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 men who did not consume diet soda. 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 of 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* 2012;96:1419–28.

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; Nutra-Sweet Co; personal communication, 14 July 2006); the average content of aspartame in a 1-L bottle of diet cola is $\sim$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 ($\sim$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 re-interpretation 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

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 ascer-

tained 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/m²)	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

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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; discretionary 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]					P-trend[3]	P-heterogeneity[4]
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Quartile 5		
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; discretionary 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]					P-trend[3]	P-heterogeneity[4]
	None	<1 serving/wk	1–3.9 servings/wk	4–6.9 servings/wk	≥1 serving/d		
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; discretionary 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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Letters to the Editor

Vitamin supplements and mortality in older people

Dear Sir:

Macpherson et al (1) carried out a meta-analysis of multivitamin and multimineral (MVMM) tablet trials and found no effect of MVMMs on average mortality. However, their study may suffer from ecological fallacy. Ecological fallacy means that study-level (group-level) analysis can lead to different conclusions than do corresponding individual-level analyses (2). For this reason, examination of individual-level data is recommended, whenever feasible, to avoid the potential for the ecological fallacy introduced by study-level analyses (2).

Macpherson et al (1) calculated that the average age of the participants in the studies was 62 y. However, ages ranged from 17 to 86 y in the included trials (1). It is probable that the effects of all vitamins and minerals are not identical at the lower and upper ends of such a wide age range. Therefore, pooling diverse trials with young and old people to a single average MVMM effect may camouflage effects of some individual vitamins or minerals, for example, on the oldest people. In the case of vitamin E there is strong empirical evidence of effect modification by age.

In an individual-level analysis of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study data, we found that among participants aged 50–62 y at baseline with a dietary vitamin C intake above the median, vitamin E increased mortality by 19% (95% CI: 5%, 35%; based on 1021 deaths). However, among participants aged 66–69 y at baseline with a dietary vitamin C intake above the median, vitamin E decreased mortality by 41% (95% CI: 21%, 56%; based on 195 deaths) (3).

Furthermore, because the follow-up time in the ATBC Study was up to 8 y, the participants became substantially older during the trial so that the baseline age was not a proper way to characterize them over the entire follow-up period. Therefore, the modification of vitamin E effects was also analyzed by using the follow-up age as the time variable (4). Among 10,837 ATBC Study participants who contributed follow-up time past the age of 65 y, the survival curves of the vitamin E and no–vitamin E participants significantly diverged at 71 y. Vitamin E extended life span by ∼0.5 y at the upper limit of the follow-up age span (4).

Macpherson et al (1) write that in a meta-regression the estimate of the effect of MVMMs was not associated with the duration of supplementation. In the ATBC Study, the harm from vitamin E in the young participants was restricted to the supplementation period after 3.3 y, indicating that there can be a lag period of several years before the effects of some vitamins appear (3). Macpherson et al used the study-level average durations, which provide a poor basis for analyzing supplementation time–dependent effect modifications. Proper analysis of time-dependent effects requires individual-level data.

It is possible that some vitamins and minerals are beneficial for specific subpopulations. For example, age, sex, smoking, diet, and exercise might modify the effects of some vitamins and minerals, so that some restricted population groups might benefit (and some might be harmed). Such subgroups can be explored by analyzing individual-level data, whereas pooling study-level averages provides no information on relevant narrow subpopulations.

The meta-analysis by Macpherson et al (1) is important in discouraging ordinary middle-aged people from taking MVMMs. Nevertheless, their study should not be interpreted as evidence that none of the vitamins and minerals included in the MVMM tablets have effects on males and females in the age range of 17–86 y. It is possible that some vitamins, such as vitamin E, are useful for restricted groups of older people. Individual-level data analyses are needed for exploring such a possibility.

The author did not declare any conflicts of interest.

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Reply to H Hemilä

Dear Sir:

We thank Hemilä for his interest in our article entitled "Multivitamin-multimineral supplementation and mortality: a meta-analysis of randomized controlled trials" (1). Our primary finding was that, across a pooled sample of 91,074 participants, multivitamin-multimineral

(MVMM) supplementation had no significant effect on the risk of all-cause mortality, mortality due to cancer, or mortality due to cardiovascular disease.

Despite our overall finding, Hemilä asserts that some vitamins and minerals may be beneficial for specific subpopulations. We concur with his suggestion that variables such as age, sex, and lifestyle factors might modify the effects of some vitamins, such that differential effects may emerge in different subpopulations. However, as pointed out by Hemilä, we were unable to perform subanalyses to examine the modifying effect of these different variables given that only trial-level data were available.

If individual-level data were accessible we could have performed any number of subanalyses. A limitation of this approach is that each subanalysis involves an additional statistical comparison and thus a greater risk of a type I error. Furthermore, subgroup analysis based on post hoc examination of data can lead to erroneous conclusions (2). The findings discussed by Hemilä, relating to vitamin E mortality risk across different age groups, still require replication for this reason. To avoid these issues, we used a limited number of prespecified analyses to determine the overall effects of MVMM supplementation in the general population, rather than in specific subpopulations.

Our results were strengthened by the large number of trials included in our analyses, generating a large pooled sample size. Although there are several advantages to undertaking an individual-level data meta-analysis, such an analysis is not always feasible. For example, we excluded 7 relevant trials from our analysis simply because trial-level data were unobtainable. Given the difficulty in obtaining raw data from chief investigators (especially when many of the trials included in our analysis were more than a decade old), undertaking a patient-level meta-analysis would have further diminished the number of trials included in our analysis.

Hemilä states that our meta-analysis is "important in discouraging ordinary middle-aged people from taking MVMMs." We are not sure how this conclusion was derived from our work given that our meta-analysis did not specifically focus on middle-aged adults. Moreover, whereas we found no effect of MVMMs on mortality across adults of all ages, this does not rule out other possible benefits to health or well-being.

Before our investigation, information on the association of MVMM use and mortality had frequently been obtained from observational studies (3). Our meta-analysis showed that, across randomized controlled trials, MVMM supplementation had no effect on mortality (1). Although we acknowledge that vitamins may have different effects in different subpopulations, it was first necessary to investigate the overall effects of MVMM supplementation in the general population. Identifying a harmful effect of MVMM use across all adults would have shown greater implications than identifying a harmful effect in one of many narrow subgroups. As discussed in our meta-analysis, we call for further research into the effects of MVMM use on all aspects of human health (1). This includes examination of MVMM use in specific subpopulations.

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Limitations to the use of plasma osmolality as a hydration biomarker

Dear Sir:

In some laboratories, plasma osmolality (P_{osm}) is used as the gold standard for detecting dehydration (1), without consideration of its limitations; however, published data dispute this technique (2, 3), which prompts us to write in response to the recent article by Cheuvront et al (4) with regard to quantitative dehydration assessment. This article correctly states that P_{osm} is the key regulated variable in fluid balance, which means that P_{osm} is constantly regulated toward a central set point as the kidneys modify urine concentration and water excretion in response to diet and daily activities. We believe that this controlled regulation limits the efficacy of P_{osm} as an index of hydration change in many experimental designs. This article (4) also states that the "criticisms for adopting P_{osm} as a gold standard for dehydration assessment are minimal" (p 460). We disagree and write to describe several limitations to the use of P_{osm} as a gold standard for dehydration.

First, individuals who lose a large amount of body water (reported as % body mass loss relative to a beginning euhydrated state) may exhibit a decreased P_{osm}, contrary to anticipated hemoconcentration. For example, a summary of 2 studies (5) reported that the P_{osm} of 6 individuals (out of 39) decreased after they lost 3–8% of body mass. In a different study, men and women who consumed a 500-mL bolus of fluid acutely exhibited an increased P_{osm}, contrary to anticipated hemodilution (1); that is, after 90 min of rest, 4 of 30 P_{osm} measurements increased. These values show that P_{osm} may not reflect widely accepted physiologic principles, and that variance of P_{osm} measurements may be large.

Second, evidence suggests that P_{osm} changes are time- and protocol-specific. Unpublished observations (CX Muñoz, EC Johnson, JK DeMartini, et al, 2012) show that dehydration equivalent to 2% of body mass resulted in P_{osm} changes that were twice as large during mild cycling exercise (2.3 h; ΔP_{osm} of 9 mOsm/kg) compared with a passive exposure (5.0 h; ΔP_{osm} of 4 mOsm/kg); participants consumed no water during either trial in

a 36°C environment. It is likely that this difference occurred because exercise increased intracellular osmolality (6) and increased extracellular fluid tonicity, causing water to move into muscle tissue.

Third, Kenney et al (3) reported that mean (±SE) P_{osm} values in 7 resting, euhydrated young male subjects decreased from 281 ± 3 at baseline to 276 ± 2 mOsm/kg at 60 min after they had consumed 1.9 L of water. However, the mean P_{osm} value returned to baseline (282 ± 2 mOsm/kg) at 90 min postingestion. These findings challenge our understanding of the interactions between intracellular-extracellular fluid shifts (6) and renal compensatory mechanisms; they also suggest that further research into the time course of acute P_{osm} changes is warranted.

Fourth, 2 recent publications (7, 8) showed that a single P_{osm} or serum osmolality measurement was a poor predictor of changes in hydration status when a single, fasted morning blood sample is collected. The former article (7) involved modified fluid intake in habitually low-volume drinkers and habitually high-volume drinkers, with the outcome that P_{osm} was constant across days in men and women, whereas urinary biomarkers reflected modified water consumption. The latter publication (8) showed that serum osmolality was a poor predictor ($r^2 = 0.01$) of 24-h water retention-clearance by the kidneys. Furthermore, the NHANES (1988–1994) reported that serum osmolality values were constant across a wide range of fluid intakes (9). Men exhibited similar mean P_{osm} values (range: 279–281 mOsm/kg) regardless of total daily fluid intake, which ranged from 1.7 to 7.9 L; women exhibited similar P_{osm} values (range: 276–278 mOsm/kg) across a total daily fluid intake range of 1.3–6.1 L. These studies argue that P_{osm} is not appropriate in clinical settings, in which a single blood sample is collected during an office visit.

Furthermore, Cheuvront et al (4) recommended that a P_{osm} value of 301 ± 5 mOsm/kg be used clinically as the threshold of dehydration (p 460), as determined statistically. However, previously published data (10) show that a P_{osm} value of 301 ± 5 mOsm/kg represents a body mass loss of ~4.5% in healthy, young males; this marked level of dehydration is hardly a threshold for dehydration.

Finally, serum samples contain numerous substances (eg, sodium, chloride, potassium, bicarbonate, urea, glucose) that constitute 95% of total osmolarity. Even though they are found in small amounts (4–5%), proteins influence total osmolality considerably. Thus, the water content in a serum sample is less per unit volume than in a calibration solution, and to obtain an accurate measurement of osmolality, the empirical value should be mathematically corrected. Furthermore, normal intraindividual differences in serum protein concentration (range: 6.0–8.5 g/dL) and within-individual changes in serum protein concentration induced by factors such as physical training and heat acclimation (11) increase the statistical variance and difficulty of interpreting the meaning of P_{osm} as a hydration index.

We recommend that scientists use P_{osm} as a marker of dehydration cautiously, with careful consideration of experimental protocol (ie, dehydration compared with hypohydration, exercise compared with rest) and tight control of dietary total osmolar load and fluid volume (2, 8, 10). We recommend that P_{osm} not be used in clinical settings as a gold standard for dehydration assessment (2, 7, 8). The limitations (described above) reflect the dynamic and complex regulation of human fluid-electrolyte balance (2), which does not lend itself to generalizations.

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Reply to LE Armstrong et al

Dear Sir:

We have great respect for the authors who have expressed interest in our article, and we appreciate the opportunity to reply to their letter; however, we find little convincing evidence for their concerns.

First and foremost we wish to emphasize 2 important points from our article that were left out of the quote taken from page 460 (1). We were very careful in our review to outline why plasma osmolality (P_{osm}) should be considered a gold standard for assessing dehydration, defined as intracellular dehydration (or hypertonic-hypovolemia), and not extracellular dehydration (or isotonic-hypovolemia or volume depletion). We also point out the criticality of considering the dehydration magnitude. With these 2 very important points in mind, the criticisms that we describe as "minimal" on page 460 relate directly to articles that have neglected these important points in their misguided assertions about the limitations of using P_{osm} for assessing dehydration.

The criticisms of our review on dehydration assessment seem to involve 3 major points: *1)* disparate research findings, *2)* a P_{osm} threshold of 301 $\pm$ 5 mmol/kg for dehydration, and *3)* the contribution of protein to P_{osm}.

Disparate research findings

Six published articles or reports were used when trying to refute our review. Curiously, only 2 of those studies were designed to produce dehydration and only one directly described the potential for using P_{osm} to quantify dehydration (2). Although the remaining studies referenced do describe the normal, and extremely well-documented, physiologic response to both normal and overconsumption of water (water intake $\geq$ water losses), when carefully read they do not in any way refute the perspectives presented in our article. As a matter of interpretation, we would also suggest that the composite figure from Sawka et al (2) shows that P_{osm} responded to dehydration exactly as expected in 33 of 39 volunteers (85%). In a recent study from our laboratory (3) in which baseline values were very carefully controlled, P_{osm} increased in 36 of 36 volunteers (100%) who became dehydrated by 2.2–5.8% of body mass via sweating (exercise-heat stress). Nowhere in our article do we generalize or make claims that P_{osm} is perfect. We do argue, however, that P_{osm} is the best currently available assessment measure (gold standard) for one specific type of dehydration (intracellular).

P_{osm} threshold of 301 $\pm$ 5 mmol/kg

A full appreciation for the genesis of the 301 $\pm$ 5-mmol/kg threshold for dehydration requires knowledge of biological variation and diagnostic decision making, which goes well beyond the scope of this letter. We encourage interested readers to seek Cheuvront et al (1, 4, 5) for details. Briefly, the nosological sensitivity of P_{osm} is modest but superior to all other common body fluids used to assess dehydration. When the variance term for P_{osm} is properly considered, the range of P_{osm} values that indicate dehydration ($\geq$2% body mass) agree extremely well with many independently published observations and commonly accepted clinical thresholds for dehydration (4). Change values are better when it is practical to make 2 measures, but here again P_{osm} does extremely well (4, 5). The ΔP_{osm} remains sensitive even when water loading is used (urine osmolality:P_{osm} <1.5).

This practice is often adopted in research where "assurance" of euhydration is desired; however, it is important to recognize that it also decreases the nosological sensitivity of the 301 $\pm$ 5-mmol/kg threshold (4). Under said circumstances, a + 5-mmol/kg change in P_{osm} still affords 80% probability that intracellular dehydration has occurred (4, 5), which is remarkably consistent with the well-taught osmotic change threshold ($\sim$2% or +6 mmol/kg) for renal compensation and water acquisition (thirst) (1).

Contribution of protein to P_{osm}

In all of our articles on P_{osm} (1, 3–5), and more in press or forthcoming, we recognize and discuss its complexity. A reduction in plasma water increases the concentration of all dissolved substances. It is, of course, well known that plasma protein concentration increases linearly as plasma water is reduced (6). When assessing the potential for dehydration, the question can only be "why" it increases. The concentration of P_{osm} reflects the loss of water from the plasma and it describes the loss of body water very well (3). Both inter- and intraindividual variation in plasma protein concentrations are already a part of inter- and intraindividual P_{osm} variation (4). Therefore, plasma protein variation is already taken into consideration in the 301 $\pm$ 5-mmol/kg threshold. Thus, unless there is good reason to believe that circumstances have produced a grossly disproportionate increase in protein beyond that expected from plasma water loss, there would be no need for corrections. Studies from our laboratory and Senay's pioneering research have shown that plasma protein can be added by heat exposure as well as lost with dehydration. We acknowledge that some flux of total circulating proteins occurs, but as previously stated such protein fluxes are already part of the observed variance and diagnostic error. Any acute influence of protein flux due to exercise would also be remedied by allowing proper recovery (1). In other words, the potential for plasma protein to confound the appropriate use of P_{osm} for assessing dehydration is marginal at best.

In our review article (1), we carefully described the true limitations of using P_{osm} for dehydration assessment on page 460. The concerns expressed in the letter by Armstrong et al are clearly but curiously misplaced. We must therefore regard the limitations inferred by the title of their letter as "false."

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No and low alcohol intake may have differential effects on risk of overall and cause-specific mortality

Dear Sir:

We read with great interest the article by Vergnaud et al (1) on the relation between adherence to the World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR) guidelines and risk of death in Europe. This well-crafted, large-scale study conducted in participants in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort offers valuable data regarding the impact of the WCRF/AICR recommendations on reducing total and cause-specific mortality and suggests that the utility of these guidelines may extend beyond the scope of cancer prevention. We are, however, keen on gaining additional understanding of the results presented in their Table 4: namely, the risk of death associated with alcohol consumption.

The authors found that adherence to the WCRF/AICR recommendation for daily alcohol intake (≤2 drinks for men and 1 drink for women) was protective against all-cause mortality in men but not in women. This result was based on a scoring system that operationalized this alcohol-specific guideline into 3 categories of ethanol intake: ≤20, >20 to ≤30, and >30 g/d for men and ≤10, >10 to ≤20, and >20 g/d for women. Among the 257,421 male study participants, the men whose ethanol intake was >20 to ≤30 g/d had a significantly reduced risk of death compared with men whose consumption exceeded 30 g/d (HR: 0.80), as did men who limited their intake to ≤20 g/d compared with the same referent (HR: 0.89). However, significant associations between risk of death and the alcoholic drinks component of the WCRF/AICR recommendations were not observed among the 121,443 female study participants.

We are highly curious both to learn whether making the distinction between no and low ethanol intake would alter the results of this analysis and to see the stratification of HRs by cause of death. Whereas it is widely acknowledged that, unlike in cardiovascular disease, the lowest alcohol-related cancer risk is in fact conferred in the absence of alcohol consumption (2), there remains uncertainty regarding whether the protective effect of abstinence on cancer risk translates to survival outcomes. The most current estimate of alcohol-attributable cancer mortality in the United States to our knowledge suggests that alcohol consumption at any level not only increases cancer risk but, more critically, is a major factor behind cancer-related death in men and women (3). Interestingly, the number of alcohol-attributable deaths was highest for female breast cancer in this investigation. A meta-analysis by Bagnardi et al (4) that included 222 articles concerning alcohol consumption and cancer found that light alcohol drinking (≤1 drink/d) was associated with breast cancer death. In contrast and illustrative of the ambiguity related to drinking and cancer mortality, another recent study reported that any alcohol consumption either before or after breast cancer diagnosis had no adverse impact on survival from breast cancer, cardiovascular disease, or other cause, and that moderate consumption may even have a survival benefit (5).

The robust data set of Vergnaud et al presents an opportunity for additional analyses that could shed further light on the advantages or lack thereof of teetotaling in the prevention of cancer or other chronic diseases. As such, we appreciate the authors' consideration of our request that they both reoperationalize the alcohol-specific WCRF/AICR score such that 0 g/d of ethanol intake is assigned its own category and evaluate alcohol-specific mortality by cause of death and share these results.

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Reply to E Falk Libby et al

Dear Sir:

We thank Falk Libby et al for their interest in our article. We acknowledge the need for more detailed analysis of the association between individual components of the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AIRC) score, including alcohol consumption and cause-specific mortality. The association between pattern of lifetime alcohol use and cause of death in the European Prospective Investigation into Cancer and Nutrition (EPIC) study has been addressed in detail by Manuela M Bergmann et al in a manuscript currently under submission. Results cannot be displayed before publication, so we encourage Falk Libby et al to pay attention to the release of this article, which will provide a comprehensive answer to their requests.

None of the authors had a conflict of interest.

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On behalf of the EPIC coauthors

The challenge of complexity and arginine metabolism

Dear Sir:

Chapeau! to Mariotti et al (1) for their attempt to put order to complexity by giving a dimension to arginine fluxes in its metabolism.

But, complexity is both a challenge and a burden. An important question relates to the lack of computation of the possible effects that arginine-derived and naturally produced inhibitors of enzymes dealing with arginine metabolism, such as asymmetric-di-methyl-arginine (ADMA), may have on peripheral tissue activity of arginases (2). Do the authors have data on acute effects of arginine ingestion on ADMA? Indeed, it has been reported that long-term ingestion of arginine supplements increases ADMA (3) and inhibition of arginases was efficient in maintaining nitric oxide (NO) production and in preventing damage related to impaired NO production in peripheral tissues (4).

Also, the expression and activity of arginases, and thus their contribution to plasma and urea by red blood cells, were not sufficiently stressed by Mariotti et al in their text or in the supplemental data. Peculiarly, in capillaries red blood cells may dramatically control and blunt arginine concentrations in plasma (5, 6) and this should also be included in a model that focuses on clusters of peripheral needs, even if the said model groups together sums of activities by different compartments. Moreover, habitual dietary arginine intake by controlling arginase expression may rule fluxes of arginine toward availability for protein syntheses or catabolism producing urea. Urea production may become misleading in evaluating adequate nitrogen intake if this is calculated on the basis of urinary urea excretion (7).

The author did not declare any conflicts of interest.

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Reply to FS Dioguardi

Dear Sir:

We appreciated the congratulations and comments received from Dioguardi regarding our recently published article, which was the first attempt to delineate the metabolism of dietary arginine, including its bioavailability and utilization for the competitive pathways that are arginase and nitric oxide (NO) synthase (1). The objective of model development was to determine the minimal structure for this nutritional system that could solve the isotopic metabolic data at hand and provide an insight into the key metabolic/compartmental structuring that explains how the body deals structurally with arginine intake.

According to the design and process of this modeling study, the effects of any potential changes in arginase or NO synthase activity during the postprandial phase (the potential existence of which was suggested by Dioguardi) are embedded in the isotopic (urea and nitrate) metabolic data and are therefore "computed" in the model predictions for the fluxes of urea and NO production. In the model, both urea and NO production indeed originate from both a plasma compartment and another compartment that aggregates all other possible sources of arginine entry into the NO synthase and arginase pathways. Because the parsimony principle was applied when developing the

model, we selected the minimum structure that would include just the main features of the system to reduce model complexity to a manageable level (2, 3), and we did not represent all of the compartments of physiologic interest, such as the red blood cells mentioned by Dioguardi. In other words, a higher-order model with a more detailed structure was not required to analyze the data and the main features of the system. As Dioguardi will understand, this does not mean that red blood cells are not physiologically important with respect to arginase activity, and, as he suggested, peripheral arginase activity, which we estimated mainly as "urea synthesis from plasma dietary arginine," may in part be ascribed to this specific compartment. However, once again, any contribution of red blood cells to the dynamics of postprandial arginine metabolism is both embedded in the data and solved by the model. Of course, our model, like all models, remains a simplification of the system but has proved to be the simplest way to understand the dynamic behavior of the arginine nutritional system.

To answer the direct question posed by Dioguardi with regard to plasma asymmetric-di-methyl-arginine (ADMA), we do have these data on effects after the ingestion of arginine in this setting, and we did not observe that plasma ADMA changed after ingestion (4). Of note, Dioguardi cited a reference that reported an increase in plasma ADMA with long-term arginine supplementation, whereas our results, and those of other groups, indicated no increase in different populations and at different doses (eg, 5–9).

However, from a general standpoint, we agree that little is known about the possible changes in arginine metabolism with regard to NO compared with urea in individuals given large amounts of arginine over the long term, and that changes in arginase activity have emerged as a critical determinant of arginine-NO homeostasis and vascular health (10). Our study was not designed to address these potential long-term effects or to analyze the related underlying possible mechanisms. By using the integrative methodology detailed here, future studies may be able to investigate whether, and to what extent, the key parameters of the system are affected by a long-term increase in arginine intake and should also be able to determine how the system is altered in prepathological conditions (such as with the metabolic syndrome) and in different dietary and nutritional situations.

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Describing a taxonomy of cognitive processes for clinical trials assessing cognition

Dear Sir:

Stonehouse et al (1) reported that DHA supplementation improved both memory and reaction time in healthy, young adults. This randomized, placebo-controlled, double-blind clinical trial had many strengths and was, for the most part, technically sound. However, we question the atheoretical manner in which the cognitive tests were grouped into broader cognitive abilities.

In an accompanying editorial, Dangour and Allen (2) questioned the applicability of the cognitive tests used by Stonehouse et al (1). They stated that considerable variability exists in the cognitive tests used between clinical trials and that this significantly hampers comparisons between studies (2). Dangour and Allen proposed that experts in the field should urgently agree on a set of cognitive tests to be used consistently across clinical trials (2). We agree that efforts need to be made to facilitate cross-study comparisons. Yet, consensus as to a standardized set of cognitive tasks is unlikely to be agreed on given the plethora of cognitive tests available and the fact that individual preferences for specific cognitive tests vary greatly. Moreover, because different cognitive tests are suited to different populations and interventions, cognitive tests are often appropriately selected on a case-by-case basis. We propose a less radical solution to aid cross-study comparisons in this area.

Even if researchers cannot agree on the cognitive tests used, consensus should be reached on the types of cognitive functions that exist. This would then enable reviewers and readers of published studies to better understand the scope of the tests chosen against the full spectrum of cognitive processes that have been reliably discovered. At present, many clinical trials combine cognitive tests into broader cognitive abilities without justification from existing literature or factor analytic investigation. This appears to be the case in the study by Stonehouse et al (1), whereby cognitive tests are combined into cognitive domains of episodic memory, working memory, attention, and processing speed without explicit justification for this grouping. This significantly hampers comparisons between studies because the cognitive composites are seemingly arbitrary and may never be created again in the same way. We suggest that a standardized and evidence-based approach to grouping cognitive test data will aid comparisons between studies. An empirically supported model for grouping cognitive test data already exists but seems to be ignored by the field of clinical nutrition.

On the basis of 70 y of factor analytical work on cognition, Carroll (3) published a seminal book on human cognitive abilities. Through extensive factor analysis of >460 data sets, his work provides a solid empirical and science-based approach to better understanding the structure of cognition. Such is the significance of this publication to the area of applied psychometrics that it has been compared in importance to Sir Isaac Newton's *Mathematical Principles of Natural Philosophy* (4).
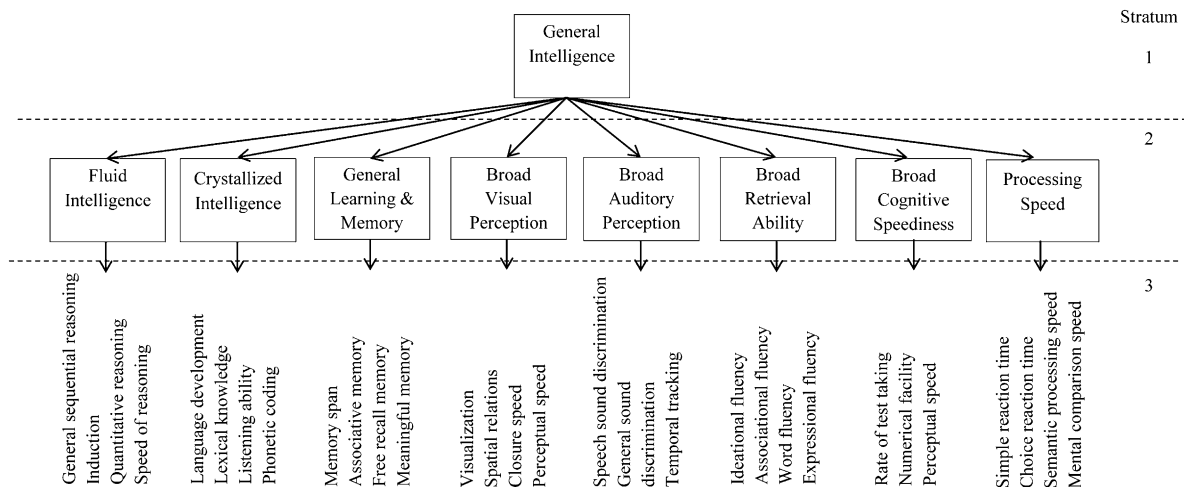

FIGURE 1. The structure of cognitive abilities based on the work of Carroll (3). Note that the figure is designed to give a snapshot of the model and only some of the 69 narrow cognitive abilities are shown. Adapted with permission from Cambridge University Press.

Carroll's work provides an empirically verified taxonomy of human cognitive abilities (4). In essence, Carroll (3) outlined a 3-strata hierarchical model of cognitive ability (**Figure 1**). At the broadest level, stratum 3 consists of a general intelligence factor, which subsumes the following 2 strata. The second stratum includes 8 broad cognitive abilities. Stratum 1 includes a group of 69 narrow, well-defined abilities. All of the cognitive abilities can be classified as belonging to one of the following domains: language, reasoning, memory and learning, visual perception, auditory perception, idea production, cognitive speed, knowledge and achievement, and miscellaneous abilities (3). These cognitive abilities can also be broken down into additional narrow abilities. For example, memory and learning can be further broken down into associative memory, meaningful memory, free recall memory, visual memory, and learning abilities. It is easy to group cognitive test scores into these "true" cognitive abilities because the taxonomy was derived through extensive factor analysis of existing cognitive tests used throughout the past century. Carroll also provides descriptions of each cognitive ability. We therefore suggest that researchers use this taxonomy to group cognitive test score data or at least report how their measures map onto this framework. This will allow significantly better comparison across clinical studies assessing cognition.

The findings reported by Stonehouse et al (1) are of great interest, but as pointed out by others, heterogeneity in cognitive outcomes between studies is significantly limiting advancements in this field. It is surprising that researchers continue to group cognitive tasks into seemingly arbitrary cognitive abilities when a comprehensive evidence-based approach exists. Carroll's work provides "a common nomenclature for professional communication" (4). From a practice perspective, this nomenclature allows for comparison and grouping of cognitive tests across studies. This cognitive taxonomy is widely accepted and used in the field of psychology, and we suggest that it also be appropriately applied in clinical trial research.

Neither of the authors had a conflict of interest.

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Reply to MP Pase and C Stough

Dear Sir:

In an editorial commenting on our recently published study (1), which showed beneficial cognitive effects as a consequence of 6 mo of supplementation with DHA in healthy, young adults, Dangour and Allen (2) expressed major concerns over the heterogeneity of the tests being used to assess the cognitive function of adults in clinical trials. They illustrated their point by noting that a wide selection of cognitive tests had been used across the 10 relevant studies published in the Journal in 2011–2012, and that no 2 studies had adopted the same primary endpoints. What they failed to mention was that only one of these studies used any form of computer-administered cognitive testing. The other studies collected data in written or verbal form. In our own case, we used a sophisticated computerized cognitive assessment system (COMPASS; Northumbria University) that was purpose designed to deliver multiple, parallel versions of a wide range of classic, standard, and bespoke cognitive tasks. The tasks used in the study were then chosen with reference to the recommendations of the European Food Safety Authority's recent guidance on the cognitive tests that are suitable for assessing the effects of nutritional interventions (3) and previous work in this area by an expert panel under the auspices of the International Life Sciences Institute (4). The potential benefits of assessing cognitive function with a computer are self-evident and include the collection of accurate information on the speed of performing tasks and responding to stimuli. This information represents a fundamental measure of brain function and is always either equally informative or complementary to information on the accuracy of task performance. Beyond this, on a purely practical level, computerized testing also allows the standardized presentation of properly randomized stimuli, it removes the person-to-person interactions with a researcher that can bias and obfuscate data, and it allows the closely controlled collection of a large amount of data within a short period of time. We are literally surrounded in our everyday lives by powerful personal computers, and computerized cognitive testing can be readily adopted both in the laboratory and in more ecologically valid environments. Given the above, it is somewhat baffling that our own study was picked out for the editorial observations on the heterogeneity of testing across the field.

Dangour and Allen concluded their editorial by suggesting that experts in cognitive testing urgently need to reach a consensus on a small set of outcomes to use across future trials. Pase and Stough, in response, suggest that because consensus in this regard is unlikely, Carroll's "Three Stratum Theory" (5) could provide a taxonomy for cognitive processes that could then inform a "standardized and evidence based approach to grouping cognitive test data." By Pase and Stough's account, our own "atheoretical" collapsing of task outcomes into "arbitrary" composite scores (which, in reality, were based on a previous factor analysis of a similar group of tasks) could be replaced by simply grouping or describing the task outcomes from a study with reference to the 8 broad cognitive ability domains and 69 narrow, well-defined abilities in Carroll's model. Whereas this seems, on the face of it, to be a plausible suggestion, there are actually several major obstacles standing in the way of adopting this approach. From a purely practical perspective, a major problem would be deciding how a given task outcome maps onto one or more of Carroll's factor analysis–derived "abilities." Presumably, this process would require further factor analysis of multiple data sets. From a more theoretical perspective, Carroll's model could also best be described as a "work in progress." As he himself noted in his preface, the model was merely a starting point for future investigators and was formulated by looking backward. Carroll also acknowledged the inadequacy of some of the data that he had to work with. For instance,

he noted that the literature on memory and learning "leaves much to be desired" and listed the many gaps in the data that would need to be filled to arrive at a complete picture of this domain. In consequence, Carroll's model has not been the fixed and stationary taxonomy that Pase and Stough would seem to be suggesting. Rather, it has been in a continuous state of modification since its initial publication. More recently, it has, for instance, been integrated with other models and has been modified and added to as new data and analytic techniques have become available (6). As an example, up to 6 new broad cognitive ability domains have been suggested as additions to Carroll's original 8 domains (6). It is also notable that Carroll started work on his opus magnum in 1979 and worked on it for 14 y, synthesizing the findings of factor analyses from a vast body of data. Although he himself was a pioneer in the application of computer technology to his complex analyses, the data that he worked with were collected without the benefit of any such technology.

As McGrew noted recently (6), Carroll's work represented a "tipping point that provided the first working map of the human cognitive ability terrain, a terrain warranting additional exploration and refined cartographic efforts." McGrew went on to urge the integration of current and future research into the emerging taxonomy. However, in this task we still seem to be laboring, certainly within the clinical trials field, with the astrolabes, quadrants, and verniers of the early map makers. Simply adopting the ubiquitous technology of our own age would necessarily make for much more accurate mapping tools, and therefore better maps. Although I applaud the ambition of Pase and Stough's suggestion, I think the necessary first step toward their ultimate goal, and indeed greater standardization of cognitive tests, is the wider adoption of sensitive computerized testing techniques within the clinical trials field. The resulting data can then contribute to the factor-analytic process of further refining the map of human cognitive ability.

The author had no conflicts of interest.

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Erratum

Gibert A, Kruizinga AG, Neuhold S, Houben GF, Canela MA, Fasano A, Catassi C. Might gluten traces in wheat substitutes pose a risk in patients with celiac disease? A population-based probabilistic approach to risk estimation. Am J Clin Nutr 2013;97:109–16.

Because of a copyediting error, data are missing in Table 3 for "Distribution" under "Scenario 3." In the first 2 columns, under "Combination of the 4 countries," the "Mean ± SD" value should be 0.18 ± 0.04, and the "95th Percentile" value should be 0.24.

Erratum

Kien CL, Bunn JY, Tompkins CL, Dumas JA, Crain KI, Ebenstein DB, Koves TR, Muoio DM. Substituting dietary monounsaturated fat for saturated fat is associated with increased daily physical activity and resting energy expenditure and with changes in mood. Am J Clin Nutr 2013;97:689–97.

On page 693, the second sentence in the third paragraph of the Results section contains a copyediting error in which the word "or" was mistakenly used: "15 or 17 subjects" should read "15 of 17 subjects" instead.

Erratum

Clemente-Postigo M, Queipo-Ortuño MI, Boto-Ordoñez M, Coin-Aragüez L, Roca-Rodriguez MdM, Delgado-Lista J, Cardona F, Andres-Lacueva C, Tinahones FJ. Effect of acute and chronic red wine consumption on lipopolysaccharide concentrations. Am J Clin Nutr 2013;97:1053–61.

On page 1053, footnote 2 should include the following additional funding information: "The study was also supported by CP07/00095 from the ISCIII, and MdMR-R was a recipient of a fellowship from ISCIII (Rio Hortega CM11/00030), Spanish Ministry of Economy and Competitiveness, Madrid, Spain."

doi: 10.3945/ajcn.113.066357.

Erratum

Schernhammer ES, Bertrand KA, Birmann BM, Sampson L, Willett WC, Feskanich D. Consumption of artificial sweetener– and sugar-containing soda and risk of lymphoma and leukemia in men and women. Am J Clin Nutr 2012;96:1419–28.

The supplemental data for this article were inadvertently missed during production and were therefore not posted online. The supplemental data file (Table 1) is now available online.

doi: 10.3945/ajcn.113.066365.

Erratum

Wilkinson SB, Tarnopolsky MA, MacDonald MJ, MacDonald JR, Armstrong D, Phillips SM. Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. Am J Clin Nutr 2007;85:1031–40.

On page 1039, an error appears in the legend to Figure 5. The solid circle line should represent skim milk, and the open circle line should represent the soy-protein beverage. The first sentence of the figure legend should read as follows: "Mean (±SEM) total amino acid (TAA) chemical net balance (NB) after consumption of a nonfat milk-protein beverage (●) or an isonitrogenous, isoenergetic, macronutrient-matched (750 kJ, 18.2 g protein, 1.5 g fat, and 23 g carbohydrate) soy-protein beverage (○)."

doi: 10.3945/ajcn.113.067389.