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One-Carbon Metabolism–Related Micronutrients Intake and Risk for Hepatocellular Carcinoma: A Prospective Cohort Study

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Abstract

Deficient intake of micronutrients involved in one-carbon metabolism (e.g., choline, methionine, vitamin B₁₂, and folic acid) leads to hepatocellular carcinoma (HCC) development in rodents, but it is under-investigated in humans. We investigated the association between one-carbon metabolism–related micronutrient intake and HCC risk in a prospective cohort of 494,860 participants with 16 years of follow-up in the NIH-AARP study. Dietary intakes and supplement use were ascertained at baseline using a food-frequency questionnaire. Total intake (diet plus supplements) of the following one-carbon metabolism–related micronutrients were calculated: folate, methionine, and vitamins B₂ (riboflavin), B₃ (niacin), B₆, and B₁₂. These micronutrients were examined both individually and simultaneously, with adjustment for covariates. Cox proportional hazard models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs). Over the 16-year follow-up period, 647 incident HCC cases were diagnosed. When examined individually, higher total vitamin B₃ intake was associated with a lower HCC risk (HR_{Q5vsQ1}=0.60; 95% CI=0.42–0.85; *P*_{trend}=0.008), and the association remained significant when all six micronutrients were examined simultaneously (HR_{Q5vsQ1}=0.32; 95% CI=0.18–0.55; *P*_{trend}<0.0001). Among participants with >3 years of follow-up, higher total vitamin B₃ intake was again associated with lower risk (HR_{Q5vsQ1}=0.37; 95% CI=0.20–0.68; *P*_{trend}=0.001), whereas higher total vitamin B₆ intake was associated with higher risk (HR_{Q5vsQ1}=2.04; 95% CI=1.02–

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The authors declare no potential conflicts of interest

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4.07; $P_{\text{trend}}=0.04$). Restricted cubic spline analyses showed a dose-response inverse association between total vitamin B₃ intake and HCC risk, and dose-response positive association between total vitamin B₆ intake and HCC risk. The study suggests that higher vitamin B₃ intake is associated with lower HCC risk, whereas higher vitamin B₆ intake is associated with increased risk.

Keywords

cancer; hepatocellular carcinoma; HCC; one-carbon metabolism; micronutrients; risk

Introduction

Hepatocellular carcinoma (HCC) is a frequently fatal primary malignancy of the liver, with an estimated 5-year survival rate of about 18% in the United States.^{1, 2} The major risk factors of HCC in the United States include metabolic syndrome, nonalcoholic fatty liver disease (NAFLD), chronic hepatitis B and C virus (HBV and HCV) infections, alcoholic liver disease, pre-existing diabetes mellitus, and obesity.³ Despite these well-established HCC risk factors and efforts aimed at reducing exposure to these risk factors, the incidence and mortality of HCC are both projected to increase in several population subgroups in the United States.^{4, 5} Current efforts aimed at addressing the increasing incidence of HCC in the United States include the now widespread use of direct-acting antiviral agents for treatment of HCV which can lead to viral clearance in more than 90% of HCV-affected patients,⁶ reducing exposure to HCV among intravenous drug users through needle exchange programs,⁷ and increasing public awareness of the link between HCC and metabolic syndrome, which is potentially modifiable and particularly prevalent among Hispanic Americans and non-Hispanic whites.³ However, there remains a need to identify additional modifiable risk factors of HCC to improve strategies for risk prevention.

Animal studies have implicated deficient intake of micronutrients involved in one-carbon metabolism in the development of HCC.⁸⁻¹¹ One-carbon metabolism involves the transfer of methyl groups derived from both dietary sources and endogenous biosynthesis for the formation of the universal methyl-donor, S-adenosylmethionine, which is then used for methylation of DNA and other cellular moieties.¹²⁻¹⁴ Methyl groups derived from S-adenosylmethionine for methylation reactions are obtained primarily from dietary micronutrients, such as choline, betaine, methionine, folate (vitamin B₉), and other B vitamins.¹²⁻¹⁵ Indeed, studies have shown that DNA methylation patterns are influenced directly by the availability of dietary methyl-donors.¹⁵⁻¹⁸ The importance of dietary methyl-donors in DNA methylation and hepatic tumorigenesis has been studied extensively in rodents. Mice deprived of methyl-donating micronutrients often develop altered gene expression,¹⁹⁻²² hypo-DNA methylation in liver tissue,^{10, 23} and spontaneous liver tumors.^{8, 9, 11, 19, 21} Specifically, mice fed with diets that are deficient in choline, methionine, vitamin B₁₂, and folic acid often develop liver tumors, including HCC.^{8, 9} It has therefore been suggested that similar phenomena may occur in humans with habitually low intake of methyl-donating micronutrients, but this is under investigated in humans.^{13, 14}

Our group previously examined the association between folate and alcohol intake, and risk for HCC using data from the National Institutes of Health and formerly the American Association of Retired Persons (NIH-AARP) Diet and Health Study, after 11 years of prospective follow-up.²⁴ This prior work showed that higher folate intake is associated with a less deleterious effect of alcohol consumption on HCC risk. However, the study did not include other micronutrients involved in one-carbon metabolism, besides folate. In general, few studies have investigated the association between one-carbon-related micronutrient intake and HCC risk.^{24–30} The majority of these studies were focused on a single nutrient, often folate, and the findings are mixed.^{24, 28–30} Only one prospective cohort study among Finnish male smokers²⁶ and two case-control studies among Chinese participants^{25, 27} investigated the association between one-carbon-related micronutrient intake as a functional group of biochemically-related nutrients in regards to HCC risk. To date, no study has comprehensively examined one-carbon metabolism-related micronutrient intake in relation to HCC risk in a US population. We therefore build upon our previous work²⁴ by examining associations between multiple one-carbon metabolism-related micronutrient intake and risk of HCC development in the NIH-AARP Diet and Health Study, with an additional five years of follow-up data.

Materials and Methods

Study Population

A detailed description of the NIH-AARP Diet and Health Study has been published³¹; thus, only a brief summary follows. Between 1995 and 1996, study invitations and questionnaires were sent to 3.5 million AARP members aged 50 to 71 years who resided in six states (California, Florida, Louisiana, New Jersey, North Carolina, and Pennsylvania) and two metropolitan areas (Atlanta, Georgia and Detroit, Michigan). The questionnaires sought information about respondents' demographics, socio-economic factors, anthropometric measurements, dietary habits, and several health-related behaviors. A total of 617,119 (18%) questionnaires were returned, among which 566,398 had satisfactory responses, as described in detail elsewhere.³¹ For the present study, we excluded proxy respondents (n=15,760), prevalent cancer cases (n=51,346), and individuals who reported extreme levels of total caloric intake (>2 times the sex-specific interquartile range; n=4,432). These exclusions left a final analytic sample of 494,860. All participants provided written informed consent. The study was approved by the National Cancer Institute's Special Studies Institutional Review Board.

Cohort Follow-up and Case Ascertainment

Incident cancer cases that developed during follow-up were identified through linkage with state cancer registries in the eight study catchment areas and three additional states where the cohort members tended to relocate (Arizona, Nevada, and Texas). Incident HCC cases were identified using the *International Classification of Diseases for Oncology, Third Edition* (ICD-O-3) topographic code C22.0 and morphology codes 8170– 8175.²⁴ Time to HCC development was calculated from the date the questionnaire was scanned to the date of cancer diagnosis, date of death, date last known to be alive, or date of last follow-up (December 31, 2011), whichever occurred first.

Dietary Intake and Covariates Assessment

Alcohol consumption has been shown to modulate the association between certain one-carbon metabolism–related micronutrient intake and HCC risk.²⁴ In the NIH-AARP Diet and Health Study, dietary intakes of various foods and beverages, including alcohol, were ascertained at baseline with a self-administered 124-item food-frequency questionnaire (FFQ).³² The FFQ asked participants to report usual frequency and portion sizes of various foods and beverages over the past year. Nutrient contents of the food items was determined through linkage of the FFQ responses to a food composition database maintained by the US Department of Agriculture.³² The data on dietary supplement use were obtained with the FFQ and included dose and frequency of supplement use. Intakes of the following one-carbon–related micronutrients from food sources were available for analyses: folate, methionine, and vitamins B₂ (riboflavin), B₃ (niacin), B₆, and B₁₂. For dietary supplements, data were available for all micronutrients, except methionine. Association analyses were performed for dietary intake only and for total intake (diet plus supplements). For dietary intake, each nutrient was adjusted for total calorie intake based on the residual method, with additional inclusion of total calories in the statistical models.³³ For total intake, supplemental intake was added to the corresponding dietary micronutrients after calorie-adjustment of the dietary portion. Other participant characteristics ascertained at baseline included age, sex, race/ethnicity, highest level of education, smoking history, alcohol consumption, self-reported history of diabetes mellitus, and body mass index (BMI, kg/m²).

Data Availability

The study data can be made available to researchers upon request to and approval by the NIH-AARP Diet and Health Cohort Study. For instructions on how to apply for data, researchers can visit <https://www.nihaarpstars.com/>.

Statistical Analysis

Differences in baseline characteristics were compared using means for continuous variables and proportions for categorical variables. Multivariable-adjusted Cox proportional hazard models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs). Prior to fitting the proportional hazard models, the proportionality assumption was assessed for each variable (both the main exposure variables and covariates) using the time-dependent method and were determined to have been satisfied ($P>0.05$). Each of the calorie-adjusted micronutrients was categorized into quintiles, with the lowest quintile (Q1) as the reference group. Linear trend was assessed by expressing the quintile variable as a continuous variable in the statistical models. The association analyses were performed in three ways. First, each of the micronutrients was examined separately, with adjustment for the following risk factors of HCC: age (continuous), sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other), personal history of diabetes mellitus (yes, no), smoking history (never, former, current), alcohol use (none, <1, 1–3, 3 drinks/day), total calorie intake (continuous), BMI (<25, 25–29, 30 kg/m²), highest level of education (<high school, high school graduate, some college/post-high school education, college graduate/post-graduate), frequency of physical activity (0, <1, 1–2, 3–4, 5+ times/week), multivitamin use (yes, no), red meat intake (0–23, 24–41, 42–63, 64–99, >99 grams/day), and Healthy Eating Index

scores (a marker of overall quality of diet; 10–59, 60–66, 67–71, 72–76, >76). Secondly, all six micronutrients were placed in a single model, with adjustment of the risk factors listed above. Because one-carbon metabolism–related micronutrients are thought to influence disease risk by donating methyl groups for methylation reactions,^{12–14} we also calculated a “methyl-donor index” as a composite measure of dietary methyl intake by standardizing the nutrient intake levels on the log-scale ($[\text{nutrient value} - \text{mean}] / \text{standard deviation}$) then summed across all six micronutrients, as described previously.³⁴ The methyl-donor index also was adjusted for total calorie intake using the residual method then categorized into quintiles. Each of the three sets of analyses was performed separately for dietary intake only and for total intake (diet plus supplements).

In sensitivity analyses, we restricted the study sample to participants with more than 3 years of follow-up to control for potential confounding by subclinical malignancy at baseline. In this sub-cohort, we examined associations between the one-carbon metabolism–related micronutrient intake and HCC risk in the most elaborate model that included all six micronutrients and the HCC risk factors. We also performed restricted cubic spline regression analyses with three knots for micronutrients that were found to be significantly associated with HCC risk to test for non-linear relationships with the dependent variable (HCC).³⁵ Stratified analyses also were performed in sub-cohorts defined by sex, BMI, alcohol intake, and physical activity level, and included assessment of statistical interaction on the multiplicative scale. All statistical tests were two-sided and a $P < 0.05$ was considered statistically significant. Analyses were performed with SAS v9.4 (SAS Institute, Cary, NC, USA).

Results

Over the 16-year follow-up period (1995–2011), we identified 647 incident HCC cases diagnosed among the 494,860 participants. The median time to HCC diagnosis was 9.1 years. The overall median time of cohort follow-up was 15.5 years. Table 1 presents differences in baseline characteristics by quintiles of total intake (diet plus supplements) of one-carbon metabolism–related micronutrients. The participants in the lowest quintile (Q1) of folate intake included higher proportions of men, non-Hispanic whites and current smokers and were more likely to be obese ($> 30 \text{ kg/m}^2$), whereas those in the highest quintile (Q5) were more likely to report a personal history of diabetes mellitus, multivitamin use and have a college degree. Participants in the lowest quintile of vitamin B₃ (niacin) were more likely to be current smokers and consume more than three alcoholic beverages a day but less likely to have a history of diabetes mellitus, whereas those in the highest quintile were more likely to be non-Hispanic white, report multivitamin use, and engage in physical activity three or more times a week. The participants in the lowest quintile of vitamin B₆ also were more likely to be current smokers, consume more than three alcoholic beverages a day and obese, but less likely to report a history of diabetes mellitus, whereas those in the highest quintile were more likely to report multivitamin use, have a college degree, and engage in physical activity three or more times a week but less likely to consume high amounts of red meat ($> 64 \text{ grams/day}$) and tended to have higher Healthy Eating Index scores. Other differences are presented in Table 1.

Multivariable-adjusted HRs and 95% CIs for associations between dietary intakes of one-carbon metabolism-related micronutrient and HCC risk are shown in Table 2. The micronutrients were examined both individually and simultaneously, in separate models, with adjustment for risk factors of HCC. When examined individually, higher dietary intake of vitamin B₃ was associated with a lower risk of HCC (HR_{Q5vsQ1}=0.57; 95% CI=0.44–0.72; $P_{\text{trend}} < 0.0001$). There also was a suggestion of an increased HCC risk associated with higher dietary intake of vitamin B₂ (HR_{Q5vsQ1}=1.20; 95% CI=0.94–1.54; $P_{\text{trend}} = 0.04$). When all six micronutrients were examined simultaneously, the association for dietary vitamin B₃ intake remained significant (HR_{Q5vsQ1}=0.47, 95% CI=0.36–0.65; $P_{\text{trend}} < 0.0001$), but no association was found for dietary vitamin B₂. Furthermore, higher dietary folate intake was associated with a lower HCC risk in the model that assessed all six micronutrients (HR_{Q5vsQ1}=0.73; 95% CI=0.54–0.98; $P_{\text{trend}} = 0.03$). Dietary vitamin B₆ intake also was associated with an increased HCC risk in the fourth quintile (HR_{Q4vsQ1}=1.40; 95% CI=1.02–1.92), but not the fifth quintile (HR_{Q5vsQ1}=1.42; 95% CI=0.98–2.05), with a significant linear trend across quintiles ($P_{\text{trend}} = 0.03$). Additionally, higher dietary methionine intake was associated with lower HCC risk in the highest quintile, with a non-significant linear trend (HR_{Q5vsQ1}=0.59; 95% CI=0.38–0.93; $P_{\text{trend}} = 0.11$). No association was observed for dietary vitamin B₁₂ intake or the methyl-donor index.

Table 3 shows results for associations between total intakes of the one-carbon metabolism-related micronutrients and HCC risk. As with the diet only analyses, total vitamin B₃ intake was associated with a lower HCC risk in both the individual model (HR=0.60; 95% CI=0.42–0.85; $P_{\text{trend}} = 0.008$) and the model that evaluated all six micronutrients simultaneously (HR=0.32; 95% CI=0.18–0.55; $P_{\text{trend}} < 0.0001$). There were no associations for total intakes of folate, methionine, or vitamins B₂, B₆, B₁₂, or the methyl-donor index.

To minimize confounding by subclinical or undiagnosed HCC at baseline, we restricted the analyses to participants with more than three years of follow-up (N=453,384; cases n=559). For these analyses, all six micronutrients were evaluated concurrently, with adjustment for HCC risk factors. Associations were examined for dietary intake only (Table 4) and total intake (Table 5), and the results were similar. For dietary intake, higher vitamin B₃ intake was associated with a lower HCC risk (HR_{Q5vsQ1}=0.51; 95% CI=0.37–0.70; $P_{\text{trend}} < 0.0001$). By contrast, higher dietary vitamin B₆ intake was associated with an increased HCC risk in the fourth quintile (HR_{Q4vsQ1}=1.51; 95% CI=1.07–1.23) but not the fifth quintile (HR_{Q5vsQ1}=1.44; 95% CI=0.97–2.15; $P_{\text{trend}} = 0.03$) (Table 4). For total intake, higher vitamin B₃ intake was again associated with a lower HCC risk (HR_{Q5vsQ1}=0.37; 95% CI=0.20–0.68; $P_{\text{trend}} = 0.001$), while higher vitamin B₆ intake was associated with an increased risk (HR_{Q5vsQ1}=2.04; 95% CI=1.02–4.07; $P_{\text{trend}} = 0.04$) (Table 5). No association was observed for the other micronutrients or the methyl-donor index. To test for non-linearity of the associations between total intakes of vitamins B₃ and B₆, and HCC risk in the sample with more than three years of follow-up, restricted cubic spline regression analyses were performed in models that included all six micronutrients and covariates. The spline curves showed a dose-response positive association between higher total vitamin B₃ intake and lower HCC risk (Figure 1A), and a dose-response association between higher total vitamin B₆ intake and increased HCC risk (Figure 1B).

Stratified analyses were performed by sex, BMI, alcohol intake, and physical activity level among the participants with more than three years of follow-up, since this group is less prone to confounding by subclinical disease at baseline. These analyses involved evaluation of total intake of all six micronutrients simultaneously, and the results did not show statistical interaction by sex, BMI, alcohol intake, or physical activity for any of the micronutrients or the methyl-donor index (Supplementary Tables 1–4). However, there were instances where associations were observed in certain subgroups. As with the previous results, higher total vitamin B₃ intake was associated with a lower HCC risk both among men (HR_{Q5vsQ1}=0.43; 95% CI=0.22–0.85; *P*_{trend}=0.01) and women (HR_{Q5vsQ1}=0.39; 95% CI=0.22–0.52; *P*_{trend}=0.003) (Supplementary Tables 1). Higher total vitamin B₆ intake was associated with increased HCC risk in women (HR_{Q5vsQ1}=6.21; 95% CI=1.12–34.45; *P*_{trend}=0.005), but not men (HR_{Q5vsQ1}=1.67; 95% CI=0.75–3.72; *P*_{trend}=0.17). Total folate intake also was associated with lower HCC risk among women (HR_{Q5vsQ1}=0.31; 95% CI=0.12–0.75; *P*_{trend}=0.04), but not men (HR_{Q5vsQ1}=0.95; 95% CI=0.64–1.40; *P*_{trend}=0.92). Among participants with a BMI greater than or equal to 25 kg/m², higher total vitamin B₃ intake was associated with lower HCC risk (HR_{Q5vsQ1}=0.25; 95% CI=0.13–0.50; *P*_{trend}<0.0001), whereas higher total vitamin B₆ intake was associated with increased HCC risk (HR_{Q5vsQ1}=3.58; 95% CI=1.62–7.91; *P*_{trend}=0.0006), but no association was found among participants with BMI less than 25 kg/m² (Supplementary Table 2). Higher vitamin B₆ intake also was associated with an increased HCC risk among participants who consumed more than three alcoholic drinks per day (HR_{Q5vsQ1}=7.38; 95% CI=1.02–53.20; *P*_{trend}=0.04), but not those in the other categories of alcohol intake (Supplementary Table 3). Furthermore, higher total vitamin B₃ intake was associated with lower HCC risk among participants who engaged in physical activity less than once a week (HR_{Q5vsQ1}=0.25; 95% CI=0.12–0.75; *P*_{trend}=0.01) and those who engaged in physical activity two to three times a week (HR_{Q5vsQ1}=0.45; 95% CI=0.14–0.65; *P*_{trend}=0.0008), but not those in the other physical activity categories (Supplementary Table 4).

Discussion

It has long been suspected that micronutrients involved in one-carbon metabolism play important roles in HCC development through modulation of DNA methylation.^{10, 11, 13} In this large prospective cohort study, involving 494,860 participants with 16 years of follow-up, we found that higher vitamin B₃ (niacin) intake is associated with a lower risk of HCC, after controlling for several known and potential risk factors of HCC, including diabetes mellitus, BMI, smoking history, red meat intake, the Healthy Eating Index (a marker of the overall quality of a person's diet) and other one-carbon micronutrients. There also was a suggestion that higher vitamin B₆ intake is associated with an increased HCC risk among participants with more than three years of follow-up. Stratified analyses were performed by sex, BMI, alcohol intake, and physical activity for associations between intakes of folate, methionine, and vitamins B₂, B₃, B₆, and B₁₂, and HCC risk, but no statistical interaction was observed. Overall, the findings suggest a potential HCC risk reduction through dietary modifications that incorporate healthy sources of vitamin B₃, such as legumes, seeds, nuts, salmon, and soy products, while limiting intake of foods and supplements that contain high

amounts of vitamin B₆, such as beef liver, other types of organ meat, and some starchy vegetables (e.g., white potatoes and corn).^{36–38}

In an earlier analysis of the NIH-AARP Diet and Health Study data that was focused on assessing the modifying effect of folate intake on the association between alcohol consumption and HCC risk, and included a smaller number of HCC cases (n=435), higher total folate intake was associated with a less deleterious effect of alcohol consumption on HCC risk.²⁴ The present study included a larger number of HCC cases (n=647) accrued over an additional five years of follow-up. When examined individually, neither dietary folate nor total folate intake was associated with HCC risk. However, when examined in the presence of other one-carbon metabolism–related micronutrients, higher dietary folate intake (but not total folate) was associated with a lower HCC risk. Some blood-based studies have also reported an inverse association between folate metabolite levels and HCC risk^{28, 29}; however, the results are conflicting since other studies did not find an association between folate metabolite levels and HCC risk.^{26, 27}

It is important to note that the impact of folate status on cancer risk depends on a number of factors, including genetic variation in folate-metabolizing genes and alcohol intake. In the present study, we accounted for alcohol intake through statistical adjustment. We further evaluated the modifying effect of alcohol intake on the association between folate and HCC risk in the presence of other one-carbon metabolism–related micronutrients and did not find effect modification by alcohol intake. The difference in the results between the previous and the present study may be explained by variation in methodological approaches. In the previous study,²⁴ the modifying effect of folate intake was assessed for the association between alcohol intake and HCC risk, without considering the potential effects of other one-carbon–related micronutrients. By design, the present study assessed the modifying effect of alcohol intake on the association between folate intake and HCC risk in the presence of other one-carbon metabolism–related micronutrients as a means of simultaneous evaluation of a set of biochemically related micronutrients that have been implicated in HCC development.^{10, 13}

To our knowledge, this is the first study to report an association between higher vitamin B₃ intake and lower HCC risk. Prior studies on one-carbon metabolism–related micronutrient intake and HCC risk did not include vitamin B₃.^{24–30} In our study population, the top five dietary sources of vitamin B₃ are ready-to-eat breakfast cereal, black coffee, bread/dinner rolls, chicken (not fried) and tuna. In the human body, vitamin B₃ is metabolized into the enzymatic cofactor, nicotinamide adenine dinucleotide (NAD), which is used by over 400 enzymes for various biological functions, including posttranslational modifications of cellular proteins.^{39, 40} However, NAD is not completely stable, it is converted to nicotinamide adenine dinucleotide phosphate (NADP) and both NAD and NADP are involved in cellular oxidation reduction processes that contribute to the prevention of oxidative DNA damage and maintenance of genome integrity.^{41, 42} In the one-carbon metabolism pathway, vitamin B₃ serves as a cofactor for the enzyme dihydrofolate reductase and is used for the conversion of folate to dihydrofolate then to tetrahydrofolate, which is eventually used for the formation of the universal methyl-donor, S-adenosylmethionine that is involved in about 85% of all methylation reactions.^{10, 13} Furthermore, vitamin B₃ is

known to lower blood levels of low-density lipoprotein cholesterol (a risk factor for NAFLD and a component of metabolic syndrome, both of which are associated with HCC development) while increasing blood levels of high-density lipoproteins (which inhibit inflammatory properties of oxidized phospholipids),^{43–46} and these could also be potential mechanisms for its association with HCC. Our study shows that higher intake of dietary and total vitamin B₃ are each associated with a lower risk for HCC when the associations were examined individually as well as simultaneously with other one-carbon metabolism–related micronutrients in the model. The association for vitamin B₃ remained significant in analyses restricted to participants with more than three years of follow-up, and was significant in both men and women. A restricted cubic spline regression curve further showed a dose-response relationship between higher vitamin B₃ intake and lower HCC risk. Thus, this prospective study provides compelling evidence of a potential etiological link between vitamin B₃ intake and lower risk of HCC development.

Our study suggest also that higher intake of vitamin B₆ is associated with a higher risk of HCC among participants with more than three years of follow-up. The top five dietary sources of vitamin B₆ in our study population are bananas, white potatoes, easy-to-eat breakfast cereal (good fiber), orange/grapefruit juice, and breakfast cereal (other). This is generally consistent with what has been reported among adults in the United States (e.g., fortified cereal, poultry, bananas, avocado, and starchy vegetables).³⁸ The biological explanation for the observed association is not completely clear. However, vitamin B₆ serves as an enzymatic cofactor in several inflammatory pathways, including the kynurenine and homocysteine metabolism pathways.⁴⁷ The kynurenine pathway, in particular, is known to facilitate immune escape of malignant cells, thereby promoting tumor growth and proliferation,⁴⁸ which may be a possible mechanism for its association with HCC. In contrast to our findings, an Italian case-control study, involving 185 HCC cases and 412 controls, did not find an association between dietary vitamin B₆ intake and HCC risk.⁴⁹ A similar null finding was reported in a prospective study among Finnish male smokers.²⁶ In contrast, a nested case-control study of 297 HCC cases and 631 controls found that higher circulating levels of pyridoxal-5-phosphate, 1 of 3 phosphorylated forms of vitamin B₆,²⁷ is associated with lower HCC risk. It is worth noting that higher dietary intake of vitamin B₆ has been associated with increased risk for endometrial cancer in the NIH-AARP Diet and Health Study cohort.⁵⁰ Hence, additional studies in large prospective cohorts are needed to clarify the association between vitamin B₆ intake and HCC risk.

We did not have data on choline and betaine intake for analysis. Fore methionine, only dietary data were available, but there was no plausible association was observed with HCC risk. In a case-control study conducted among Chinese participants, Zhou *et al.*²⁵ found that higher dietary intakes of choline and betaine were each associated with lower HCC risk, but as with our study, they also did not find an association between dietary methionine intake and HCC risk. Schwartz *et al.*²⁶ also did not find an association between circulating methionine levels and HCC risk in a prospective cohort study, but a nested case-control study by Butler *et al.*²⁷ reported an association between elevated blood levels of methionine and higher HCC risk.²⁷ Furthermore, in the presented study, we did not find an association between vitamin B₁₂ intake and HCC risk. At least one blood-based study has reported higher HCC risk among individuals with elevated serum vitamin B₁₂ levels³⁰, but another

study reported a null association.²⁶ Moreover, vitamin B₂ was not associated with HCC risk in the present study, and findings from existing studies on vitamin B₂ and HCC risk are mostly null.^{26, 49} One unique feature of our study is the assessment of the methyl-donor index, as a composite measure of DNA methylation potential, but no association was found between the methyl-donor index and HCC risk.

The strengths of our study include its large sample size, long follow-up period, control for several potential confounders, and simultaneous evaluation of multiple one-carbon metabolism-related micronutrients in one model. Additionally, the analyses performed among participants with more than three years of follow-up minimizes concerns of confounding by undiagnosed HCC at baseline. The evaluation of effect modification by well-known HCC risk factors also adds to the study strengths. Limitations of our study include its predominantly non-Hispanic white population, lack of data on HBV/HCV status, and lack of data on some potentially important one-carbon metabolism-related micronutrients, such as choline and betaine. Additional limitations include our inability to account for endogenous sources of one-carbon metabolism-related micronutrients and inter-individual variation in genes involved in one-carbon metabolism. The study is further limited by the single diet assessment performed at baseline. However, the dietary questionnaire used in the NIH-AARP study was designed to assess habitual dietary patterns in the year prior to enrollment, which is often many years before cancer development—the potentially susceptibility period.

In summary, the results of this large prospective cohort study suggest that higher vitamin B₃ (niacin) intake is associated with a lower risk for HCC, whereas higher intake of vitamin B₆ is associated with a higher HCC risk. Additional studies in large prospective cohorts that incorporate a broader list of one-carbon metabolism-related micronutrients, including choline and betaine, would improve our understanding of the role of one-carbon metabolism-related micronutrient intake in HCC development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BMI	Body mass index
CI	confidence interval
FFQ	food-frequency questionnaire
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HR	hazard ratio
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NAFLD	Non-alcoholic fatty liver disease
NIH-AARP	National Institutes of Health and formerly the American Association of Retired Persons
Q1	lowest or 1st quintile
Q5	highest or 1st quintile

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Novelty and Impact:

This comprehensive investigation of the association between one-carbon metabolism-related micronutrient intake and risk of hepatocellular carcinoma (HCC) development shows that higher vitamin B₃ intake is associated with a lower risk of HCC, whereas higher intake of vitamin B₆ is associated with a higher HCC risk. HCC prevention strategies might be enhanced with dietary modifications involving healthy sources of vitamin B₃ coupled with limited intake of foods and supplements containing high amounts of vitamin B₆.

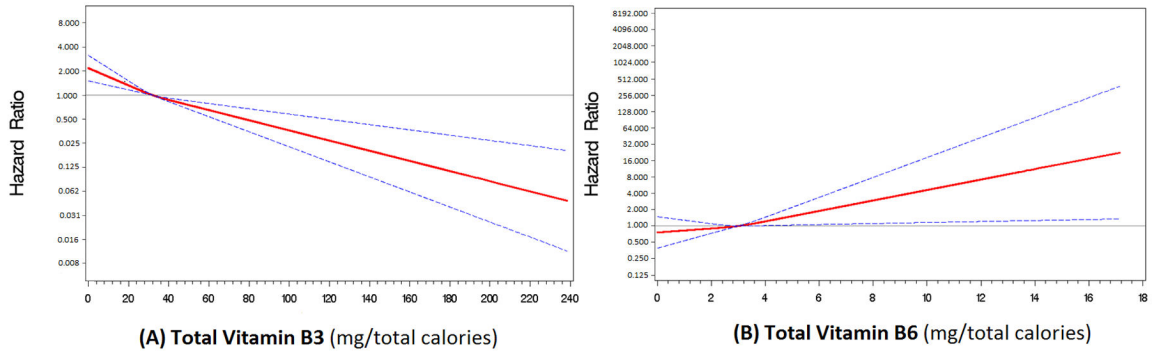


Figure 1.

Restricted cubic spline regression curves with three knots showing (A) a dose-response inverse association between total vitamin B₃ intake and hepatocellular carcinoma (HCC) risk, and (B) dose-response positive association between total vitamin B₆ intake and HCC risk (N=494,860, cases=647). Cut points for the knots were determined using the 25th, 50th, and 75th percentile values of the respective variable. The analyses were performed in models that included all six one-carbon metabolism-related micronutrient(i.e., continuous forms of total folate, methionine, and vitamins B₂, B₃, B₆ and B₁₂), with adjustments for age (continuous), sex, race/ethnicity (white, black, Hispanic, other), diabetes mellitus (yes, no), smoking status (never, former, current), alcohol use (<1, 1–3, >3 drinks per day), total caloric intake (continuous), body mass index (<25, 25–29, 30 kg/m²), education (<high school, high school graduate, some college/post-high school education, college graduate/post-graduate), physical activity (0, <1, 1–2, 3–4, 5+ times per week), multivitamin use (yes, no), red meat intake (0–23, 24–41, 42–63, 64–99, >99 grams per day), and Healthy Eating Index scores (10–59, 60–66, 67–71, 72–76, >76). Each of micronutrient was adjusted for total caloric intake for the dietary portion using the residual method before adding intake from supplements.

Table 1.

Baseline Characteristics of the Study Participants (N=494,860) by Quintiles of Total Intake of One-carbon Metabolism-Related Micronutrients: The NIH-AARP Diet and Health Study (Enrolled 1995–1996, Followed Up to 2011).

	Folate ^a		Methionine ^b		Vitamin B ₂ ^a		Vitamin B ₃ ^a		Vitamin B ₆ ^a		Vitamin B ₁₂ ^a		Total Sample
	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	Mean (SD)
Total calories, kcal	2148 (981.3)	2054 (784.7)	1072 (408.2)	2846 (804.9)	1926 (924.0)	1910 (814.6)	1874(885.0)	1903 (816.8)	2089 (931.4)	2061 (840.0)	2265 (899.8)	2060 (844.4)	1835 (806.4)
Age, years	61 (5.5)	62 (5.3)	62 (5.4)	61 (5.4)	61 (5.4)	62 (5.4)	62 (5.4)	61 (5.4)	61 (5.5)	62 (5.3)	61 (5.4)	62 (5.3)	61.5 (5.4)
Sex	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c
Male	68.7	61.5	42.3	76.6	65.2	55.7	59.3	58.2	64.7	64.7	63.0	64.9	59.7
Female	31.3	38.5	57.7	23.4	34.8	44.3	40.7	41.8	35.3	35.3	37.0	35.1	40.3
Race/Ethnicity													
Non-Hispanic white	92.7	88.2	87.4	91.4	88.2	92.1	87.9	92.4	91.4	90.6	87.6	92.4	91.2
Non-Hispanic black	4.0	4.2	5.7	3.8	5.6	3.2	6.1	3.1	4.3	4.1	5.7	3.7	3.9
Hispanic	1.2	2.9	2.1	2.2	2.3	1.9	2.3	1.9	1.5	2.3	2.6	1.5	1.9
Other	2.1	4.7	4.7	2.5	3.9	2.8	3.7	2.7	2.9	3.1	4.0	2.4	3.0
Diabetes mellitus													
No	93.0	90.6	93.0	87.6	93.7	90.5	92.6	90.4	94.4	88.3	94.2	87.2	91.0
Yes	7.0	9.4	7.0	12.4	6.3	9.5	7.4	9.6	5.6	11.7	5.8	12.8	9.0
Smoking status													
Non-smoker	25.5	39.7	37.7	32.1	30.9	36.6	36.1	34.9	27.3	39.0	33.7	33.3	35.1
Former smoker	47.6	49.9	45.6	50.3	48.5	49.2	45.2	51.2	46.2	50.8	50.0	48.6	49.2
Current smoker	23.0	6.5	12.3	13.5	16.4	10.4	14.5	10.2	22.6	6.3	12.3	14.1	11.9
Unknown ^d	3.9	3.9	4.4	4.0	4.2	3.8	4.3	3.7	3.9	3.9	4.0	4.0	3.8
Alcohol, drinks/day													
None	22.0	26.5	28.4	24.0	22.3	25.4	27.1	23.9	23.6	27.4	24.8	25.1	24.5
<1	41.3	55.3	54.6	48.7	46.3	54.5	47.6	54.4	46.1	52.2	43.3	54.1	52.7
1–3	16.4	14.9	11.9	16.7	17.4	14.5	14.4	15.6	15.3	14.2	15.3	15.2	15.3

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	Folate ^a		Methionine ^b		Vitamin B ₂ ^a		Vitamin B ₃ ^a		Vitamin B ₆ ^a		Vitamin B ₁₂ ^a		Total Sample
	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	
>3	20.4	3.3	5.1	10.5	14.0	5.6	10.9	6.1	15.0	6.2	16.6	5.6	7.5
BMI, kg/m²													
<25	30.9	39.3	41.4	25.6	32.6	37.1	33.2	36.6	33.6	36.5	39.3	28.5	34.6
25–29	39.4	38.0	35.9	40.3	39.5	37.9	38.0	38.4	38.7	39.0	37.9	39.3	38.9
30	27.4	20.2	19.7	31.8	25.3	22.7	25.9	22.7	25.2	22.0	20.0	29.8	24.2
Unknown ^d	2.4	2.6	3.0	2.3	2.6	2.4	2.9	2.3	2.5	2.5	2.7	2.4	2.4
Multivitamins use													
No	50.7	40.6	45.9	45.4	96.5	1.1	96.7	0.5	50.5	41.4	45.5	45.2	44.9
Yes	49.3	59.4	54.1	54.6	3.5	98.9	3.3	99.5	49.5	58.6	54.5	54.8	55.1
Education, years	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c	% ^c
< HS	7.8	5.1	6.5	6.8	7.4	4.6	7.9	4.4	7.5	5.9	7.0	6.5	6.0
HS graduate	22.9	15.7	21.9	18.7	21.6	16.4	22.5	16.0	22.0	18.1	19.8	19.4	19.5
Some college /post- HS	35.2	30.1	33.8	32.6	33.1	33.2	32.8	33.1	34.2	31.1	32.6	32.9	33.0
College/post- graduate	30.9	46.1	33.9	39.0	34.9	42.7	33.4	43.4	33.3	41.8	37.5	38.1	38.5
Unknown ^d	3.1	3.0	3.8	2.9	3.0	3.1	3.3	3.0	2.9	3.2	3.0	3.1	2.9
Physical Activity, times per week													
0	6.8	3.2	5.7	4.4	6.0	3.6	6.4	3.4	6.8	3.3	5.0	4.6	4.4
<1	35.2	18.9	30.3	25.0	32.2	23.9	31.6	23.4	35.4	19.0	25.7	27.4	27.1
1–2	21.9	19.1	20.5	21.6	21.5	20.8	21.0	20.8	21.9	18.6	19.5	22.2	21.5
3–4	20.7	30.6	24.9	26.3	22.8	28.6	22.8	29.2	20.5	30.7	26.1	26.0	26.7
5+	14.3	27.1	16.9	21.6	16.2	22.0	16.6	22.2	14.3	27.1	22.6	18.6	19.2
Unknown ^d	1.2	1.1	1.8	1.0	1.3	1.0	1.6	0.9	1.1	1.2	1.1	1.1	1.1
Red Meat Intake, grams/day													
0–23	7.8	30.7	49.7	4.6	15.7	23.9	20.5	22.6	11.3	26.2	26.5	9.8	20.2
24–41	13.1	20.6	33.9	5.4	17.5	20.8	20.8	19.5	16.5	19.4	20.4	13.1	20.0
42–63	17.7	18.1	14.0	9.2	19.2	19.4	20.6	18.7	19.8	18.3	19.3	16.8	20.0

	Folate ^a		Methionine ^b		Vitamin B ₂ ^a		Vitamin B ₃ ^a		Vitamin B ₆ ^a		Vitamin B ₁₂ ^a		Total Sample
	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	
64-99	23.9	16.4	2.4	19.0	21.8	18.2	20.3	18.5	23.7	17.5	18.6	22.1	20.0
>99	37.4	14.2	0.0	61.9	25.8	17.7	17.8	20.7	28.6	18.6	15.2	38.2	19.9
Healthy Eating Index Score													
10-59	49.2	5.9	19.3	25.5	33.7	14.3	31.1	13.7	55.3	4.2	25.6	20.2	20.0
60-66	25.6	12.5	19.2	22.5	24.4	17.4	22.7	17.6	24.6	10.4	21.6	20.4	20.0
67-71	14.1	19.3	19.9	19.9	18.8	19.8	18.2	20.5	12.0	17.8	19.4	20.0	20.0
72-76	7.7	26.5	20.9	17.1	14.3	22.3	15.1	23.0	5.8	27.0	18.0	19.3	20.0
>76	3.4	35.8	20.7	14.9	8.8	26.2	12.9	25.2	2.3	40.6	15.5	20.1	20.0

Abbreviations: BMI, body mass index; HS, high school; Q1, 1st quintile; Q5, 5th quintile

^aTotal intake was computed as diet plus supplements. Each micronutrient was adjusted for total caloric intake by the residual method. Quintiles were created from intake values at baseline and categorized based on distribution in the entire cohort using the 20th, 40th, 60th, and 80th percentile values as cut points.

^bMethionine was based on dietary intake only because data on supplemental intake were not available.

^cPercentages may not sum to 100% because of rounding.

^dA separate category was created for “unknown” and retained in the statistical analysis models.

Table 2.

Dietary Intake of Micronutrients Involved in One-Carbon Metabolism and Risk of Hepatocellular Carcinoma: Results from Assessment of the Micronutrients Individually and Simultaneously, With Adjustment for Covariates: The NIH-AARP Diet and Health Study (Enrolled 1995–1996, Followed Up to 2011; N=494,860, Cases=647).

Micronutrient ^a	Total N	Cases	Person-Years	Assessment of Micronutrients Individually	Assessment of Micronutrients Simultaneously
				HR (95% CI) ^b	HR (95% CI) ^c
Folate					
Q1	98,975	186	1,182,022	1.00 (ref)	1.00 (ref)
Q2	98,971	134	1,202,404	0.84 (0.66–1.06)	0.84 (0.66–1.08)
Q3	98,970	103	1,210,087	0.69 (0.53–0.89)	0.68 (0.52–0.89)
Q4	98,971	112	1,217,945	0.78 (0.60–1.02)	0.76 (0.58–1.01)
Q5	98,973	112	1,222,929	0.77 (0.58–1.01)	0.73 (0.54–0.98)
<i>P</i> _{trend} ^d				0.05	0.03
Methionine					
Q1	99,168	104	1,218,916	1.00 (ref)	1.00 (ref)
Q2	98,751	104	1,212,516	0.87 (0.65–1.16)	0.85 (0.64–1.14)
Q3	99,387	128	1,214,539	0.91 (0.67–1.24)	0.87 (0.63–1.19)
Q4	99,128	149	1,204,573	0.88 (0.64–1.22)	0.82 (0.58–1.15)
Q5	98,426	162	1,184,843	0.67 (0.45–1.00)	0.59 (0.38–0.93)
<i>P</i> _{trend} ^d				0.13	0.11
Vitamin B ₂					
Q1	98,970	145	1,200,522	1.00 (ref)	1.00 (ref)
Q2	98,973	106	1,213,390	0.90 (0.70–1.17)	
Q3	98,974	106	1,214,292	0.92 (0.71–1.20)	1.03 (0.77–1.38)
Q4	98,971	133	1,209,677	1.14 (0.88–1.47)	1.26 (0.94–1.70)
Q5	98,972	157	1,197,506	1.20 (0.94–1.54)	1.27 (0.92–1.77)
<i>P</i> _{trend} ^d				0.04	0.07
Vitamin B ₃					
Q1	98,974	183	1,197,415	1.00 (ref)	1.00 (ref)
Q2	98,972	113	1,210,855	0.69 (0.54–0.88)	0.68 (0.53–0.88)
Q3	98,970	108	1,211,608	0.63 (0.49–0.81)	0.61 (0.47–0.79)
Q4	98,970	120	1,210,293	0.65 (0.51–0.83)	0.60 (0.46–0.78)
Q5	98,974	123	1,205,216	0.57 (0.44–0.72)	0.47 (0.36–0.65)
<i>P</i> _{trend} ^d				<.0001	<.0001
Vitamin B ₆					
Q1	98,973	151	1,189,847	1.00 (ref)	1.00 (ref)
Q2	98,970	122	1,211,215	0.95 (0.74–1.23)	1.17 (0.89–1.53)
Q3	98,972	115	1,215,460	0.91 (0.70–1.18)	1.19 (0.88–1.60)
Q4	98,974	128	1,214,508	1.02 (0.78–1.33)	1.40 (1.02–1.92)

Micronutrient ^a	Total N	Cases	Person-Years	Assessment of Micronutrients Individually	Assessment of Micronutrients Simultaneously
				HR (95% CI) ^b	HR (95% CI) ^c
Q5	98,971	131	1,204,357	0.96 (0.73–1.27)	1.42 (0.98–2.05)
<i>P</i> _{trend} ^d				0.98	0.03
Vitamin B ₁₂					
Q1	98,972	131	1,215,461	1.00 (ref)	1.00 (ref)
Q2	98,971	112	1,218,847	1.04 (0.80–1.35)	1.08 (0.82–1.42)
Q3	98,973	119	1,211,187	1.10 (0.84–1.44)	1.14 (0.85–1.52)
Q4	98,972	117	1,203,134	1.01 (0.77–1.33)	1.03 (0.75–1.40)
Q5	98,972	168	1,186,757	1.22 (0.95–1.56)	1.26 (0.92–1.72)
<i>P</i> _{trend} ^d				0.17	0.43
Methyl-donor index ^e					
Q1	98,972	160	1,191,675	1.00 (ref)	N/A
Q2	98,971	115	1,215,867	0.85 (0.66–1.10)	
Q3	98,972	94	1,213,573	0.69 (0.52–0.91)	
Q4	98,973	137	1,214,058	0.96 (0.74–1.24)	
Q5	98,972	141	1,191,675	0.85 (0.65–1.10)	
<i>P</i> _{trend} ^c				0.49	

Abbreviations: HR, hazard ratio; CI, confidence interval; N/A, not applicable; Q1–Q5, calorie-adjusted quintiles of micronutrients intake (without supplements)

^aEach dietary micronutrient was adjusted for total calories intake using the residual method. Quintiles were created from intake values at baseline and categorized based on distribution in the entire cohort using the 20th, 40th, 60th, and 80th percentile values as cut points

^bAll models adjusted for age (continuous), sex, race/ethnicity (white, black, Hispanic, other), diabetes mellitus (yes, no), smoking status (never, former, current), alcohol use (<1, 1–3, >3 drinks/day), total caloric intake (continuous), body mass index (<25, 25–29, ≥30 kg/m²), education level (<high school, high school graduate, some college/post-high school education, college graduate/post-graduate), physical activity (0, <1, 1–2, 3–4, 5+ times per week), multivitamin use (yes, no), red meat intake (0–23, 24–41, 42–63, 64–99, >99 grams/day), and the Healthy Eating Index scores (10–59, 60–66, 67–71, 72–76, >76).

^cAll of the dietary micronutrients were placed in one model with adjustment for the factors listed in ^b.

^d*P*_{trend} was calculated by treating the quintile variable as a continuous variable.

^eA separate model was fitted for the methyl-donor index, adjusting for the factors listed in ^b.

Table 3.

Total Intake of One-Carbon Metabolism–Related Micronutrients and Hepatocellular Carcinoma Risk: Results for Assessment of the Micronutrients Individually and Simultaneously, with Adjustment for Covariates: The NIH-AARP Diet and Health Study (Enrolled 1995–1996; N=494,860, Cases=647).

Micronutrient ^a	Total N	Cases	Person-Years	Assessment of Micronutrients	Assessment of Micronutrients
				Individually	Simultaneously
				HR (95% CI) ^b	HR (95% CI) ^c
Folate					
Q1	98,974	170	1,176,525	1.00 (ref)	1.00 (ref)
Q2	98,969	121	1,201,809	0.87 (0.68–1.11)	0.89 (0.69–1.14)
Q3	98,973	122	1,214,979	0.92 (0.71–1.18)	0.94 (0.72–1.23)
Q4	98,972	117	1,219,050	0.89 (0.68–1.16)	0.93 (0.70–1.22)
Q5	98,972	117	1,223,023	0.83 (0.63–1.10)	0.88 (0.66–1.19)
<i>P</i> _{trend} ^d				0.27	0.53
Methionine^e					
Q1	99,168	104	1,218,916	1.00 (ref)	1.00 (ref)
Q2	98,751	104	1,212,516	0.87 (0.65–1.16)	0.88 (0.66–1.18)
Q3	99,387	128	1,214,539	0.91 (0.67–1.24)	0.93 (0.68–1.26)
Q4	99,128	149	1,204,573	0.88 (0.64–1.22)	0.90 (0.64–1.26)
Q5	98,426	162	1,184,843	0.67 (0.45–1.00)	0.68 (0.44–1.04)
<i>P</i> _{trend} ^d				0.13	0.14
Vitamin B₂					
Q1	98,974	140	1,203,248	1.00 (ref)	1.00 (ref)
Q2	98,970	136	1,207,007	1.07 (0.84–1.36)	1.20 (0.91–1.58)
Q3	98,972	118	1,208,260	0.92 (0.69–1.23)	1.12 (0.78–1.63)
Q4	98,973	125	1,211,528	1.15 (0.81–1.64)	1.39 (0.86–2.26)
Q5	98,971	128	1,205,344	1.14 (0.80–1.63)	1.06 (0.60–1.88)
<i>P</i> _{trend} ^d				0.42	0.48
Vitamin B₃					
Q1	98,971	159	1,199,363	1.00 (ref)	1.00 (ref)
Q2	98,974	142	1,207,886	0.87 (0.69–1.10)	0.88 (0.68–1.13)
Q3	98,971	111	1,209,537	0.54 (0.40–0.73)	0.45 (0.31–0.66)
Q4	98,972	104	1,209,631	0.51 (0.35–0.73)	0.30 (0.19–0.50)
Q5	98,972	131	1,208,970	0.60 (0.42–0.85)	0.32 (0.18–0.55)
<i>P</i> _{trend} ^d				0.008	<.0001
Vitamin B₆					
Q1	98,972	155	1,196,450	1.00 (ref)	1.00 (ref)
Q2	98,973	125	1,211,728	0.86 (0.67–1.11)	0.96 (0.73–1.26)
Q3	98,971	119	1,206,064	0.82 (0.61–1.11)	1.19 (0.81–1.73)
Q4	98,971	120	1,212,566	0.94 (0.65–1.38)	1.58 (0.93–2.68)

				Assessment of Micronutrients Individually	Assessment of Micronutrients Simultaneously
Q5	98,973	128	1,208,579	0.99 (0.67–1.45)	1.66 (0.88–3.15)
<i>P</i> _{trend} ^d				0.91	0.10
Vitamin B ₁₂					
Q1	98,972	143	1,212,872	1.00 (ref)	1.00 (ref)
Q2	98,972	123	1,203,764	0.91 (0.71–1.17)	0.93 (0.71–1.22)
Q3	98,973	121	1,210,731	0.89 (0.67–1.19)	1.05 (0.75–1.46)
Q4	98,971	111	1,209,232	0.95 (0.68–1.34)	1.17 (0.76–1.80)
Q5	98,972	149	1,198,787	1.20 (0.86–1.67)	1.65 (1.03–2.65)
<i>P</i> _{trend} ^d				0.21	0.09
Methyl-donor index ^f					
Q1	98973	160	1,191,675	1.00 (ref)	N/A
Q2	98971	115	1,215,867	0.85 (0.62–1.12)	
Q3	98972	100	1,213,985	0.71 (0.52–0.93)	
Q4	98972	134	1,214,058	0.91 (0.69–1.28)	
Q5	98972	137	1,200,154	0.88 (0.62–1.15)	
<i>P</i> _{trend} ^c				0.49	

Abbreviations: HR, hazard ratio; CI, confidence interval; N/A, not applicable; Q1–Q5, calorie-adjusted quintiles of micronutrients intake.

^aTotal intake was computed as diet plus supplements. Each micronutrient was adjusted for total caloric intake by the residual method. Quintiles were created from intake values at baseline and categorized based on distribution in the entire cohort using the 20th, 40th, 60th, and 80th percentile values as cut points.

^bAll models adjusted for age (continuous), sex, race/ethnicity (white, black, Hispanic, other), diabetes mellitus (yes, no), smoking status (never, former, current), alcohol use (<1, 1–3, >3 drinks/day), total caloric intake (continuous), body mass index (<25, 25–29, 30 kg/m²), education level (<high school, high school graduate, some college/post-high school education, college graduate/post-graduate), physical activity (0, <1, 1–2, 3–4, 5+ times per week), multivitamin use (yes, no), red meat intake (0–23, 24–41, 42–63, 64–99, >99 grams/day), and the Healthy Eating Index scores (10–59, 60–66, 67–71, 72–76, >76).

^cAll of the micronutrients were placed in 1 model, adjusting for the factors listed in ^b.

^d*P*_{trend} was calculated by treating the quintile variable as a continuous variable.

^eMethionine intake was based on diet alone because data on supplemental methionine intake were not available.

^fA separate model was fitted for the methyl-donor index, adjusting for the factors listed in ^b.

Table 4.

Simultaneous Evaluation of Dietary Micronutrients Involved in One-Carbon Metabolism and Risk of Hepatocellular Carcinoma Among Participants With >3 Years of Follow-up: The NIH-AARP Health and Diet Study (N=453,384, Cases=559).

Nutrient ^a	>3 Years of Follow-up			
	Total N	Cases	Person-Years	HR (95% CI) ^b
Folate				
Q1	90,675	145	1,170,410	1.00 (ref)
Q2	90,676	102	1,190,574	0.90 (0.69–1.17)
Q3	90,679	111	1,199,420	0.70 (0.52–0.94)
Q4	90,677	105	1,202,773	0.80 (0.59–1.08)
Q5	90,677	96	1,206,317	0.78 (0.56–1.07)
<i>P</i> trend ^c				0.07
Methionine				
Q1	91,086	87	1,206,169	1.00 (ref)
Q2	90,610	93	1,199,551	0.92 (0.67–1.26)
Q3	91,067	112	1,201,320	0.94 (0.67–1.31)
Q4	90,754	124	1,191,207	0.83 (0.57–1.22)
Q5	89,867	143	1,171,248	0.65 (0.40–1.06)
<i>P</i> trend ^c				0.21
Vitamin B2				
Q1	90,673	124	1,187,860	1.00 (ref)
Q2	90,681	90	1,197,686	0.97 (0.72–1.30)
Q3	90,680	88	1,199,105	0.97 (0.71–1.32)
Q4	90,674	119	1,196,439	1.25 (0.91–1.72)
Q5	90,676	138	1,188,405	1.27 (0.89–1.81)
<i>P</i> trend ^c				0.08
Vitamin B3				
Q1	90,677	152	1,187,428	1.00 (ref)
Q2	90,675	97	1,196,448	0.69 (0.52–0.90)
Q3	90,681	92	1,198,361	0.59 (0.45–0.79)
Q4	90,673	107	1,195,082	0.62 (0.47–0.83)
Q5	90,678	111	1,192,176	0.51 (0.37–0.70)
<i>P</i> trend ^c				<0.0001
Vitamin B6				
Q1	90,678	124	1,180,011	1.00 (ref)
Q2	90,678	106	1,197,252	1.23 (0.92–1.64)
Q3	90,675	100	1,200,155	1.25 (0.90–1.72)
Q4	90,674	116	1,199,025	1.51 (1.07–2.13)
Q5	90,679	113	1,193,051	1.44 (0.97–2.15)

Nutrient ^a	>3 Years of Follow-up			
	Total N	Cases	Person-Years	HR (95% CI) ^b
<i>P</i> trend ^c				0.03
Vitamin B12				
Q1	90,675	108	1,198,617	1.00 (ref)
Q2	90,680	98	1,201,801	1.07 (0.79–1.44)
Q3	90,675	104	1,197,856	1.13 (0.83–1.55)
Q4	90,678	99	1,191,020	0.97 (0.70–1.36)
Q5	90,676	150	1,180,200	1.25 (0.89–1.76)
<i>P</i> trend ^c				0.47
Methyl-donor index ^d				
Q1	90,677	130	1,177,974	1.00 (ref)
Q2	90,676	100	1,203,114	0.91 (0.69–1.20)
Q3	90,677	80	1,200,701	0.72 (0.54–0.98)
Q4	90,678	126	1,201,078	1.09 (0.83–1.44)
Q5	90,676	123	1,186,628	0.92 (0.69–1.22)
<i>P</i> trend ^c				0.94

Abbreviations: HR, hazard ratio; CI, confidence interval; N/A, not applicable; Q1–Q5, calorie-adjusted quintiles of micronutrients intake (supplements not included).

^aEach micronutrient was adjusted for total caloric intake by the residual method. Quintiles were created from intake values at baseline and categorized based on distribution in the entire cohort using the 20th, 40th, 60th, and 80th percentile values as cut points.

^bAll models adjusted for age (continuous), sex, race/ethnicity (white, black, Hispanic, other), diabetes mellitus (yes, no), smoking status (never, former, current), alcohol use (<1, 1–3, >3 drinks/day), total caloric intake (continuous), body mass index (<25, 25–29, 30 kg/m²), education level (<high school, high school graduate, some college/post-high school education, college graduate/post-graduate), physical activity (0, <1, 1–2, 3–4, 5+ times per week), multivitamin use (yes, no), red meat intake (0–23, 24–41, 42–63, 64–99, >99 grams/day), and the Healthy Eating Index scores (10–59, 60–66, 67–71, 72–76, >76).

^c*P*trend was calculated by treating the quintile variable as a continuous variable.

^dA separate model was fitted for the methyl-donor index, adjusting for the factors listed in ^b.

Table 5.

Simultaneous Evaluation of Total Intake of Micronutrients Involved in One-Carbon Metabolism and Risk of Hepatocellular Carcinoma Among Participants With >3 Years of Follow-up: The NIH-AARP Health and Diet Study (N=453,384, Cases=559).

Nutrient ^a	Total N	>3 Years of Follow-up		
		Cases	Person-Years	HR (95% CI) ^b
Folate				
Q1	90,073	144	1,162,399	1.00 (ref)
Q2	90,510	102	1,188,470	0.86 (0.66–1.14)
Q3	90,877	110	1,202,050	0.97 (0.73–1.29)
Q4	90,947	107	1,206,311	0.96 (0.71–1.29)
Q5	90,977	96	1,210,263	0.82 (0.59–1.13)
<i>P</i> trend ^c				0.45
Methionine ^d				
Q1	91,086	87	1,206,169	1.00 (ref)
Q2	90,610	93	1,199,551	0.96 (0.70–1.30)
Q3	91,067	112	1,201,320	1.01 (0.72–1.40)
Q4	90,754	124	1,191,207	0.93 (0.65–1.34)
Q5	89,867	143	1,171,248	0.76 (0.48–1.19)
<i>P</i> trend ^c				0.27
Vitamin B2				
Q1	90,657	118	1,189,976	1.00 (ref)
Q2	90,659	117	1,193,762	1.16 (0.86–1.56)
Q3	90,775	103	1,195,174	1.06 (0.71–1.59)
Q4	90,753	107	1,198,515	1.22 (0.72–2.06)
Q5	90,540	114	1,192,067	0.89 (0.48–1.64)
<i>P</i> trend ^c				0.93
Vitamin B3				
Q1	90,477	129	1,185,852	1.00 (ref)
Q2	90,707	122	1,194,687	0.90 (0.68–1.19)
Q3	90,745	99	1,196,460	0.53 (0.36–0.78)
Q4	90,814	90	1,196,634	0.35 (0.21–0.60)
Q5	90,641	119	1,195,861	0.37 (0.20–0.68)
<i>P</i> trend ^c				0.001
Vitamin B6				
Q1	90,425	125	1,182,756	1.00 (ref)
Q2	90,828	114	1,198,705	1.09 (0.81–1.47)
Q3	90,696	99	1,192,976	1.25 (0.82–1.89)
Q4	90,833	105	1,199,714	1.84 (1.04–3.27)
Q5	90,602	116	1,195,343	2.04 (1.02–4.07)

Nutrient ^a	>3 Years of Follow-up			
	Total N	Cases	Person-Years	HR (95% CI) ^b
<i>P</i> trend ^c				0.04
Vitamin B12				
Q1	90,868	120	1,199,916	1.00 (ref)
Q2	90,635	102	1,190,496	0.90 (0.67–1.20)
Q3	90,725	109	1,197,578	1.10 (0.77–1.58)
Q4	90,741	92	1,196,258	1.13 (0.71–1.80)
Q5	90,415	136	1,185,246	1.64 (0.92–2.91)
<i>P</i> trend ^c				0.08
Methyl-donor index ^e				
Q1	90,677	130	1,182,152	1.00 (ref)
Q2	90,676	100	1,199,074	0.83 (0.63–1.12)
Q3	90,677	80	1,199,128	0.64 (0.52–1.03)
Q4	90,678	126	1,199,875	1.18 (0.74–1.53)
Q5	90,676	123	1,189,265	0.92 (0.58–1.26)
<i>P</i> trend ^c				0.91

Abbreviations: HR, hazard ratio; CI, confidence interval; N/A, not applicable; Q1–Q5, calorie-adjusted quintiles of micronutrients intake.

^aTotal intake was computed as diet plus supplement use. Each micronutrient was adjusted for total calories intake by the residual method. Quintiles were created from intake values at baseline and categorized based on distribution in the entire cohort using the 20th, 40th, 60th, and 80th percentile values as cut points.

^bAll models adjusted for age (continuous), sex, race/ethnicity (white, black, Hispanic, other), diabetes mellitus (yes, no), smoking status (never, former, current), alcohol use (<1, 1–3, >3 drinks/day), total caloric intake (continuous), body mass index (<25, 25–29, 30 kg/m²), education level (<high school, high school graduate, some college/post-high school education, college graduate/post-graduate), physical activity (0, <1, 1–2, 3–4, 5+ times per week), multivitamin use (yes, no), red meat intake (0–23, 24–41, 42–63, 64–99, >99 grams/day), and the Healthy Eating Index scores (10–59, 60–66, 67–71, 72–76, >76)

^c*P*trend was calculated by treating the quintile variable as a continuous variable.

^dMethionine intake levels were based on dietary intake only because data on supplemental use was not available.

^eA separate model was fitted for the methyl-donor index, adjusting for the factors listed in ^b.