively low case counts.

We assessed the consistency of our overall findings with various secondary analyses. Considering a combined endpoint of 703 cases of important vascular events, comprised of non-fatal MI, nonfatal stroke, and CVD death, resulted in RRs that were similar to those for total CVD. We examined whether

Table 3. Relative Risks (RRs) and 95% Confidence Intervals of Total Cardiovascular Disease (CVD) and Individual Cardiovascular Endpoints according to Categories of Strawberry Intake among 38,176 Women

	Category of Strawberry Intake				P, trend*
	None (n = 9,780)	1–3 servings/mth (n = 16,010)	1 serving/wk (n = 9,451)	≥2 servings/wk (n = 2,935)	
Total CVD	282†	394	236	92	
Age-, treatment-adjusted RR‡	1.00 (ref)	0.90 (0.77–1.05)	0.86 (0.72–1.02)	1.08 (0.85–1.38)	0.28
+ Lifestyle factors§	1.00 (ref)	0.99 (0.84–1.17)	0.93 (0.78–1.13)	1.24 (0.96–1.59)	0.08
+ Lifestyle, clinical factors	1.00 (ref)	1.00 (0.85–1.18)	0.94 (0.78–1.13)	1.23 (0.95–1.59)	0.09
+ Lifestyle, clinical, dietary factors¶	1.00 (ref)	1.01 (0.85–1.19)	0.95 (0.77–1.17)	1.27 (0.94–1.72)	0.06
Myocardial Infarction	83	107	70	29	
Age-, treatment-adjusted RR‡	1.00 (ref)	0.84 (0.63–1.12)	0.89 (0.65–1.23)	1.23 (0.80–1.90)	0.17
+ Lifestyle factors§	1.00 (ref)	0.98 (0.72–1.33)	1.12 (0.79–1.58)	1.55 (0.97–2.48)	0.041
+ Lifestyle, clinical factors	1.00 (ref)	0.99 (0.73–1.35)	1.12 (0.79–1.58)	1.59 (0.99–2.54)	0.035
+ Lifestyle, clinical, dietary factors¶	1.00 (ref)	1.03 (0.74–1.41)	1.21 (0.82–1.79)	1.84 (1.04–3.24)	0.025
Stroke	87	134	83	35	
Age-, treatment-adjusted RR‡	1.00 (ref)	0.99 (0.75–1.30)	1.00 (0.74–1.35)	1.39 (0.93–2.08)	0.07
+ Lifestyle factors§	1.00 (ref)	1.01 (0.76–1.34)	1.07 (0.78–1.46)	1.59 (1.05–2.40)	0.017
+ Lifestyle, clinical factors	1.00 (ref)	1.02 (0.77–1.36)	1.07 (0.78–1.46)	1.59 (1.06–2.41)	0.017
+ Lifestyle, clinical, dietary factors¶	1.00 (ref)	0.99 (0.74–1.33)	0.99 (0.70–1.41)	1.40 (0.86–2.28)	0.11
Cardiovascular Death	69	81	39	15	
Age-, treatment-adjusted RR‡	1.00 (ref)	0.74 (0.53–1.02)	0.55 (0.37–0.82)	0.68 (0.38–1.19)	0.27
+ Lifestyle factors§	1.00 (ref)	0.91 (0.65–1.28)	0.60 (0.39–0.92)	0.85 (0.47–1.55)	0.58
+ Lifestyle, clinical factors	1.00 (ref)	0.92 (0.66–1.29)	0.59 (0.39–0.92)	0.79 (0.43–1.46)	0.41
+ Lifestyle, clinical, dietary factors¶	1.00 (ref)	0.91 (0.64–1.29)	0.58 (0.36–0.93)	0.76 (0.38–1.55)	0.63

* Test for trend based on ordinal variable containing median value for each category.

† Number of cases.

‡ Adjusted for age, randomized aspirin treatment, randomized vitamin E treatment, randomized beta-carotene treatment, and total energy intake.

§ Adjusted for the covariates above plus lifestyle factors: body mass index, exercise, alcohol intake, smoking, post-menopausal hormone use, and parental history of myocardial infarction <60 years.

|| Adjusted for the covariates above plus clinical factors: hypertension, hypercholesterolemia, and diabetes.

¶ Adjusted for the covariates above plus dietary components related to strawberry intake: fruit and vegetables, fiber, folate, vitamin C, potassium, saturated fat, and total flavonoid intake.

18,633 women at baseline who were not obese (BMI <30 kg/m²) and free of diabetes, hypertension, and hypercholesterolemia may differ in their association between strawberry intake and the risk of CVD. There were only 252 cases of CVD in this healthy group of women during nearly 11 years of follow-up. In multivariate models adjusted for age, lifestyle, clinical, and dietary factors, the RRs (95% confidence intervals) of CVD were 1.00 (ref), 0.94 (0.68–1.30), 0.81 (0.53–1.24), and 1.49 (0.82–2.69) for corresponding strawberry intake of none, 1–3 servings/month, 1 serving/week, and ≥2 servings/week (P, trend = 0.06). The exclusion of the first 2 years of follow-up to minimize any impact of underlying diseases did not change the RRs of CVD. Finally, we examined whether the association between strawberry intake and risk of developing CVD differed according to those women who on average consumed <7 versus ≥7 servings/day of fruits and vegetables. We found neither any evidence for an interaction (P, interaction = 0.74) nor any distinct differences in the RRs of CVD for strawberry intake regardless of baseline fruit and vegetable intake.

In a subgroup of 26,966 women who provided baseline bloods, Table 4 presents the cross-sectional association between categories of strawberry intake and the likelihood of

having elevations in various plasma lipid parameters and C-reactive protein. Overall, 22.1% of women had a TC of ≥240 mg/dL, 13.9% had an LDL cholesterol ≥160 mg/dL, 29.9% had a beneficial HDL cholesterol of ≥60 mg/dL, 19.7% had a non-HDL cholesterol of ≥190 mg/dL, 9.8% had a TC/HDL cholesterol ratio of ≥6 units, and 37.0% had a C-reactive protein level of ≥3 mg/L. Regardless of how we adjusted for potential confounders, we found no overall association between levels of strawberry intake and the likelihood of having elevated levels of plasma lipids (all P, trend > 0.05). For C-reactive protein, women consuming ≥2 servings/week of strawberries had a borderline significant 14% lower likelihood of a C-reactive protein ≥3 mg/L that was not evident in models adjusted for age and randomized treatment (P, trend = 0.35) but emerged only after adjustment for lifestyle, clinical, and dietary factors (P, trend = 0.012).

DISCUSSION

In this large prospective study of middle-aged and older women, we found little evidence for an association between

Table 4. Relative Risks (RRs) and 95% Confidence Intervals of Having Elevations in Various Lipid Parameters and C-Reactive Protein according to Categories of Strawberry Intake in a Cross-Sectional Study among 26,965 Women

	Category of Strawberry Intake				P, trend*
	None (n = 6,743)	1–3 servings/month (n = 11,498)	1 serving/week (n = 6,693)	≥2 servings/week (n = 2,032)	
Total Cholesterol ≥240 mg/dL	22.5†	21.7	22.2	22.1	
Age-, treatment-adjusted RR‡	1.00 (ref)	0.95 (0.88–1.02)	0.95 (0.88–1.03)	0.95 (0.84–1.07)	0.58
Multivariate-adjusted RR§	1.00 (ref)	1.02 (0.94–1.11)	0.99 (0.90–1.10)	0.96 (0.82–1.13)	0.50
LDL Cholesterol ≥160 mg/dL	14.6	13.3	14.2	13.9	
Age-, treatment-adjusted RR‡	1.00 (ref)	0.89 (0.81–0.97)	0.93 (0.84–1.03)	0.91 (0.78–1.05)	0.53
Multivariate-adjusted RR§	1.00 (ref)	0.95 (0.86–1.04)	0.98 (0.87–1.10)	0.91 (0.75–1.09)	0.41
HDL Cholesterol ≥60 mg/dL	29.0	30.8	29.5	28.4	
Age-, treatment-adjusted RR‡	1.00 (ref)	1.11 (1.04–1.19)	1.06 (0.98–1.14)	1.02 (0.91–1.14)	0.61
Multivariate-adjusted RR§	1.00 (ref)	1.02 (0.94–1.11)	0.97 (0.88–1.06)	0.95 (0.82–1.10)	0.39
Non-HDL Cholesterol ≥190 mg/dL	20.7	19.1	19.7	19.4	
Age-, treatment-adjusted RR‡	1.00 (ref)	0.89 (0.83–0.96)	0.89 (0.82–0.98)	0.88 (0.77–1.00)	0.17
Multivariate-adjusted RR§	1.00 (ref)	0.99 (0.90–1.08)	0.96 (0.86–1.07)	0.89 (0.75–1.05)	0.18
TC/HDL Ratio ≥6 units	10.9	9.2	9.6	10.3	
Age-, treatment-adjusted RR‡	1.00 (ref)	0.80 (0.90–1.07)	0.96 (0.86–1.06)	0.89 (0.75–1.05)	0.48
Multivariate-adjusted RR§	1.00 (ref)	0.96 (0.85–1.07)	0.96 (0.83–1.11)	1.02 (0.83–1.27)	0.60
C-reactive protein ≥3 mg/L	37.2	36.6	37.6	36.7	
Age-, treatment-adjusted RR‡	1.00 (ref)	0.96 (0.90–1.02)	0.98 (0.91–1.05)	0.94 (0.84–1.04)	0.35
Multivariate-adjusted RR§	1.00 (ref)	1.03 (0.95–1.11)	0.99 (0.90–1.09)	0.86 (0.75–0.99)	0.012

* Test for trend based on ordinal variable containing median value for each quintile.

† Percent of women with elevations in each lipid parameter and C-reactive protein.

‡ Adjusted for age, randomized aspirin treatment, randomized vitamin E treatment, randomized beta-carotene treatment, and total energy intake.

§ Adjusted for the covariates above plus lifestyle, clinical and dietary factors: body mass index, exercise, alcohol intake, smoking, post-menopausal hormone use, parental history of myocardial infarction <60 years, hypertension, hypercholesterolemia, diabetes, and the intake of fruits and vegetables, fiber, folate, vitamin C, potassium, saturated fat, and total flavonoids.

relatively low levels of baseline strawberry intake and the subsequent risk of developing cardiovascular disease (CVD). Though overall strawberry consumption was low in this population of women in the 1990s, those consuming greater amounts of strawberries followed a healthier lifestyle in terms of behavioral and dietary risk factors, but also tended to have slightly higher rates of chronic conditions such as diabetes. Strawberry intake was not associated with either lipid levels or the likelihood of exceeding standard clinical thresholds. However, our study did preliminarily suggest that consuming even modest amounts of strawberries may reduce the likelihood of having an elevated level of C-reactive protein.

To our knowledge, this is the first large observational cohort study that has considered strawberry intake in the context of the primary prevention of CVD. Strawberries are a rich source of several key nutrients that have been implicated as having a possible protective role in the prevention of CVD, including fiber [14], folate [15, 16], vitamin C [17], potassium [18], and flavonoids such as quercetin and kaempferol [19]. Alternatively, other components or characteristics of strawberries that may lend itself to having cardioprotective properties. One study examined antioxidant activity in 8 healthy elderly women after they consumed 240 grams of strawberries with a standard morning meal [3]. Strawberry consumption markedly increased the antioxidant capacity of serum up to 2 hours after consumption. In another study examining eight strawberry cultivars for

their flavonoid content and their antioxidant capacities [20], however, there was no significant association with antioxidant activity. The potentially high antioxidant capacity attributed to strawberry intake may instead reflect a strong plasma response to its high vitamin C content. Strawberries also appear to be rich in anthocyanin [21], a less common flavonoid, which is highly bioavailable based upon a short-term feeding study of 6 subjects with 200 g strawberries [22] While various polyphenols in strawberries have antioxidant activity, cardiovascular benefits may alternatively occur through the interactions of these compounds with the signaling pathways of immune and inflammatory cells. More studies are necessary to better elucidate these potential mechanisms.

Women who consume greater amounts of strawberries may follow a more prudent heart-healthy dietary pattern. This was supported by the analyses in our study, as the association between strawberry intake and CVD was most strongly confounded by lifestyle and dietary factors. While our observation that those women consuming ≥2 serving/week of strawberries may be less likely to have elevated levels of C-reactive protein is preliminary, as there are no published clinical studies that have carefully investigated the short- and long-term biologic effects on strawberry intake. This underscores the need to better understand the potential mechanisms through which strawberry intake may impact important biomarkers and clinical measurements of CVD risk.

Low levels of strawberry intake in this initially healthy cohort of US female health professionals in 1992 may explain our lack of association between strawberries and either incident CVD or measured lipids and C-reactive protein. Further, it remains unclear what constitutes a high level of strawberry intake. In the WHS, too few women consumed ≥ 2 servings/week of strawberries for us to consider higher intake levels for the risk of CVD. Our relatively narrow comparison of high versus low strawberry intake may have therefore precluded us from detecting any association with CVD. However, those women consuming the most strawberries in the present study still exhibited a more prudent dietary pattern, suggesting that despite a narrow range of intake the difference remains relevant in the US population. Because previous clinical studies of strawberries have considered considerably higher levels of intake, it remains unclear what the nature of the association is between strawberry intake and either the risk of CVD or levels of relevant biomarkers.

Additional information is needed on temporal trends in strawberry intake as well as other fruits and vegetables, including other commonly consumed berries also rich in antioxidants that may alternatively be associated with the risk of CVD. Apple intake was previously examined in the WHS, in which an unadjusted significant linear trend across categories of intake was attenuated upon multivariate adjustment (p , trend = 0.74) [19]. We also considered blueberry intake in additional analyses, for which only 10.4% of women consumed ≥ 1 serving/week (1 serving = 1/2 cup blueberries). Higher levels of blueberry intake were not associated with either the risk of developing CVD (p , trend = 0.35) or the likelihood of having a C-reactive protein ≥ 3 mg/L (p , trend = 0.99).

Some important methodological considerations should be considered. First, inherent misclassification for a single baseline measurement of dietary factors may have biased our results, as multiple dietary assessments tend to generate a stronger magnitude of effect [23]. Follow-up information is not yet available for strawberry intake after baseline. Second, strawberry consumption may also be susceptible to residual confounding by other uncontrolled lifestyle or dietary factors that are associated with CVD. Women consuming more strawberries were older, heavier, and more likely to be hypertensive and diabetic, suggesting that greater strawberry consumption may reflect dietary responses to early clinical manifestations of CVD rather than long-term dietary patterns amenable to primary prevention. However, we comprehensively controlled for potential confounding by known risk behavioral, clinical, and dietary factors, so we would expect the impact of residual confounding to be relatively minor. Third, we did not measure of any reliable markers of oxidative status such as F_2 -isoprostane in this study, which would have allowed us to examine whether the observation that high strawberry intake may positively impact C-reactive protein levels was due to antioxidant activity. Finally, generalizability may be of concern as this

study was limited to predominantly White female health professionals. However, we see no *a priori* reason why any biologic mechanisms underlying a potential association would differ versus other population groups.

CONCLUSIONS

We found no overall association between strawberry intake and the risk of CVD in middle-aged and older women. However, strawberries remain an important part of the broader spectrum of fruits and vegetables that have been associated with a lower risk of CVD [1,2]. Though higher levels of strawberry intake were not cross-sectionally associated with lipids and C-reactive protein in this study, women consuming ≥ 2 servings/day of strawberry may be less likely to have elevated C-reactive protein levels. However, this finding remains speculative. Additional research is essential to better elucidate the potential biologic mechanisms through which strawberries may influence biomarkers and clinical measurements related to CVD in a variety of study settings.

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