CONSUMPTION OF ARTIFICIAL SWEETENER AND SUGAR CONTAINING SODA AND THE RISK OF LYMPHOMA AND LEUKEMIA IN MEN AND WOMEN

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Abbreviations:

AARP American Association for Retired Persons

ADH	Alcohol dehydrogenase type I
ADI	Acceptable daily intake
BMI	Body mass index
CI	Confidence intervals
CLL	Chronic lymphocytic leukemia
EFSA	European Food and Safety Agency
FDA	Food & Drug Administration
FFQ	Food frequency questionnaire
HPFS	Health Professionals Follow-Up Study
IARC	International Agency for Cancer Research
IRB	Institutional Review Board
NHL	non-Hodgkin lymphomas
NHS	Nurses' Health Study
RR	Rate ratio
SLL	Small lymphocytic lymphoma
US	United States
WHO	World Health Organization

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ABSTRACT

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

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

Design. We repeatedly assessed diet in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS). Over 22 years, we identified 1,324 non-Hodgkin lymphomas (NHL), 285 multiple myelomas, and 339 leukemias. We calculated incidence rate ratios (RRs) and 95% confidence intervals (CIs) using Cox proportional hazards models.

Results. When combining the two cohorts, there was no significant association of soda intake and risks of NHL and multiple myeloma. Among men, however, ≥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), compared to 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 higher consumption of regular, sugar-sweetened soda in men, but not in women. On the other hand, when genders 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/day of diet soda when pooling the two cohorts, 1.42; 95% CI=1.00, 2.02).

Conclusion. While our findings preserve the possibility for a detrimental effect of a constituent of diet soda, such as aspartame, on select cancers, the inconsistent gender effects and the occurrence of an apparent cancer risk in individuals consuming regular soda, do not permit ruling out chance as an explanation.

INTRODUCTION

Aspartame (L-alpha-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, it is used as a sweetener and flavor-enhancer in over 6,000 foods worldwide. The annual amount of aspartame currently used in diet soda in the United States (US) is 4,500 metric tons (G Crosby, Nutra Sweet Co, personal communication July 14, 2006); the average content of aspartame in a one-liter bottle of diet cola is approximately 560 mg, whereas (diet) orange soda contains as much as 930 mg per liter (2-4). As the annual aspartame used across all applications in the US was estimated at 5,000 to 5,500 metric tons (C Heinzinger, Nutra Sweet Co, personal communication July 18, 2006), diet soda accounts for the large majority (~86%) of all aspartame in foods.

Despite many previous experimental studies evaluating and confirming the safety of aspartame, making it one of the most extensively tested food ingredients in the history of food additives, health-related concerns continue to be debated. Most notably, some question the relevance of animal studies – which, in general, show no harm – with regard to human safety (5, 6). Prior evidence (7) and a reinterpretation of long-term carcinogenicity studies in rats (1), however, suggest that aspartame may be carcinogenic, specifically that it may cause brain tumors. Moreover, aspartame, especially in liquids (8), quickly breaks down into its three 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 mega-experiment of 1,800 rats tested at aspartame doses much lower than the currently acceptable daily intake for humans (10) reported a dose-dependent increase in

lymphomas, leukemias and transitional renal cell tumors. This 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), concluding that there is "no reason to revise the previously established acceptable daily intake (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 not supportive of an association between aspartame intake with cancer risk (12-14). However, studies have been limited by their exposure assessment, which assessed aspartame intake only at one point in time. We, therefore, conducted a prospective analysis of diet soda and aspartame consumption in relation to the cancers with elevated risks in the Italian mega-experiment (10), e.g. lymphoma and leukemia, 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 four years. Transitional renal cell cancers were too few (n=33 in HPFS, n=34 in NHS) to analyze separately. Because we have been assessing diet soda and intake of foods high in aspartame since aspartame was first allowed into the food supply, our analyses largely capture lifetime aspartame exposure in two 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 to 55 years 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 to 75 years 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 two years 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 then again in 1986 in both cohorts. Diet was subsequently reassessed every four years.

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 (IRB) of the Brigham and Women's Hospital in Boston and the HPFS received IRB approval from the Harvard School of Public Health in Boston.

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 from 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 histologic subtype based on the current World Health Organization (WHO) classification system (16) using morphology and immunophenotype information in the medical records and pathology reports. 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 Morton and colleagues' proposed translation from previous classification systems to the current WHO standard (17). Over the follow-up period (1984-2006 in the NHS; 1986-2006 in the HPFS), we confirmed 571 non-Hodgkin lymphomas (NHL) in the HPFS men and 753 in the NHS women, of which 399 and 581, respectively, could be classified by histologic subtype from the medical record. As expected, the large majority of these were B cell origin lymphomas (374 in men; 553 in women). Of these, 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 the men and only 28 in the women, precluding 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 medical record. 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 with a semiquantitative food frequency questionnaire (FFQ) on which participants reported their frequency of consumption over the previous year for specified amounts of approximately 130 foods. The nine frequency categories ranged from never to six or more times per day. Frequency of diet soda consumption was assessed per 12 fluid ounce (355 ml; equivalent to one bottle, glass, or can) serving for three items: diet cola with caffeine, diet cola without caffeine, and other diet soda. These three types were summed for analysis of total diet soda consumption. Consumption of regular sugar-sweetened soda was similarly assessed. For analysis, we condensed the nine reported frequencies from the FFQ into five categories

ranging from zero to ≥1 serving/day to accommodate the distribution of soda consumption in these cohorts, though we were also able to examine ≥2 servings/day of diet soda in analyses of NHL.

The use of aspartame sweeteners added at the table (i.e., NutraSweet®, Equal®) 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 from 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, though none contained aspartame in the early years and only 4% of the brands contained aspartame at the end of follow-up and therefore were not included in the total intake. Other possible sources of aspartame (e.g., artificially sweetened yogurt or ice cream) were not assessed, though their contributions were likely small in comparison to that from soda. For analysis, we created five aspartame categories, with zero intakes as the lowest category and cohort-specific exact quartiles for the remaining categories.

Nutrient intakes that are correlated with total energy intake were adjusted for total energy using regression analysis (18). To generate estimates of long-term diet, diet soda consumption and other food and nutrient intakes were cumulatively averaged in the statistical analyses, i.e., 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 discriminating between levels of dietary intakes (19, 20). In a comparison of the FFQ with two 1-week diet records collected from 127 HPFS participants, the correlation was 0.73 for diet sodas (20).

Non-dietary Measures

All non-dietary 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. Body mass index (BMI, kg/m^2) was calculated from current weight and the height reported on the initial cohort questionnaire. For physical activity, we calculated total met-hours per week, a measure of energy expenditure, from reported hours of participation and the assigned metabolic equivalent score for each activity listed on the questionnaire (21). The activity data were cumulatively averaged in statistical analyses. For the NHS cohort, questions on menopausal status and 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 from the return date of their baseline questionnaire (1984 for NHS, 1986 for HPFS) and were censored at the first report of cancer, at death, or the end of follow-up (January 1, 2006 for HPFS and June 1, 2006 for NHS).

We used Cox proportional hazards models to compute incidence rate ratios (RR), 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 two-way interactions between these two time scales, we stratified analyses jointly by age in months at start of follow-up and calendar year of the current questionnaire cycle. We calculated multivariable RRs by adjusting models simultaneously for the dietary and non-dietary covariates. Analyses of diet 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, hence 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 using the random effects method of DerSimonian and Laird and pooled results when appropriate (22).

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 of diet soda/day were younger, on average, than those with less frequent consumption (**Table 1**). After adjusting for age, those with 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 among those with any soda consumption (r=-0.52 in men, -0.56 in women). At the baseline dietary assessment, 55% of the men and 62% of the women reported diet soda consumption with mean intakes of 6.0 and 6.6 servings/week, respectively. Consumption of diet soda declined slightly over time, particularly in women. At the final dietary assessment in 2002, 53% of the men and 54% of the women reported diet soda consumption with mean intakes of 5.7 and 5.3 servings/week, respectively. Mean daily

aspartame intake among consumers at the final dietary assessment was114 mg in HPFS and 102 mg in 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 will be discussed. In men, risk of NHL was significantly elevated for those who consumed ≥1 serving/day of diet soda (RR=1.31; 95% CI, 1.01, 1.72) compared with those who reported no consumption (**Table 2**). Risk was even greater for ≥2 servings/day 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). In an examination of NHL subtypes, ≥1 serving/day of diet soda 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 any subtype examined even at intakes of ≥2/day (RR=1.12; 95% CI, 0.81, 1.56; P_{trend}=0.65; $P_{heterogeneity}$ = 0.24).

For multiple myeloma, risk increased linearly with increasing consumption of diet soda in men (P_{trend}=0.009) and was significantly elevated for those consuming ≥1 serving/day (RR=2.02; 95% CI, 1.20, 3.40). Diet soda was not associated with risk of multiple myeloma in women, and significant heterogeneity was observed between cohorts for linear trend ($P_{heterogeneity}$=0.04) and for risk in the high category of ≥1 serving/day ($P_{heterogeneity}$=0.01).

For leukemia, risk was elevated in the higher intake categories of diet soda in both men and women, though these sex-specific results were not significant. Statistical power was improved when pooling the two cohorts, which yielded a linear trend (P_{trend}=0.05) and an

increased risk of leukemia for those consuming ≥1 serving/day of diet soda (RR=1.42; 95% CI, 1.00, 2.02). Restricting to myeloid leukemia (which represents the majority of all leukemias in our data set) produced similar results (pooled RR=1.31; 95% CI, 0.85, 2.03 for ≥1 serving/day; P_{trend}=0.06).

Although incidence rates remained stable across follow-up, in analyses stratified by follow-up time (1986 (men)/1984 (women) – 1996, and 1996 –2006), 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 follow-up period for multiple myeloma (data not shown).

Though aspartame was approved for use in the U.S. in 1981 and was used as the sole artificial sugar sweetener in Diet Coke® (the most commonly used diet soda at the time) beginning in 1983, most other diet sodas in the 1980's 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 reduced statistical power, we observed increased risks in men for all three outcomes with higher intakes of aspartame (**Table 3**) similar to those we observed with diet soda. Among 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 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 be due to the recognized higher enzymatic activity of alcohol dehydrogenase type I (ADH) in men, possibly inducing higher conversion rates from methanol to the carcinogenic substrate formaldehyde. Because 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), thus higher formaldehyde conversion rates if they consumed large amounts of diet soda, and consequently higher cancer risk. For NHL, ≥2 servings/day of diet soda was associated with an increased risk (RR=2.34; 95% CI, 1.46, 3.76; P_{trend}=0.004) among men consuming <6 g/day of alcohol (median intake) but not among men with higher alcohol consumption (RR=0.96; 95% CI, 0.48, 1.90; P_{trend}=0.99; Supplemental Table 1). The interaction between diet soda and alcohol was significant ($P_{interaction}$=0.03). Risks of multiple myeloma and leukemia associated with ≥1 serving/day of diet soda were also higher among the men with lower alcohol intake. For women, risks associated with diet soda did not differ by alcohol consumption for any of the outcomes although few women in 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, though 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 an increased risk of NHL associated with ≥1 serving/day of regular soda (RR=1.66; 95% CI, 1.10, 2.51; P_{trend}=0.03; **Table 4**) after adjusting for diet soda consumption. Risk was also increased for multiple myeloma, though 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

306 to explain these positive associations as neither sucrose, fructose nor total sugar intake was
307 associated these outcomes (data not shown). No association was observed between regular soda
308 and leukemia in men or any of the outcomes in women, though power was low for assessing
309 risks associated with regular soda since consumption was low in these cohorts. Finally, results
310 for associations between diet and regular soda and cancer risk were similar when considering no
311 soda intake of any kind as the reference category (data not shown).

312 Further, risks seemed to vary slightly depending on whether cola-type or other soda was
313 consumed, with a suggestion for higher risks of multiple myeloma among those with higher
314 intakes of cola-type diet soda, and for leukemia among those with higher intakes of other (non-
315 cola-type) diet soda. Case numbers were too small, however, particularly among the regular soda
316 consumers, to draw any meaningful conclusions (data not shown/Supplemental Table 1).
317

318 **DISCUSSION**

319 In the most comprehensive long-term epidemiologic study to evaluate the association between
320 aspartame intake and cancer risk in humans to date, we observed a positive association between
321 diet soda and total aspartame intake and risks of NHL and multiple myeloma in men, and
322 leukemia among both men and women. Higher consumption of regular, sugar sweetened soda
323 was associated with higher risk of NHL and multiple myeloma in men, but not in women.
324 Though we lacked statistical power to examine associations with less common NHL subtypes in
325 men, we observed similar associations between diet soda and major subtypes of NHL, including
326 B-cell NHL and CLL/SLL. Among women, no associations were observed for all NHL or its
327 common subtypes (i.e., 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 aspartame's approval by the US Food & Drug Administration (FDA). Four experimental studies evaluated potential cancer risk, three of which contributed to FDA's approval of the substance for use in foods (1, 28). Further, several small, placebo-controlled studies have been conducted in humans on the effects of aspartame intake on hormones and blood levels of the three main compounds of aspartame. These studies have also evaluated aspartame's safety in specific subpopulations such as healthy infants and children as well as diabetics (i.e., groups likely to consume more aspartame than the general population) and have generally found short-term aspartame intake to be safe at various doses (1). However, few long-term studies have been conducted, with the longest duration being 18 weeks in diabetics (29) in which no serious adverse events were reported. Although data from longer-term studies in humans were lacking, the larger body of shorter-term and animal evidence appeared to support no health effects of aspartame, ultimately leading to FDA's approval for its use in foods. Today, it is used as a sweetener and flavor-enhancer in over 6,000 foods worldwide.

While a small 9-month feeding study conducted in the US in 2005 did not demonstrate higher tumor rates in their genetically altered mice (30), in 2006, an Italian research team reported findings from the largest aspartame feeding study in rats to date (10, 31). Following their Sprague-Dawley rats throughout their entire lifespan (an average of three years) constantly being fed with aspartame 0-5 g/kg body weight per day, until their natural death, the risk of several cancers was significantly elevated in animals that had been fed increasing doses of aspartame compared to animals that received the same feed without aspartame. Specifically, the incidence of leukemia and lymphomas was found to be significantly higher among animals fed

with aspartame at doses as low as 20 mg/kg bw. Furthermore, increases in transitional cell carcinomas of the pelvis and ureter as well as bladder were noted.

Subsequently, human data were presented from a one-time assessment of soda, fruit juice, and iced tea consumption among 566,990 participants in the NIH-American Association for Retired Persons (AARP) Diet and Health Study (12). Overall, aspartame intake was not associated with the risk of lymphoma, leukemia, or brain tumors in this observational study with 5 years of follow-up. However, given their single exposure assessment and short follow-up, concerns about validity 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 the risk of specific cancers or overall cancer risk. In the NHS and HPFS, we previously reported a non-significantly elevated risk for 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 upon ingestion into these amino acids as well as methanol, which are then absorbed into the systemic circulation. Although early toxicology studies demonstrated no genotoxic effects of aspartame, more recent studies (i.e., post regulatory approval) have not been entirely consistent, with one reporting an interaction of aspartame and its metabolites with DNA in an *in vitro* model (32) and another study showing the potential for aspartame to induce DNA strand breaks in the 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, generating 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 found animal protein to be associated with an increased risk of NHL among 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 recently been classified as a definite carcinogen (9). This classification is largely based on occupational exposure to formaldehyde, with the most common routes of exposure being inhalation, skin and eye contact. Even though the literature is ambiguous, it appears possible that the ADI for aspartame could translate into levels of methanol and/or formaldehyde potentially higher than what one would currently consider acceptable daily intake. For example, if the roughly 600 mg aspartame contained in 1 liter of diet soda translate into 60 mg methanol (38, 39), or 60 mg formaldehyde, this could in certain cases exceed their respective ADI's. In humans, the ADI for formaldehyde has been estimated at 0.15 mg/kg body weight per day (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 per day (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 common to both diet and regular soda, for example other ingredients in soda or the packaging material of soda containers (42, 43). Ultimately, it is also conceivable that our results are reflective of not a single, but multiple unknown agents, or a chance finding unrelated to the chemical contents of sodas.

The sex differences we observed in our data deserve consideration. One possible explanation, of course, is that our findings in men are due to chance. However, given the consistency and dose-response relationships we observed, other possible explanations must be considered. They could be 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 lifestyle or occupation). Another, more speculative explanation could be that men are more susceptible to the effects of aspartame, perhaps due to differences in enzyme activity: the only human enzyme capable of metabolizing methanol, one of the breakdown products of aspartame, to formaldehyde is alcohol dehydrogenase (ADH) (44). Previous studies reported that ADH activity was significantly higher in males than in females (45) and that increasing alcohol consumption was associated with decreasing ADH activity in men (24, 46), hence slowing down methanol's conversion to formaldehyde and formate (47, 48). Specifically, Frezza et al. report that chronic alcohol consumption lead to 37 to 46 percent reduction in ADH activity in the men, with a smaller reduction of ADH activity (11 to 20 percent) also seen in women with chronic alcohol use (24). While it is still being debated whether methanol by itself is carcinogenic in humans (49), in 2006, the International Agency for

Cancer Research (IARC) classified formaldehyde as a class one definite carcinogen, with likely carcinogenic effects for leukemia, as well as other tumors (9). When we examined the influence of alcohol intake on the observed associations, risks appeared significantly higher among men who consumed the least amounts of alcohol. This lends some support to differences in enzyme activity as a potential explanation of the apparent sex differences in our results related to diet soda and aspartame intake. They could, however, likely not explain sex differences we observed related to regular soda intake and risk of cancer.

A limitation of our study is that measurement of aspartame intake is necessarily imperfect, for two primary reasons. First, we did not have complete assessment of each single dietary item that may contain aspartame; however, we are confident that we capture close to 95% of all aspartame intake by adding diet soda consumption and aspartame consumption via sweetener packets (19, 20). Other sources are minor contributors to overall aspartame intake. Secondly, 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 cumulative average aspartame intake based on repeated FFQs. Alternative methods (i.e., using biomarkers) are expensive and may be inferior to repeated questionnaires that take into account changes in dietary habits over time. Moreover, compared to most dietary factors, aspartame is 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. Further, despite comparable age, mean aspartame intake in our cohorts was lower than that reported in another large US cohort [e.g., mean intake of 114 mg/day in HPFS and 102 mg/day in NHS in 2002; in the NIH-AARP Diet and Health Study, mean overall aspartame intake 200 mg/day] (12). This difference could in part

441 be explained by differences in the detail of the questionnaire regarding portion size and
442 frequency of intake, or the timing when questionnaires were administered.

443 Detailed covariate information available in the NHS and HPFS allowed us to take into
444 account many sources of potential confounding. For all cancers, results from multivariable
445 models were very similar to those from models that adjusted for age and time period only,
446 suggesting little evidence for confounding by the factors considered. Residual confounding, or
447 confounding by unmeasured factors, however, cannot be ruled out. We did observe an increased
448 risk of NHL in men with a higher intake of regular sugar-sweetened soda, though sugar itself
449 was not associated with increased risk, whereas aspartame intake supported the positive
450 association between diet soda and NHL. Also, given the limited case numbers and modest
451 intakes of soda in our cohorts, in certain instances, we were unable to explore associations with
452 higher intakes of soda.

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

458 In sum, these observational data provide some support for findings from a recent animal
459 experiment, suggesting positive associations between aspartame intake and NHL, multiple
460 myeloma, and leukemia, particularly among men. Because this is the first large-scale
461 observational human study to report associations between diet soda and aspartame intake and
462 these cancer types, our results necessarily require confirmation in other large cohorts. Future
463 studies should also evaluate the potential for higher enzymatic activity and – by extension –

464 higher chronic low-dose formaldehyde exposure from aspartame intake in men to account for the
465 observed gender differences in these associations.
466

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AUTHOR CONTRIBUTIONS

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Table 1. Age and age-standardized characteristics of the study populations within categories for frequency of diet soda consumption at baseline in 1986 among men in the Health Professionals Follow-up Study and in 1984 among women in the Nurses' Health Study.

	Diet Soda[1]					
	Men			Women		
	none	**1-3.9/week**	**≥ 1/day**	**none**	**1-3.9/week**	**≥ 1/day**
(median per week)	(0)	(2.9)	(11.0)	(0)	(3.0)	(11.0)
number of participants	21,328	8,023	8,259	29,206	13,091	17,427
	mean [SD]					
Age, yrs	55.3 [10.0]	54.3 [9.5]	51.5 [8.9]	51.5 [7.3]	50.8 [7.1]	49.5 [7.0]
Regular soda[1]/week	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[2], mg/day	3.6 [13.7]	55.9 [30.5]	268 [186]	2.9 [12.0]	54.7 [29.8]	248 [177]
Fruit+vegetables/day	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[3] g/day	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[3] g/day	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/day	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/day	2039 [630]	1930 [600]	1980 [630]	1784 [537]	1714 [519]	1738 [536]
Activity[4] met-h/week	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]
	percent					
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[5]	na	na	na	23	24	22

na = not applicable

[1] frequency of diet soda and regular sugar-sweetened soda consumption based upon a 12 fluid ounce (355 ml) serving, equivalent to one glass, bottle, or can

[2] aspartame was assessed in 1994 in both cohorts rather than at baseline

[3] nutrient intake adjusted for total energy intake

[4] metabolic equivalent energy expenditure from discretionary physical activity

[5] use of hormone replacement therapy among postmenopausal women

Table 2. Relative risks of non-Hodgkin lymphoma, multiple myeloma, and leukemia by frequency of diet soda consumption among men in the Health Professionals Follow-up Study, 1986-2006, and among women in the Nurses' Health Study, 1984-2006.

	Diet Soda[1]						
	none	**< 1/week**	**1-3.9/week**	**4-6.9/week**	**≥ 1/day**	**p for trend[2]**	**p for heterogeneity[3]**
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	172	122	124	53	100		
RR (95% CI): simple model[4]	1.00	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[5]	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	189	167	173	87	137		
RR (95% CI): simple model[4]	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[5]	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							
RR (95% CI): multivariable[5]	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	40	27	23	12	29		
RR (95% CI): simple model[4]	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[5]	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	39	28	40	23	24		
RR (95% CI): simple model[4]	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[5]	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							
RR (95% CI): multivariable[5]	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		
RR (95% CI):	simple model[4]	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[5]	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		
RR (95% CI):	simple model[4]	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[5]	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								
RR (95% CI):	multivariable[5]	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

RR = relative risk; CI = confidence interval

[1] frequency of diet soda consumption based upon a 12 fluid ounce (355 ml) serving, equivalent to one glass, bottle, or can

[2] test for linear trend using median values within each category of diet soda consumption

[3] test for heterogeneity between linear models for men and women

[4] adjusted for age and questionnaire cycle

[5] 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)

[6] $p < 0.05$ in test for heterogeneity between relative risks for men and women in the same diet soda category

We used Cox proportional hazards models to compute RRs, 95% CIs, and P for trends. We tested for heterogeneity between main results for men and women using the random effects method of DerSimonian and Laird.

Table 3. Relative risks of non-Hodgkin lymphoma, multiple myeloma, and leukemia by categories of aspartame intake among men in the Health Professionals Follow-up Study and women in the Nurses' Health Study, 1994-2006.

	Aspartame[1]						
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**p for Trend[2]**	**p for Heterogeneity[3]**
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	95	55	65	49	69		
RR (95% CI): simple model[4]	1.00	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[5]	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	172	114	110	91	86		
RR (95% CI): simple model[4]	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[5]	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							
RR (95% CI): multivariable[5]	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)[f]	0.12	0.006
Multiple Myeloma							
Men							
cases	10	17	11	14	13		
RR (95% CI): simple model[4]	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[5]	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	45	14	25	25	15		
RR (95% CI): simple model[4]	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[5]	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								
RR (95% CI):	multivariable[5]	1.00	0.86 (0.53,1.41)[f]	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		23	14	23	19	18		
RR (95% CI):	simple model[4]	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[5]	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		34	21	32	21	21		
RR (95% CI):	simple model[4]	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[5]	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								
RR (95% CI):	multivariable[5]	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

RR = relative risk; CI = confidence interval

[1] aspartame intake is from diet soda and from packets used at the table; categories are for zero intake plus quartiles of intakes greater than zero

[2] test for linear trend using median values within each category of aspartame intake

[3] test for heterogeneity between linear models for men and women

[4] adjusted for age and questionnaire cycle

[5] 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)

[6] $p < 0.05$ in test for heterogeneity between relative risks for men and women in the same aspartame category

We used Cox proportional hazards models to compute RRs, 95% CIs, and P for trends. We tested for heterogeneity between main results for men and women using the random effects method of DerSimonian and Laird.

Table 4. Multivariable relative risks of non-Hodgkin lymphoma, multiple myeloma, and leukemia by frequency of regular sugar-sweetened soda consumption among men in the Health Professionals Follow-up Study, 1986-2006, and among women in the Nurses' Health Study, 1984-2006.

		Regular Sugar-sweetened Soda[1]					p for trend[2]	p for heterogeneity[3]
		none	< 1/week	1-3.9/week	4-6.9/week	≥ 1/day		
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	181	185	137	36	32		
	RR (95% CI)[4]	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	293	293	121	25	21		
	RR (95% CI)[4]	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)[4]	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	47	39	32	5	8		
	RR (95% CI)[4]	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	62	56	24	8	4		
	RR (95% CI)[4]	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)[4]	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	71	65	31	11	8		
	RR (95% CI)[4]	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	56	55	31	7	4		
	RR (95% CI)[4]	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)[4]	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

RR = relative risk; CI = confidence interval

[1] frequency of regular sugar-sweetened soda consumption based upon a 12 fluid ounce (355 ml) serving equivalent to one bottle, glass, or can
[2] test for linear trend using median values within each category of regular soda consumption
[3] test for heterogeneity between linear models for men and women
[4] 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)
[5] $p < 0.05$in test for heterogeneity between relative risks for men and women in the same category for regular soda

We used Cox proportional hazards models to compute RRs, 95% CIs, and P for trends. We tested for heterogeneity between main results for men and women using the random effects method of DerSimonian and Laird.