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Consumption of artificial sweetener– and sugar-containing soda and risk of lymphoma and leukemia in men and women[1–4]

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ABSTRACT

Background: Despite safety reports of the artificial sweetener aspartame, health-related concerns remain.

Objective: We prospectively evaluated whether the consumption of aspartame- and sugar-containing soda is associated with risk of hematopoetic cancers.

Design: We repeatedly assessed diet in the Nurses' Health Study (NHS) and Health Professionals Follow-Up Study (HPFS). Over 22 y, we identified 1324 non-Hodgkin lymphomas (NHLs), 285 multiple myelomas, and 339 leukemias. We calculated incidence RRs and 95% CIs by using Cox proportional hazards models.

Results: When the 2 cohorts were combined, there was no significant association between soda intake and risks of NHL and multiple myeloma. However, in men, ≥1 daily serving of diet soda increased risks of NHL (RR: 1.31; 95% CI: 1.01, 1.72) and multiple myeloma (RR: 2.02; 95% CI: 1.20, 3.40) in comparison with in men without diet soda consumption. We observed no increased risks of NHL and multiple myeloma in women. We also observed an unexpected elevated risk of NHL (RR: 1.66; 95% CI: 1.10, 2.51) with a higher consumption of regular, sugar-sweetened soda in men but not in women. In contrast, when sexes were analyzed separately with limited power, neither regular nor diet soda increased risk of leukemia but were associated with increased leukemia risk when data for men and women were combined (RR for consumption of ≥1 serving diet soda/d when the 2 cohorts were pooled: 1.42; 95% CI: 1.00, 2.02).

Conclusion: Although our findings preserve the possibility of a detrimental effect of a constituent of diet soda, such as aspartame, on select cancers, the inconsistent sex effects and occurrence of an apparent cancer risk in individuals who consume regular soda do not permit the ruling out of chance as an explanation. *Am J Clin Nutr* doi: 10.3945/ajcn.111.030833.

INTRODUCTION

Aspartame (L-α-aspartyl-L-phenylalanine methyl ester) is an artificial sweetener used in many low-calorie, low-carbohydrate, sugar-free products. Aspartame was first approved for restricted use in dry foods in 1981 (1), first used in carbonated beverages in 1983, and approved for general purposes in 1996. Today, aspartame is used as a sweetener and flavor enhancer in >6000 foods worldwide. The annual amount of aspartame currently used in diet soda in the United States is 4500 tons (G Crosby; NutraSweet Co; personal communication, 14 July 2006); the average content of aspartame in a 1-L bottle of diet cola is ~560 mg, whereas (diet) orange soda contains as much as 930 mg/L (2–4). Because the annual aspartame used across all applications in the US was estimated at 5000–5500 tons (C Heinzinger; NutraSweet Co; personal communication, 18 July 2006), diet soda accounts for the large majority (~86%) of all aspartame in foods.

Despite many previous experimental studies that evaluated and confirmed the safety of aspartame, which have made aspartame one of the most extensively tested food ingredients in the history of food additives, health-related concerns continue to be debated. Most notably, the relevance of animal studies, which, in general, have shown no harm, with regard to human safety has been questioned (5, 6). However, previous evidence (7) and a reinterpretation of long-term carcinogenicity studies in rats (1) have suggested that aspartame may be carcinogenic (specifically, that it may cause brain tumors). Moreover, aspartame, especially in liquids (8), quickly breaks down into its 3 main ingredients (methanol, aspartic acid, and phenylalanine) if stored near or above room temperature (3), and the formaldehyde metabolized from methanol is a documented human carcinogen (9). A recent megaexperiment in 1800 rats tested at aspartame doses much

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[5] Abbreviations used: ADH, alcohol dehydrogenase type I; ADI, acceptable daily intake; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; EFSA, European Food and Safety Agency; FDA, Food and Drug Administration; FFQ, food-frequency questionnaire; HPFS, Health Professionals Follow-Up Study; NHL, non-Hodgkin lymphoma; NHS, Nurses' Health Study.

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lower than the currently acceptable daily intake (ADI)[5] for humans (10) reported a dose-dependent increase in lymphomas, leukemias, and transitional renal cell tumors. This report provoked a review by several European agencies, including the European Food Safety Authority Panel on Food Additives, Flavors, Processing Aids and Materials and the European Food and Safety Agency (EFSA), which concluded that there is "no reason to revise the previously established ADI for aspartame of 40 mg/kg body weight" (11). In the United States, the ADI for aspartame is set at 50 mg/kg body weight (6).

Human data on aspartame intake and cancer risk are scarce and largely have not been supportive of an association between aspartame intake and cancer risk (12–14). However, studies have been limited by their exposure assessment, which assessed aspartame intake only at one point in time. Therefore, we conducted a prospective analysis of diet soda and aspartame consumption in relation to the cancers with elevated risks in the Italian mega-experiment (10) (eg, lymphoma and leukemia) by using data from the Nurses' Health Study (NHS) and Health Professionals Follow-Up Study (HPFS) cohorts that included updated assessments of diet and beverage consumption every 4 y. Transitional renal cell cancers were too few (n = 33 in the HPFS and n = 34 in the NHS) to analyze separately. Because we have been assessing diet soda and intakes of foods high in aspartame since aspartame was first allowed into the food supply, our analyses largely capture lifetime aspartame exposure in 2 large populations of middle-aged and older adults. To clarify whether any associations are likely to be attributed to aspartame, we also examined regular soda and its association with these outcomes.

SUBJECTS AND METHODS

The NHS began in 1976 when 121,701 female registered nurses, 30–55 y of age, responded to a mailed questionnaire. The HPFS was established in 1986 with 51,529 male health professionals (dentists, veterinarians, pharmacists, optometrists, podiatrists, and osteopaths) who were 40–75 y of age. On the initial questionnaire in both cohorts, participants provided a medical history and information on lifestyle and risk factors related to cancer and other health outcomes. Follow-up questionnaires have been mailed every 2 y to update individual characteristics and to identify incident diagnoses. Dietary intake, including detailed soda consumption, was assessed as part of the 1984 questionnaire in the NHS women and again in 1986 in both cohorts. Diet was subsequently reassessed every 4 y.

Participants were excluded from the study populations if they did not respond to the baseline dietary questionnaire or had reported any previous diagnosis of cancer. A total of 77,218 women and 47,810 men contributed to these analyses. The NHS was approved by the Institutional Review Board of the Brigham and Women's Hospital, and the HPFS received Institutional Review Board approval from the Harvard School of Public Health.

Lymphoma and leukemia cases

On each biennial questionnaire, participants were asked to report all incident cancer diagnoses. We also identified incident cancers from state tumor registries and deaths that were ascertained from family members, the postal service, and the National Death Index (15). To confirm diagnoses, for each cancer report we sought permission to obtain medical records. For reported lymphomas, we determined the histologic subtype on the basis of the current WHO classification system (16) by using morphology and immunophenotype information in medical records and pathology reports. The immunophenotype was not required for diagnoses of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) or follicular lymphoma, which can be reliably diagnosed by morphology alone. For early diagnoses before immunophenotyping was routinely performed, we used the proposed translation of Morton et al (17) from previous classification systems to the current WHO standard. Over the follow-up period (1984–2006 in the NHS; 1986–2006 in the HPFS), we confirmed 571 non-Hodgkin lymphomas (NHLs) in HPFS men and 753 NHLs in NHS women, of which 399 and 581 individuals, respectively, could be classified by histologic subtype from medical records. As expected, the large majority of these were B cell–origin lymphomas (374 in men; 553 in women). Of these lymphomas, the primary histologic subtypes were CLL/SLL (184 in men; 210 in women), follicular lymphoma (54 in men; 132 in women), and diffuse large B cell lymphoma (55 in men; 111 in women). Only 27 cases of Hodgkin lymphoma were confirmed in men, and only 28 cases of Hodgkin lymphoma were confirmed in women, which precluded any meaningful analysis of this outcome. We also identified 131 and 154 multiple myelomas in men and women, respectively, of which 97% were confirmed by using medical records. Of the 186 and 153 leukemias in men and women, respectively, 228 myeloid and only 8 monocytic types were identified.

Diet soda and diet assessment

Diet was assessed by using a semiquantitative food-frequency questionnaire (FFQ) on which participants reported their frequency of consumption over the previous year for specified amounts of ~130 foods. The 9 frequency categories ranged from never to ≥6 times/d. The frequency of diet soda consumption was assessed per 12-fl oz (355 mL; equivalent to one bottle, glass, or can) serving for the following 3 items: diet cola with caffeine, diet cola without caffeine, and other diet soda. These 3 types were summed for the analysis of total diet soda consumption. The consumption of regular sugar-sweetened soda was similarly assessed. For analysis, we condensed the 9 reported frequencies from the FFQ into 5 categories that ranged from 0 to ≥1 serving/d to accommodate the distribution of soda consumption in these cohorts, although we were also able to examine ≥2 servings diet soda/d in analyses of NHLs.

The use of aspartame sweeteners added at the table [ie, NutraSweet and Equal (manufactured by The NutraSweet Company, formerly Searle and Co)] was initially included on the FFQ in 1994 and was assessed as individual serving packets. Total aspartame intake was calculated as the sum from diet soda and packets (20 mg). The aspartame content of each soda item on the FFQ was assigned as a weighted average of the representative sodas in that category (70–180 mg/serving). Participants also reported their consumption of breakfast cereal by brand name, although no breakfast cereals contained aspartame in the early years, and only 4% of the brands contained aspartame at the end

of follow-up; therefore, the consumption of breakfast cereal was not included in the total intake. Other possible sources of aspartame (eg, artificially sweetened yogurt or ice cream) were not assessed, although their contributions were likely small compared with that from soda. For analysis, we created 5 aspartame categories with zero intakes as the lowest category and cohort-specific exact quartiles for the remaining categories.

Nutrient intakes that were correlated with the total energy intake were adjusted for total energy by using regression analysis (18). To generate estimates of the long-term diet, diet soda consumption and other food and nutrient intakes were cumulatively averaged in the statistical analyses (ie, after every dietary assessment, intakes were updated with the mean of all reported intakes up to that time). In validation studies, the FFQ has been shown to be a suitable instrument for the discrimination between dietary intakes (19, 20). In a comparison of the FFQ with two 1-wk diet records collected from 127 HPFS participants, the correlation was 0.73 for diet sodas (20).

Nondietary measures

All nondietary covariate measures, including weight, smoking status, and cigarettes smoked per day, discretionary physical activity, and multivitamin use were assessed on most biennial questionnaires and updated in statistical analyses. BMI (in kg/m^2) was calculated from the current weight and the height reported on the initial cohort questionnaire. For physical activity, we calculated total metabolic equivalent task–hours per week as a measure of energy expenditure from reported hours of participation and the assigned metabolic equivalent score for each activity listed on the questionnaire (21). Activity data were cumulatively averaged in statistical analyses. For the NHS cohort, questions on menopausal status and the use of hormone replacement therapy were also part of every biennial assessment.

Statistical analysis

Men and women were analyzed separately to examine possible sex differences. Participants contributed person-time to the analyses from the return date of their baseline questionnaire (1984 for the NHS; 1986 for the HPFS) and were censored at the first report of cancer, death, or end of follow-up (1 January 2006 for the HPFS and 1 June 2006 for the NHS).

We used Cox proportional hazards models to compute incidence RRs by comparing risk of the outcome in each upper exposure category with that in the lowest reference category. To control as finely as possible for confounding by age, calendar time, and any possible 2-way interactions between these 2 time scales, we stratified analyses jointly by age in months at the start of follow-up and calendar year of the current questionnaire cycle. We calculated multivariable RRs by adjusting models simultaneously for dietary and nondietary covariates. Analyses of diet soda and regular soda were also mutually adjusted for each of these exposures. Adjustment for diabetes and waist-to-hip ratio did not alter our results, and thus, these variables were not retained in the final models. To assess a dose-response effect, a P value for linear trend was determined by entering the medians within exposure categories into the model as a single continuous value. We also conducted stratified analyses to determine whether the influence of aspartame intake was modified by alcohol intake or BMI and tested for significant interaction by comparing the difference in -2 log likelihood from models with and without interaction terms to a chi-square distribution. We tested for heterogeneity between main results for men and women by using the random-effects method of DerSimonian and Laird (22) and pooled results when appropriate.

RESULTS

A total of 47,810 men contributed 784,461 person-years to this analysis, and 77,218 women contributed 1,493,935 person-years. Both men and women in the highest category of ≥1 serving diet soda/d were younger, on average, than subjects with less frequent consumption (**Table 1**). After adjustment for age, subjects with a higher intake of diet soda had higher BMI ($r = 0.23$ in men; $r = 0.21$ in women) and animal protein intake and were less likely to smoke. The correlation between regular sugar-sweetened and diet soda consumption was inverse in subjects with any soda consumption ($r = -0.52$ in men; $r = -0.56$ in women). At the baseline dietary assessment, 55% of men and 62% of women reported diet soda consumption with mean intakes of 6.0 and 6.6 servings/wk, respectively. The consumption of diet soda declined slightly over time, particularly in women. At the final dietary assessment in 2002, 53% of men and 54% of women reported diet soda consumption with mean intakes of 5.7 and 5.3 servings/wk, respectively. The mean daily aspartame intake in consumers at the final dietary assessment was 114 mg in the HPFS and 102 mg in the NHS.

Age-adjusted and multivariable models were similar for the associations between diet soda and NHL, multiple myeloma, and leukemia; hence, only the multivariable results are discussed. In men, risk of NHL was significantly elevated for subjects who consumed ≥1 serving diet soda/d (RR: 1.31; 95% CI: 1.01, 1.72) compared with in subjects who reported no consumption (**Table 2**). Risk was even greater for the consumption of ≥2 servings diet soda/d, and the association showed a linear trend (RR: 1.69; 95% CI: 1.17, 2.45; P-trend = 0.02; data not shown in Table 2). In an examination of NHL subtypes, the intake of ≥1 serving diet soda/d compared with all lower intakes was associated with elevated risks of confirmed B cell origin NHL (RR: 1.34; 95% CI: 1.01, 1.78) and CLL/SLL (RR: 1.36; 95% CI: 0.91, 2.04; NS). There were too few outcomes for a meaningful examination of other subtypes. In contrast to men, there was no evidence of an association between diet soda consumption and risk of all NHL in women or for any subtype examined even at ≥2 intakes/d (RR: 1.12; 95% CI: 0.81, 1.56; P-trend = 0.65, P-heterogeneity = 0.24).

For multiple myeloma, risk increased linearly with increased consumption of diet soda in men (P-trend = 0.009) and was significantly elevated for subjects who consumed ≥1 serving/d (RR: 2.02; 95% CI: 1.20, 3.40). Diet soda was not associated with risk of multiple myeloma in women, and a significant heterogeneity was observed between cohorts for the linear trend (P-heterogeneity = 0.04) and for risk in the high category of ≥1 serving/d (P-heterogeneity = 0.01).

For leukemia, risk was elevated in the higher intake categories of diet soda in both men and women, although these sex-specific results were not significant. The statistical power was improved when the 2 cohorts were pooled, which yielded a linear trend (P-trend = 0.05) and increased risk of leukemia for subjects who

TABLE 1
Age and age-standardized characteristics of study populations within categories for frequency of diet soda consumption at baseline in 1986 in men in the HPFS and in 1984 in women in the NHS[1]

	Diet soda[2]					
	Men			Women		
	None	1–3.9 servings/wk	≥1 serving/d	None	1–3.9 servings/wk	≥1 serving/d
Median diet soda intake (/wk)	0	2.9	11.0	0	3.0	11.0
Participants (*n*)	21,328	8023	8259	29,206	13,091	17,427
Age (y)	55.3 ± 10.0[3]	54.3 ± 9.5	51.5 ± 8.9	51.5 ± 7.3	50.8 ± 7.1	49.5 ± 7.0
Regular sugar-sweetened soda (/wk)[2]	2.6 ± 4.5	1.4 ± 2.6	1.1 ± 2.8	2.4 ± 4.6	0.9 ± 1.9	0.7 ± 2.3
Aspartame (mg/d)[4]	3.6 ± 13.7	55.9 ± 30.5	268 ± 186	2.9 ± 12.0	54.7 ± .8	248 ± 177
Fruit and vegetables (/d)	5.3 ± 2.8	5.5 ± 2.7	5.7 ± 2.9	5.0 ± 2.5	5.3 ± 2.4	5.5 ± 2.7
Saturated fat (g/d)[5]	24.5 ± 6.4	24.0 ± 5.8	25.0 ± 6.4	21.9 ± 4.8	22.0 ± 4.3	22.6 ± 4.1
Animal protein (g/d)[5]	65.4 ± 17.4	69.2 ± 17.1	71.1 ± 18.9	49.3 ± 13.1	53.6 ± 13.5	55.8 ± 14.7
Alcohol (g/d)	11.6 ± 16.0	11.3 ± 14.7	10.9 ± 15.4	6.8 ± 11.5	6.7 ± 10.6	7.3 ± 11.7
Energy (kcal/d)	2039 ± 630	1930 ± 600	1980 ± 630	1784 ± 537	1714 ± 519	1738 ± 536
Activity (MET-h/wk)[6]	19.0 ± 23.1	21.2 ± 23.9	21.5 ± 25.1	12.7 ± 16.5	14.3 ± 17.3	14.3 ± 17.8
BMI (kg/m^2)	24.9 ± 2.9	25.7 ± 3.0	26.6 ± 3.3	23.9 ± 4.3	25.5 ± 4.7	26.5 ± 5.1
Height (cm)	178 ± 6.7	178 ± 6.7	178 ± 6.7	164 ± 6.1	164 ± 6.1	164 ± 6.1
Current smoker (%)	12	8	8	30	19	22
Multivitamin user (%)	40	43	43	35	38	38
Postmenopausal (%)	NA	NA	NA	49	49	49
HRT user (%)[7]	NA	NA	NA	23	24	22

[1] HPFS, Health Professionals Follow-Up Study; HRT, hormone replacement therapy; MET-h, metabolic equivalent task hours; NA, not applicable; NHS, Nurses' Health Study.
[2] Frequency of diet soda and regular sugar-sweetened soda consumption on the basis of a 12–fl oz (355 mL) serving that was equivalent to one glass, bottle, or can.
[3] Mean ± SD (all such values).
[4] Aspartame was assessed in 1994 in both cohorts rather than at baseline.
[5] Nutrient intake adjusted for total energy intake.
[6] Metabolic equivalent energy expenditure from discretionary physical activity.
[7] Use of HRT in postmenopausal women.

consumed ≥1 serving diet soda/d (RR: 1.42; 95% CI: 1.00, 2.02). Restriction to myeloid leukemia (which represented the majority of all leukemias in our data set) produced similar results (pooled RR for ≥1 serving/d: 1.31; 95% CI: 0.85, 2.03; *P*-trend = 0.06).

Although incidence rates remained stable across the follow-up, in analyses stratified by follow-up time (1986–1996 for men; 1984–1996 for women, and 1996–2006 for both men and women), overall, risks associated with soda consumption appeared to be stronger in the second half of follow-up for NHL and leukemia, and they were similar regardless of the follow-up period for multiple myeloma (data not shown).

Although aspartame was approved for use in the United States in 1981 and was used as the sole artificial sugar sweetener in Diet Coke soda (The Coca-Cola Company), which was the most commonly used diet soda at the time, beginning in 1983, most other diet sodas in the 1980s used both aspartame and saccharin for sweetness. Aspartame became most broadly used in sodas in 1992 when its patent expired and the price dropped significantly. Therefore, we conducted a secondary analysis of aspartame intake beginning with the 1994 FFQ, which also included our initial assessment of aspartame use from packets used at the table. Despite a reduced statistical power, we observed increased risks in men for all 3 outcomes with higher intakes of aspartame (**Table 3**) that were similar to risks we observed with diet soda. In men in the highest quintile of aspartame intake, RRs were 1.64 (95% CI: 1.17, 2.29; *P*-trend = 0.002) for NHL, 3.36 (95% CI: 1.38, 8.19; *P*-trend = 0.05) for multiple myeloma, and 1.56 (95% CI: 0.79, 3.06; *P*-trend = 0.17) for leukemia. No associations were observed for aspartame in women. There was significant heterogeneity between men and women for NHL and multiple myeloma in the linear trend (*P*-heterogeneity = 0.006 and 0.049, respectively) and in the highest quintile of aspartame intake (*P*-heterogeneity = 0.008 and 0.002, respectively).

We hypothesized that the sex differences we observed may have been due to the recognized higher enzymatic activity of alcohol dehydrogenase type I (ADH) in men, which possibly induced higher conversion rates from methanol to the carcinogenic substrate formaldehyde. Because the concurrent ingestion of ethanol inhibits methanol metabolism (23), we conducted analyses stratified by alcohol intake. We assumed that men with lower regular alcohol consumption would have more unbound ADH activity (24) and, thus, higher formaldehyde conversion rates if they consumed large amounts of diet soda and, consequently, higher cancer risk. For NHL, ≥2 servings diet soda/d was associated with increased risk (RR: 2.34; 95% CI: 1.46, 3.76; *P*-trend = 0.004) in men who consumed <6 g alcohol/d (median intake) but not in men with a higher alcohol consumption (RR: 0.96; 95% CI: 0.48, 1.90; *P*-trend = 0.99; *see* Table 1 under "Supplemental data" in the online issue). The interaction between diet soda and alcohol was significant (*P*-interaction = 0.03). Risks of multiple myeloma and leukemia associated with ≥1 serving diet soda/d were also higher in men with a lower alcohol intake. For women, risks associated with

TABLE 2
RRs of non-Hodgkin lymphoma, multiple myeloma, and leukemia by frequency of diet soda consumption in men in the HPFS (1986–2006) and in women in the NHS (1984–2006)[1]

	Diet soda[2]						
	None	<1 serving/wk	1–3.9 servings/wk	4–6.9 servings/wk	≥1 serving/d	*P*-trend[3]	*P*-heterogeneity[4]
Person-years (thousands)							
Men	262.2	137.4	161.5	83.2	140.1	—	—
Women	369.9	177.7	345.4	198.0	303.0	—	—
Non-Hodgkin lymphoma							
Men							
Cases (*n*)	172	122	124	53	100	—	—
Simple model	1.00 (—)[5]	1.14 (0.90, 1.45)	1.09 (0.86, 1.38)	0.99 (0.72, 1.35)	1.30 (1.01, 1.68)	0.11	—
Multivariable	1.00 (—)	1.12 (0.88, 1.43)	1.06 (0.83, 1.34)	0.96 (0.69, 1.32)	1.31 (1.01, 1.72)	0.11	—
Women							
Cases (*n*)	189	167	173	87	137	—	—
Simple model	1.00 (—)	1.00 (0.81, 1.23)	0.90 (0.73, 1.11)	0.83 (0.64, 1.08)	0.98 (0.78, 1.22)	0.73	—
Multivariable	1.00 (—)	0.98 (0.79, 1.22)	0.90 (0.72, 1.11)	0.85 (0.65, 1.10)	1.00 (0.78, 1.26)	0.999	—
Pooled							
Multivariable	1.00 (—)	1.04 (0.89, 1.22)	0.96 (0.82, 1.13)	0.89 (0.72, 1.09)	1.13 (0.94, 1.34)	0.28	0.24
Multiple myeloma							
Men							
Cases (*n*)	40	27	23	12	29	—	—
Simple model	1.00 (—)	1.15 (0.70, 1.90)	0.99 (0.59, 1.67)	1.04 (0.54, 2.00)	1.86 (1.14, 3.05)	0.02	—
Multivariable	1.00 (—)	1.17 (0.70, 1.96)	1.04 (0.61, 1.78)	1.08 (0.55, 2.12)	2.02 (1.20, 3.40)	0.01	—
Women							
Cases (*n*)	39	28	40	23	24	—	—
Simple model	1.00 (—)	0.77 (0.47, 1.26)	0.95 (0.61, 1.49)	1.04 (0.62, 1.75)	0.86 (0.51, 1.44)	0.94	—
Multivariable	1.00 (—)	0.71 (0.43, 1.17)	0.86 (0.54, 1.37)	0.95 (0.55, 1.63)	0.79 (0.45, 1.36)	0.79	—
Pooled							
Multivariable	1.00 (—)	0.91 (0.63, 1.30)	0.94 (0.66, 1.33)	1.00 (0.65, 1.52)	1.29 (0.89, 1.89)[6]	0.10	0.04
Leukemia							
Men							
Cases	52	33	49	19	33	—	—
Simple model	1.00 (—)	1.08 (0.69, 1.68)	1.50 (1.01, 2.23)	1.23 (0.72, 2.11)	1.49 (0.95, 2.34)	0.10	—
Multivariable	1.00 (—)	1.07 (0.68, 1.68)	1.51 (1.00, 2.28)	1.29 (0.75, 2.24)	1.47 (0.92, 2.35)	0.13	—
Women							
Cases	33	31	37	21	31	—	—
Simple model	1.00 (—)	1.01 (0.62, 1.66)	1.06 (0.66, 1.70)	1.17 (0.67, 2.03)	1.35 (0.82, 2.22)	0.17	—
Multivariable	1.00 (—)	1.04 (0.63, 1.73)	1.05 (0.64, 1.72)	1.21 (0.68, 2.17)	1.36 (0.80, 2.31)	0.20	—
Pooled							
Multivariable	1.00 (—)	1.06 (0.75, 1.48)	1.30 (0.95, 1.78)	1.26 (0.84, 1.87)	1.42 (1.00, 2.02)	0.05	0.93

[1] Cox proportional hazards models were used to compute RRs (95% CIs) and *P*-trend values. Heterogeneity between main results for men and women was tested by using the random-effects method of DerSimonian and Laird (22). Simple model values were adjusted for age and questionnaire cycle. Multivariable values were adjusted for age; questionnaire cycle; sugar-sweetened soda consumption; fruit and vegetable consumption; multivitamin use; intakes of alcohol, saturated fat, animal protein, and total energy; race; BMI; height; discretional physical activity; smoking history; and menopausal status and use of hormone replacement therapy (women only). HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[2] Frequency of diet soda consumption on the basis of a 12–fl oz (355 mL) serving that was equivalent to one glass, bottle, or can

[3] Test for linear trend using median values within each category of diet soda consumption.

[4] Test for heterogeneity between linear models for men and women.

[5] RR; 95% CI in parentheses (all such values).

[6] $P < 0.05$ in the test for heterogeneity between RRs for men and women in the same diet soda category.

diet soda did not differ by alcohol consumption for any of the outcomes, although few women in the NHS consume high amounts of alcohol.

We also conducted analyses stratified by baseline BMI because of its strong positive association with diet soda consumption but observed no meaningful effect modification in any of our endpoints, although the power was low for a critical evaluation (data not shown).

All analyses of diet soda were controlled for regular sugar-sweetened soda consumption. In the multivariable models for men, we observed increased risk of NHL associated with ≥1 serving regular soda/d (RR: 1.66; 95% CI: 1.10, 2.51; *P*-trend = 0.03; **Table 4**) after adjustment for diet soda consumption. Risk was also increased for multiple myeloma, although results were not significant (RR: 1.76; 95% CI: 0.77, 4.03; *P*-trend = 0.37). The sugar in regular soda did not seem to explain these positive associations because neither sucrose, fructose, nor total sugar intake was associated these outcomes (data not shown). No association was observed between regular soda and leukemia in men or any of the outcomes in women, although the power was low for the assessment of risks associated with regular soda because the consumption was low in these cohorts. Finally,

TABLE 3
RRs of non-Hodgkin lymphoma, multiple myeloma, and leukemia by categories of aspartame intake in men in the HPFS and in women in the NHS, 1994–2006[1]

	Aspartame[2]						
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Quartile 5	*P*-trend[3]	*P*-heterogeneity[4]
Range (mg/d)							
Men	0	<19	19–59	60–142	≥143	—	—
Women	0	<19	19–55	56–128	≥129	—	—
Person-years (thousands)							
Men	100.4	58.3	57.6	57.6	58.6	—	—
Women	224.8	147.0	147.0	147.7	147.6	—	—
Non-Hodgkin lymphoma							
Men							
Cases (*n*)	95	55	65	49	69	—	—
Simple model	1.00 (—)[5]	0.93 (0.66, 1.30)	1.15 (0.83, 1.58)	0.98 (0.69, 1.38)	1.59 (1.15, 2.19)	0.002	—
Multivariable	1.00 (—)	0.92 (0.65, 1.29)	1.13 (0.82, 1.57)	0.98 (0.68, 1.40)	1.64 (1.17, 2.29)	0.002	—
Women							
Cases (*n*)	172	114	110	91	86	—	—
Simple model	1.00 (—)	0.97 (0.76, 1.23)	0.99 (0.78, 1.26)	0.87 (0.67, 1.12)	0.95 (0.73, 1.24)	0.61	—
Multivariable	1.00 (—)	0.94 (0.74, 1.20)	0.96 (0.75, 1.22)	0.83 (0.64, 1.08)	0.91 (0.69, 1.20)	0.48	—
Pooled							
Multivariable	1.00 (—)	0.93 (0.76, 1.13)	1.02 (0.83, 1.24)	0.88 (0.71, 1.09)	1.16 (0.93, 1.43)[6]	0.12	0.006
Multiple myeloma							
Men							
Cases (*n*)	10	17	11	14	13	—	—
Simple model	1.00 (—)	2.80 (1.26, 6.20)	1.62 (0.67, 3.92)	2.56 (1.13, 5.84)	2.85 (1.23, 6.62)	0.07	—
Multivariable	1.00 (—)	3.33 (1.48, 7.49)	1.70 (0.68, 4.23)	2.96 (1.25, 6.96)	3.36 (1.38, 8.19)	0.05	—
Women							
Cases (*n*)	45	14	25	25	15		
Simple model	1.00 (—)	0.43 (0.23, 0.78)	0.82 (0.50, 1.34)	0.89 (0.54, 1.45)	0.61 (0.34, 1.11)	0.47	—
Multivariable	1.00 (—)	0.40 (0.22, 0.74)	0.76 (0.46, 1.27)	0.83 (0.50, 1.39)	0.59 (0.32, 1.09)	0.48	—
Pooled							
Multivariable	1.00 (—)	0.86 (0.53, 1.41)[6]	0.92 (0.59, 1.44)	1.16 (0.75, 1.81)[6]	1.03 (0.62, 1.72)[6]	0.44	0.049
Leukemia							
Men							
Cases (*n*)	23	14	23	19	18	—	—
Simple model	1.00 (—)	0.88 (0.45, 1.73)	1.62 (0.89, 2.93)	1.56 (0.84, 2.90)	1.68 (0.89, 3.17)	0.07	—
Multivariable	1.00 (—)	0.89 (0.45, 1.77)	1.69 (0.91, 3.12)	1.55 (0.81, 2.94)	1.56 (0.79, 3.06)	0.17	—
Women							
Cases (*n*)	34	21	32	21	21		
Simple model	1.00 (—)	0.88 (0.51, 1.51)	1.45 (0.89, 2.36)	1.06 (0.61, 1.84)	1.29 (0.74, 2.25)	0.36	—
Multivariable	1.00 (—)	0.85 (0.48, 1.48)	1.34 (0.81, 2.21)	0.95 (0.54, 1.66)	1.04 (0.58, 1.85)	0.94	—
Pooled							
Multivariable	1.00 (—)	0.86 (0.56, 1.33)	1.47 (1.00, 2.17)	1.17 (0.77, 1.79)	1.23 (0.80, 1.91)	0.31	0.35

[1] Cox proportional hazards models were used to compute RRs (95% CIs) and *P*-trend values. Heterogeneity between main results for men and women was tested by using the random-effects method of DerSimonian and Laird (22). Simple model values were adjusted for age and questionnaire cycle. Multivariable values were adjusted for age; questionnaire cycle; total sugar intake; fruit and vegetable consumption; multivitamin use; intakes of alcohol, saturated fat, animal protein, and total energy; race; BMI; height; discretional physical activity; smoking history; and menopausal status and use of hormone replacement therapy (women only). HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[2] Aspartame intake was from diet soda and packets used at the table; categories are for zero intake plus quartiles of intakes greater than zero.

[3] Test for linear trend by using median values within each category of aspartame intake.

[4] Test for heterogeneity between linear models for men and women.

[5] RR; 95% CI in parentheses (all such values).

[6] $P < 0.05$ in the test for heterogeneity between RRs for men and women in the same aspartame category.

results for associations between diet and regular soda and cancer risk were similar when no soda intake of any kind as the reference category was considered (data not shown).

In addition, risks seemed to vary slightly depending on whether cola-type or other soda was consumed, with a suggestion for higher risks of multiple myeloma in subjects with higher intakes of cola-type diet soda and for leukemia in subjects with higher intakes of other non–cola-type diet soda. However, case numbers were too small, particularly in regular soda consumers, to draw any meaningful conclusions (data not shown; *see* Table 1 under "Supplemental data" in the online issue).

DISCUSSION

In the most comprehensive long-term epidemiologic study, to our knowledge, to evaluate the association between aspartame intake and cancer risk in humans, we observed a positive association between diet soda and total aspartame intake and risks

TABLE 4
Multivariable RRs of non-Hodgkin lymphoma, multiple myeloma, and leukemia by frequency of regular sugar-sweetened soda consumption in men in the HPFS (1986–2006) and in women in the NHS (1984–2006)[1]

	Regular sugar-sweetened soda[2]						
	None	<1 serving/wk	1–3.9 servings/wk	4–6.9 servings/wk	≥1 serving/d	*P*-trend[3]	*P*-heterogeneity[4]
Person-years (thousands)							
Men	264.9	222.9	190.4	57.1	49.1	—	—
Women	623.6	489.3	262.5	62.0	56.5	—	—
Non-Hodgkin lymphoma							
Men							
Cases (*n*)	181	185	137	36	32	—	—
RR (95% CI)	1.00 (—)	1.18 (0.95, 1.46)	1.23 (0.97, 1.57)	1.27 (0.87, 1.87)	1.66 (1.10, 2.51)	0.03	—
Women							
Cases (*n*)	293	293	121	25	21	—	—
RR (95% CI)	1.00 (—)	1.15 (0.97, 1.36)	0.99 (0.79, 1.24)	0.94 (0.62, 1.44)	1.01 (0.63, 1.62)	0.59	—
Pooled							
RR (95% CI)	1.00 (—)	1.16 (1.01, 1.33)	1.09 (0.93, 1.29)	1.11 (0.84, 1.48)	1.34 (0.98, 1.83)	0.05	0.27
Multiple myeloma							
Men							
Cases (*n*)	47	39	32	5	8	—	—
RR (95% CI)	1.00 (—)	1.10 (0.70, 1.74)	1.28 (0.78, 2.11)	0.80 (0.30, 2.10)	1.76 (0.77, 4.03)	0.37	—
Women							
Cases (*n*)	62	56	24	8	4	—	—
RR (95% CI)	1.00 (—)	1.03 (0.70, 1.51)	0.96 (0.57, 1.59)	1.54 (0.70, 3.38)	1.07 (0.36, 3.16)	0.58	—
Pooled							
RR (95% CI)	1.00 (—)	1.06 (0.79, 1.42)	1.11 (0.78, 1.59)	1.18 (0.64, 2.17)	1.47 (0.76, 2.83)	0.31	0.81
Leukemia							
Men							
Cases (*n*)	71	65	31	11	8	—	—
RR (95% CI)	1.00 (—)	0.97 (0.68, 1.40)	0.64 (0.41, 1.01)	0.96 (0.49, 1.89)	0.92 (0.42, 2.02)	0.61	—
Women							
Cases (*n*)	56	55	31	7	4	—	—
RR (95% CI)	1.00 (—)	1.27 (0.85, 1.88)	1.62 (1.01, 2.60)	1.73 (0.76, 3.96)	1.39 (0.47, 4.07)	0.21	—
Pooled							
RR (95% CI)	1.00 (—)	1.10 (0.84, 1.43)	0.99 (0.72, 1.38)[5]	1.22 (0.72, 2.06)	1.06 (0.56, 2.00)	0.68	0.23

[1] Cox proportional hazards models were used to compute RRs (95% CIs) and *P*-trend values. Heterogeneity between main results for men and women was tested by using the random-effects method of DerSimonian and Laird (22). RRs (95% CIs) were adjusted for age; questionnaire cycle; diet soda consumption; fruit and vegetable consumption; multivitamin use; intakes of alcohol, saturated fat, animal protein, and total energy; race; BMI; height; discretional physical activity; smoking history; and menopausal status and use of hormone replacement therapy (women only). HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[2] Frequency of regular sugar-sweetened soda consumption on the basis of a 12-fl oz (355 mL) serving that was equivalent to one bottle, glass, or can.

[3] Test for linear trend using median values within each category of regular soda consumption.

[4] Test for heterogeneity between linear models for men and women.

[5] $P < 0.05$ in the test for heterogeneity between RRs for men and women in the same category for regular soda.

of NHL and multiple myeloma in men and leukemia in both men and women. A higher consumption of regular sugar-sweetened soda was associated with higher risk of NHL and multiple myeloma in men but not in women. Although we lacked statistical power to examine associations with less common NHL subtypes in men, we observed similar associations between diet soda and major subtypes of NHL, including B cell NHL and CLL/SLL. In women, no associations were observed for all NHLs or NHL common subtypes (ie, CLL/SLL, follicular lymphoma, and diffuse large B cell lymphoma).

Because of the reported effect of aspartic acid on neuronal necrosis in the brains of rodents (25–27), carcinogenicity studies in animals were reviewed carefully before the approval of aspartame by the US Food and Drug Administration (FDA). Four experimental studies evaluated potential cancer risk, 3 of which contributed to the FDA's approval of the substance for use in foods (1, 28). In addition, several small placebo-controlled studies have been conducted in humans on the effects of aspartame intake on hormones and blood concentrations of the 3 main compounds of aspartame. These studies have also evaluated the safety of aspartame in specific subpopulations such as in healthy infants and children as well as in patients with diabetes (ie, groups who are likely to consume more aspartame than the general population) and have generally shown short-term aspartame intake to be safe at various doses (1). However, few long-term studies have been conducted, the longest duration of which was 18 wk in patients with diabetes (29) in which no serious adverse events were reported. Although there was a lack of data from longer-term studies in humans, the larger body of shorter-term and animal evidence appeared to support no health effects of aspartame, which ultimately led to the FDA's approval of the use of aspartame in foods. Today, aspartame is used as a sweetener and flavor enhancer in >6000 foods worldwide.

Although a small 9-mo feeding study conducted in the US in 2005 did not demonstrate higher tumor rates in genetically altered mice (30), in 2006, an Italian research team reported findings from the largest aspartame feeding study in rats to date (10, 31). The team followed Sprague-Dawley rats throughout their entire life span (an average of 3 y), while the rats were constantly fed 0–5 g aspartame · kg body weight^{-1} · d^{-1}, until their natural deaths and showed risks of several cancers were significantly elevated in animals that had been fed increasing doses of aspartame in comparison with animals that received the same feed without aspartame. Specifically, the incidence of leukemia and lymphomas was shown to be significantly higher in animals fed aspartame at doses as low as 20 mg/kg body weight. Furthermore, increases in transitional cell carcinomas of the pelvis, ureter, and bladder were noted.

Subsequently, human data were presented from a one-time assessment of soda, fruit juice, and iced tea consumption in 566,990 participants in the NIH–American Association for Retired Persons Diet and Health Study (12). Overall, aspartame intake was not associated with risk of lymphoma, leukemia, or brain tumors in this observational study with 5 y of follow-up. However, because of the single-exposure assessment and short follow-up in the study, concerns about the validity of the results remain. Moreover, relatively small case numbers limited the ability to explore potential sex differences. Few studies have evaluated associations between diet soda and other surrogates for aspartame intake and risk of specific cancers or overall cancer risk. In the NHS and HPFS, we previously reported a nonsignificantly elevated risk of pancreatic cancer associated with greater diet soda consumption (14). In a small case-control study conducted in Italy (230 gastric cancer cases, 326 pancreatic cancer cases, and 454 endometrial cancer cases), no increased risk of any of the tumors examined was observed in relation to aspartame intake (13).

The potential carcinogenicity of aspartame is biologically plausible. Aspartame is the methyl ester of a dipeptide of phenylalanine and aspartic acid, and it is broken down on ingestion into these amino acids as well as methanol, which are then absorbed into the systemic circulation. Although early toxicology studies showed no genotoxic effects of aspartame, more-recent studies (ie, postregulatory approval) have not been entirely consistent, with one study that reported an interaction of aspartame and its metabolites with DNA in an in vitro model (32) and another study that showed the potential for aspartame to induce DNA strand breaks in bone marrow cells of mice (33).

Nitrosation was reported as the putative mechanism behind the hypothesized association between aspartame and brain tumors (34). Extremely high nitrite concentrations may react with a variety of amino acids, including aspartame, which generate compounds with mutagenic properties under certain conditions. However, these mechanisms are not unique to aspartame. The primary food sources of phenylalanine and aspartic acid are meats, fish, and dairy foods, and diet soda adds a minor amount to the total. In a previous analysis, we showed animal protein to be associated with increased risk of NHL in women in the NHS (35). In the current analysis, the disease associations we observed with aspartame intake were not confounded by animal protein intake.

It has also been speculated that methanol, through its metabolization to formaldehyde, may cause an increase in lymphomas and leukemias in rats (10). Some animal studies have shown that both methanol and formaldehyde administered in water increased the rate of lymphoma and leukemias in female rats (36, 37). Moreover, in humans, formaldehyde has been classified as a definite carcinogen (9). This classification was largely based on occupational exposure to formaldehyde, with the most common routes of exposure being inhalation, skin, and eye contact. Although the literature is ambiguous, it appears possible that the ADI for aspartame could translate into amounts of methanol and formaldehyde that are potentially higher than currently considered ADIs. For example, if the ~600 mg aspartame contained in 1 L diet soda translates into 60 mg methanol (38, 39) and 60 mg formaldehyde, these amounts could, in certain cases, exceed their respective ADIs. In humans, the ADI for formaldehyde has been estimated at 0.15 mg · kg body weight^{-1} · d^{-1} (40), and for methanol, which can also stem from other dietary sources, the US Food Additives and Contaminants Committee recommended a maximum concentration of 8 ppm in food, which is the equivalent of 2.28 mg · kg body weight^{-1} · d^{-1} (41).

In the light of some elevated cancer risks associated also with regular soda consumption in our data, alternative hypothetical explanations might relate to factors that are common to both diet and regular soda (eg, other ingredients in soda or packaging materials of soda containers) (42, 43). Ultimately, it is also conceivable that our results are reflective of multiple unknown agents rather than a single agent or a chance finding unrelated to the chemical contents of sodas.

The sex differences we observed in our data deserve consideration. One possible explanation is that our findings in men were due to chance. However, because of the consistency and dose-response relations we observed, other possible explanations must be considered. The results could have been related to uncontrolled confounding by yet-to-be-discovered risk factors for lymphoma and leukemia, which are associated with soda consumption in men but not women (perhaps related to their lifestyles or occupations). Another, more speculative explanation could be that men are more susceptible to the effects of aspartame, perhaps because of differences in enzyme activity; the only human enzyme that is capable of metabolizing methanol, one of the breakdown products of aspartame, to formaldehyde is ADH (44). Previous studies reported that ADH activity was significantly higher in men than in women (45), and increased alcohol consumption was associated with decreased ADH activity in men (24, 46), which slowed down the conversion of methanol to formaldehyde and formate (47, 48). Specifically, Frezza et al (24) report that chronic alcohol consumption lead to a 37–46% reduction in ADH activity in men, with a smaller reduction of ADH activity (11–20%) also seen in women with chronic alcohol use. Although it is still being debated whether methanol, by itself, is carcinogenic in humans (49), in 2006 the International Agency for Cancer Research classified formaldehyde as a class 1 definite carcinogen, with likely carcinogenic effects for leukemia and other tumors (9). When we examined the influence of alcohol intake on the observed associations, risks appeared significantly higher in men who consumed the least amounts of alcohol. These data provided some support of differences in enzyme activity as a potential explanation of the apparent sex differences in our results related to diet soda and aspartame intake. However, differences in ADH activity cannot explain the sex

differences we observed that were related to regular soda intake and risk of cancer.

A limitation of our study is that the measurement of aspartame intake is necessarily imperfect for 2 primary reasons. First, we did not have complete assessment of each single dietary item that may have contained aspartame; however, we are confident that we captured close to 95% of all aspartame intake by adding diet soda consumption and aspartame consumption via sweetener packets (19, 20). Other sources of aspartame intake are minor contributors to overall aspartame intake. Second, the assessment of aspartame intake is imperfect because there are multiple sources in the diet that must be self-reported. However, we assessed long-term aspartame intake by deriving the cumulative average aspartame intake on the basis of repeated FFQs. Alternative methods (ie, by using biomarkers) are expensive and may be inferior to repeated questionnaires that take into account changes in dietary habits over time. Moreover, compared with most dietary factors, aspartame was measured relatively well, especially for the majority of the follow-up period, during which its use was restricted to a limited number of dietary products. In addition, despite comparable ages, the mean aspartame intake in our cohorts was lower than that reported in another large US cohort (eg, the mean intake of 114 mg/d in the HPFS and 102 mg/d in the NHS in 2002; in the NIH–American Association for Retired Persons Diet and Health Study, the mean overall aspartame intake was 200 mg/d) (12). This difference could in part be explained by differences in the details of the questionnaire regarding the portion size and frequency of intake or the timing when questionnaires were administered.

Detailed covariate information available in the NHS and HPFS allowed us to take into account many sources of potential confounding. For all cancers, results from multivariable models were very similar to those from models that adjusted for age and time period only, which suggested little evidence for confounding by the factors considered. However, residual confounding or confounding by unmeasured factors could not be ruled out. We observed increased risk of NHL in men with a higher intake of regular sugar-sweetened soda, although sugar itself was not associated with increased risk, whereas aspartame intake supported the positive association between diet soda and NHL. Also, because of the limited case numbers and modest intakes of soda in our cohorts, in certain instances, we were unable to explore associations with higher intakes of soda.

One of the major strengths of our study was the prospective nature of the study. Exposure and covariate information is not subject to recall bias because it is collected before disease onset. Another, rather unique strength of this study, besides its large sample size, was that we effectively captured lifetime exposure to aspartame because we have been assessing diet soda consumption intake since aspartame was first allowed into the food supply.

In conclusion, these observational data provide some support for findings from a recent animal experiment that suggested positive associations between aspartame intake and NHL, multiple myeloma, and leukemia, particularly in men. Because this is, to our knowledge, the first large-scale observational human study to report associations between diet soda and aspartame intake and these cancer types, our results necessarily require confirmation in other large cohorts. Future studies should also evaluate the potential for higher enzymatic activity and, by extension, higher chronic low-dose formaldehyde exposure from aspartame intake in men to account for the observed sex differences in these associations.

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