

## Alcohol Consumption, Folate Intake, Hepatocellular Carcinoma, and Liver Disease Mortality

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### Abstract

**Background:** Excessive alcohol consumption is a well-established risk factor for liver disease and hepatocellular carcinoma (HCC). Previous studies have found that increased alcohol consumption can lead to lower absorption of folate. Conversely, higher folate intake has been inversely associated with liver damage and HCC. In the current study, we investigate the effect of alcohol consumption and folate intake on HCC incidence and liver disease mortality in the NIH–American Association of Retired Persons Diet and Health Study.

**Methods:** The study population included 494,743 participants who reported at baseline their dietary intake for the previous year. Alcohol and folate were analyzed with hazards ratios (HR) and 95% confidence intervals (CI) using multivariate Cox proportional hazards regression models adjusted for age, sex, race, education, smoking, body mass index, and diabetes. HCC incidence ( $n = 435$ ) was determined through 2006 via linkage with cancer registries, and liver disease mortality ( $n = 789$ ) was determined through 2008 via linkage to the U.S. Social Security Administration Death Master File and the National Death Index Plus by the National Center for Health Statistics.

**Results:** Consumption of more than three drinks per day was positively associated with both HCC incidence (HR: 1.92; 95%CI: 1.42–2.60) and liver disease mortality (HR: 5.84; 95%CI: 4.81–7.10), whereas folate intake was associated with neither outcome. Folate, however, modified the relationship between alcohol and HCC incidence ( $P_{\text{interaction}} = 0.03$ ), but had no effect on the relationship between alcohol and liver disease mortality ( $P_{\text{interaction}} = 0.54$ ).

**Conclusions:** These results suggest that higher folate intake may ameliorate the effect of alcohol consumption on the development of HCC.

**Impact:** Folate intake may be beneficial in the prevention of alcohol-associated HCC. *Cancer Epidemiol Biomarkers Prev*; 22(3); 415–21. ©2013 AACR.

### Introduction

Since 1980, the incidence of hepatocellular carcinoma (HCC) has increased in the United States (1). Higher rates have been linked to improved survival of persons with chronic liver disease and to increased prevalence of hepatitis C virus (HCV; refs. 2–6). In the United States, other well-known risk factors for HCC and liver disease include alcohol consumption, chronic infection with hepatitis B virus (HBV), certain rare genetic disorders,

and the related conditions of diabetes, obesity, and metabolic syndrome (7–10). It has been estimated that approximately one third of HCC cases in the United States are linked to excessive alcohol consumption, but the mechanism by which alcohol causes HCC remains uncertain (10, 11). One possible mechanism is by lowering the levels of folate, as alcohol is known to interact with the absorption and extraction of folate. Chronic alcohol consumption has been shown to cause low levels of folate that becomes more pronounced with advancing tumor stage (11–14). Circulating folate is important in methionine synthesis and lower levels of folate can lead to inhibition of this synthesis, which may increase the likelihood of gene mutation or modify gene expression progressing to cancer (11, 15).

The extent to which folate might counteract the deleterious effects of alcohol on liver damage has not been widely studied, although a previous study reported that increased blood levels of folate were inversely associated with liver damage and HCC (16). Therefore, the purpose of the current study was to investigate the association of alcohol consumption and folate intake, both independently and together on HCC incidence and liver disease

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mortality in the NIH–American Association of Retired Persons (AARP) Diet and Health Study.

## Material and Methods

### Study population

The NIH–AARP Diet and Health Study has been described in detail elsewhere (17). Briefly, this large prospective cohort was established in 1995 to 1996 by inviting the participation of 3.5 million AARP members aged 50 to 71 years who lived in 6 states (California, Florida, Louisiana, New Jersey, North Carolina, and Pennsylvania) and 2 metropolitan areas (Atlanta, Georgia and Detroit, Michigan). AARP members were mailed a self-administrated questionnaire that included questions on demographics, dietary habits, and lifestyle characteristics. A total of 617,119 questionnaires were returned, of which 566,398 unique questionnaires were completed in satisfactory detail. Proxy respondents ( $N = 15,760$ ), persons with prevalent cancer ( $N = 51,230$ ), persons with information only on death from prevalent cancer ( $N = 229$ ), and persons with no follow-up time or extreme calorie intakes ( $N = 4,436$ ) were excluded from the current analysis, resulting in the inclusion of data from 494,743 individuals. Extreme calorie intakes were considered those beyond twice the interquartile range of sex-specific Box–Cox–transformed intakes. Participants gave informed consent by virtue of completing and returning the questionnaire. The study was approved by the National Cancer Institute Special Studies Institutional Review Board (Rockville, MD).

### Cohort follow-up

Addresses for cohort members were updated annually by matching the cohort database to the National Change of Address database maintained by the U.S. Postal Service, and by specific change of address requests from participants, updated addresses returned from yearly mailings, and the Maximum Change of Address database (Anchor Computer). Vital status was ascertained by periodic linkage of the cohort to the Social Security Administration Death Master File, cancer registry linkage, questionnaire responses, and responses to other mailings.

Incident HCC cases were identified by linkages with the state cancer registries of the original recruitment areas as well as 3 additional states (Arizona, Nevada, and Texas). HCC topography and morphology were determined using version 3 of the International Classification of Disease for Oncology (ICD-O; topography code C22; morphology codes 8170–8175; ref. 18). As previously reported, 90% of all incident cancer cases in the NIH–AARP cohort were identified by using linkage to cancer registries (19). Follow-up time to HCC incidence was calculated from the date the questionnaire was scanned until the date of diagnosis, relocation out of the catchment area, date of death, or December 31, 2006, whichever came first. Eighteen participants who were identified as having an incident HCC and as dying from liver disease were included solely in the analysis of HCC incidence and not in the analysis of liver disease mortality.

Liver disease mortality was ascertained via linkage with the U.S. Social Security Administration Death Master File and the National Death Index Plus by the National Center for Health Statistics (NCHS). Underlying causes of death from death certificates were provided as ICD-9 and -10 codes. Chronic liver disease deaths were identified using the definition of the NCHS as chronic liver disease, fibrosis and cirrhosis of liver, alcoholic liver disease, chronic hepatitis, and cirrhosis of liver without mention of alcohol (ICD-9: 571.0–571.9; ICD-10: K70, K73, K74). Follow-up time to death from liver disease was calculated from the date the questionnaire was returned until the date of death, or December 31, 2008, whichever came first.

### Alcohol, folate, and covariate assessment

The baseline questionnaire contained questions on demographic factors, anthropometry, reproductive factors, medical history, and diet. Dietary intake was ascertained using a 124-item Food Frequency Questionnaire, an early version of the Diet History Questionnaire developed and validated by the National Cancer Institute (20). Participants were asked to report the frequency of intake and portion size of their usual dietary intake of foods and beverages over the past year. The intake of alcohol was measured using 10 frequency categories ranging from never to 6 times or more per day and 3 portion sizes for beer (<12 ounces, one to two 12 ounce cans, >two 12 ounce cans), wine or wine coolers (<4 ounces, 4–8, >8), and liquor or mixed drinks (<1 shot, 1–2, >2). The food items, portion sizes, and nutrient database were constructed using the U.S. Department of Agriculture's 1994–96 Continuing Survey of Food Intakes by Individuals (21). Total folate intake was calculated as energy-adjusted dietary intake using the residual method plus unadjusted supplemental intake via multivitamin intake (22).

### Statistical analyses

Categorization of folate exposure was based on the total daily adjusted folate intake at baseline divided into tertiles within the entire cohort. The total daily alcohol consumption was categorized into number of alcoholic drinks consumed per day (no drinks per day, <1, 1–3, and >3). In the initial analysis, Cox proportional hazards regression models were used to determine hazards ratios (HRs) and 95% confidence intervals (CI) for association between alcohol or adjusted folate and HCC incidence and liver disease mortality adjusted for age, sex, race, education, diabetes, smoking, and body mass index (BMI). Adjustment for HBV and HCV infection status was not possible due to lack of information. In addition, HRs were calculated for the number of alcoholic drinks consumed per day stratified by tertiles of total adjusted folate consumption. The nondrinkers were included as a separate covariate in the model, because the questionnaire was based on intake over the past year and therefore did not ascertain previous drinking patterns. Similarly, in the trend analysis, less than 1 drink per day served as the referent group, but was overall adjusted for nondrinkers. Likelihood ratio tests for

interaction across tertiles of adjusted folate consumption were computed on the basis of cross-product terms with alcohol use. Excluding nondrinkers, alcohol consumption was also stratified by type of beverage (beer, wine, and liquor). Statistical significance was set at  $P < 0.05$  based on 2-sided tests. All statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc).

## Results

A total of 435 individuals developed HCC through 2006 and 789 individuals died from liver disease through 2008. The median follow-up times were 6.3 years for persons developing HCC and 7.0 years for persons dying from liver disease. The overall median follow-up time for participants in the cohort was 10.5 years. Table 1 shows the baseline characteristics of the study participants by adjusted folate intake and alcohol consumption. At baseline, the highest tertile of adjusted folate intake was associated with male sex, non-Hispanic white race/ethnicity, college, and postgraduate education. The lowest tertile of adjusted folate intake was associated with obese BMI ( $\geq 30$  kg/m<sup>2</sup>), diabetes, and current smoking. Those

who consumed more than 3 drinks per day of alcohol, were more likely to be of male sex, white non-Hispanic white race/ethnicity, have college and postgraduate education, and be current smokers.

In the analysis of the association between alcohol and HCC incidence and liver disease mortality, consuming more than 3 drinks of alcohol per day was associated significantly with both HCC incidence (HR: 1.92; 95% CI: 1.42–2.60) and liver disease mortality (HR: 5.84; 95% CI: 4.81–7.10; Table 2), compared with those drinking up to one drink per day. Nondrinkers were also at higher risk of both developing HCC (HR: 1.71; 95% CI: 1.37–2.14) and dying of liver disease (HR: 1.88; 95% CI: 1.55–2.28) than were those drinking less than 1 drink per day. The analysis of folate intake without adjustment for alcohol consumption found that the second tertile of adjusted folate intake (419.2–737.0  $\mu\text{g}/\text{day}$ ) was associated with a decreased incidence of HCC (HR: 0.77; 95% CI: 0.61–0.97). However, no association was observed at the highest tertile of adjusted folate intake (HR: 0.98; 95% CI: 0.79–1.23; Table 2). No association was evident between adjusted folate intake and liver disease mortality.

**Table 1.** Distribution of baseline characteristics across total folate intake tertiles and alcohol consumption in the NIH–AARP Diet and Health Study Cohort

Characteristics	Total folate intake ( $\mu\text{g}/\text{day}$ )			Alcohol consumption (drinks/day)			
	1st tertile <419.2	2nd tertile 419.2–736.9	3rd tertile >736.9	None	<1	1–3	>3
No. of subjects	164,750	164,748	165,245	118,926	260,769	75,821	37,188
Sex (%)							
Men	64.0	59.1	55.9	51.0	56.0	73.1	85.6
Women	36.0	40.9	44.1	49.0	44.0	26.9	14.4
Race (%)							
White non-Hispanic	91.5	90.6	91.6	87.7	91.6	94.6	94.6
Black non-Hispanic	4.4	4.1	3.2	6.3	3.5	2.0	2.5
Hispanic	1.7	2.0	2.1	1.9	2.1	1.6	1.3
Other	1.1	1.9	2.0	2.6	1.6	0.9	0.7
BMI, kg/m <sup>2</sup> (%)							
<25	29.5	34.9	38.4	31.6	34.5	39.0	32.1
25–29	42.4	41.5	40.7	38.6	41.5	44.0	46.9
$\geq 30$	24.6	20.3	18.0	25.4	21.1	14.7	18.4
Education, y (%)							
<11	7.6	5.9	4.4	9.7	5.0	3.7	5.1
12 years or completed high school	22.9	18.9	16.8	25.3	19.0	14.2	16.2
Post-high school	10.5	9.8	9.3	10.5	10.0	8.6	9.6
Some College	23.0	23.3	23.3	21.6	24.0	22.7	24.1
College and postgraduation	33.0	39.2	43.4	29.3	39.4	48.7	42.6
Diabetes (%)							
No	90.1	91.3	91.5	84.2	92.3	95.5	94.4
Yes	9.9	8.7	8.5	15.8	7.7	4.6	5.6
Cigarette smoking (%)							
Never	32.9	35.4	36.9	43.3	37.0	25.3	15.9
Former	47.8	48.7	51.0	42.1	48.3	58.7	59.1
Current	15.3	12.1	8.4	10.7	11.1	12.4	21.1

**Table 2.** Association between alcohol consumption in drinks per day and folate intake and the risk of HCC incidence and liver disease mortality in the NIH–AARP Diet and Health Study Cohort

	HCC incidence (N = 435)			Liver disease mortality (N = 789)		
	Cases	Person-years	HR (95% CI) <sup>a</sup>	Cases	Person-years	HR (95% CI) <sup>a</sup>
Alcohol consumption (drinks/day)						
None	148	1,139,882	1.71 (1.37–2.14)	208	1,374,968	1.88 (1.55–2.28)
<1	172	2,557,091	1.00 (Reference)	225	3,103,481	1.00 (Reference)
1–3	52	741,285	0.97 (0.71–1.32)	135	901,495	2.03 (1.63–2.52)
>3	59	355,629	1.92 (1.42–2.60)	217	428,149	5.84 (4.81–7.10)
Folate intake (µg/day)						
<419.2	169	1,597,053	1.00 (Reference)	287	1,927,547	1.00 (Reference)
419.2–737.0	120	1,604,194	0.77 (0.61–0.97)	289	1,943,762	1.10 (0.93–1.31)
>737.0	146	1,611,672	0.98 (0.79–1.23)	213	1,959,586	0.85 (0.71–1.02)

<sup>a</sup>adjusted for sex, age, race, education, smoking, body mass index, and diabetes.

In the analysis of alcohol and HCC incidence stratified by adjusted folate intake (Table 3), folate had a significant effect on the alcohol–HCC association ( $P_{\text{interaction}} = 0.03$ ). Among the individuals within the first and second tertile of adjusted folate intake, more than 3 drinks of alcohol per day was associated with a significantly increased risk of HCC (1st tertile  $P_{\text{trend}} = 0.002$ ; 2nd tertile  $P_{\text{trend}} = 0.003$ ). Among the individuals with the highest tertile of folate intake, however, there was no association between alcohol consumption and HCC (for 1–3 drinks/day, HR: 1.00; 95% CI: 0.60–1.66, and for >3 drinks per day HR: 0.95; 95% CI: 0.49–1.88,  $P_{\text{trend}} = 0.91$ ). Nondrinkers remained at significantly increased risk of HCC across all tertiles of adjusted folate

intake. Unlike HCC incidence, adjusted folate intake had no effect on the relationship between alcohol and liver disease mortality ( $P_{\text{interaction}} = 0.54$ ). Regardless of the level of adjusted folate intake, more than 3 drinks per day of alcohol were significantly associated with liver disease mortality. Similarly, adjusted folate intake did not affect the increased risk of liver disease mortality that we observed among the nondrinkers, relative to those who drank up to 1 drink per day. A sensitivity analysis that excluded all persons with diabetes resulted in conclusions very similar to those of the entire study population (data not shown).

Overall, the mean adjusted folate consumption in the cohort was 603.9 µg/day, with men having a slightly

**Table 3.** Association between alcohol consumption and the risk of HCC incidence and liver disease mortality, stratified by levels of folate intake among participants of the NIH–AARP Diet and Health Study Cohort

Alcohol consumption (drinks/day)	Folate intake (µg/day)						$P_{\text{interaction}}$
	<419.2		419.2–737.0		>737.0		
	Cases	HR (95%CI) <sup>a</sup>	Cases	HR (95%CI) <sup>a</sup>	Cases	HR (95%CI) <sup>a</sup>	
HCC incidence							
None	52	1.64 (1.13–2.38)	43	1.85 (1.21–2.84)	53	1.67 (1.15–2.44)	
<1	65	1.00 (Reference)	45	1.00 (Reference)	62	1.00 (Reference)	
1–3	22	1.10 (0.67–1.79)	10	0.73 (0.37–1.46)	20	1.00 (0.60–1.66)	
>3	27	2.21 (1.40–3.49)	22	2.66 (1.58–4.49)	10	0.95 (0.49–1.88)	
$P_{\text{trend}}$		0.002		0.003		0.91	0.03
Liver disease mortality							
None	70	1.37 (1.00–1.88)	75	2.18 (1.57–3.02)	63	2.35 (1.64–3.39)	
<1	93	1.00 (Reference)	74	1.00 (Reference)	58	1.00 (Reference)	
1–3	44	1.72 (1.20–2.47)	48	2.09 (1.44–3.01)	43	2.42 (1.62–3.61)	
>3	77	5.19 (3.80–7.10)	91	6.65 (4.83–9.15)	49	5.54 (3.73–8.25)	
$P_{\text{trend}}$		<0.0001		<0.0001		<0.0001	0.54

NOTE:  $P_{\text{trend}}$  based on only 3 categories: <1, 1–3, and >3 drinks of alcohol per day.

<sup>a</sup>adjusted for sex, age, race, education, smoking, body mass index, and diabetes

lower mean intake than women (589.9 vs. 624.6  $\mu\text{g}/\text{day}$ ). The consumption of alcohol was 0.91 drinks per day, with men consuming roughly 3 times more alcohol than women (1.24 vs. 0.43 drinks/day). There were, however, no large differences between the sex-specific results and the results of the overall analysis (Supplementary Table S1). Similarly, findings by alcohol type (wine, beer, and liquor) did not differ from the findings of total alcohol consumption (Supplementary Table S2). Among the individuals in the highest tertile of adjusted folate intake, there was no significant association between any type of alcohol consumption and HCC, whereas alcohol consumption of each type remained associated with liver disease mortality, regardless of folate level.

## Discussion

Alcohol has long been known to be a risk factor for chronic liver disease (13) and its most serious sequela, liver cancer (23–28). The evidence in support of the alcohol–liver cancer association led the International Agency for Research Cancer in 1988 to conclude that there was a causal relationship (12). Whether folate, which has been reported to affect the relationship between alcohol and other cancers, can also alter the relationship between alcohol and liver cancer, however, has not been as well studied. In the current manuscript, we present data from a large prospective study that suggests higher folate consumption may decrease the risk of HCC imposed by alcohol.

Folate is a water-soluble vitamin B that occurs naturally in a wide variety of foods, including among others, leafy green vegetables, citrus fruits, beans, seeds, and nuts. Folate represents an important factor in DNA methylation and replication during cell regeneration, a role that has received increasing attention in human carcinogenesis. In several animal studies, low folate levels have been linked to oxidative stress, liver damage, and liver cancer (29, 30). In a previous human study conducted by our group, folate levels in blood were inversely associated with evidence of liver damage and with development of HCC in a high-risk population (16).

Animal studies exploring the relationship between alcohol consumption and folate absorption report that alcohol seems to inhibit folate uptake via mitochondrial carriers, leading to folate deficiency (31). This deficiency further promotes alcoholic damage to the liver, which can lead to the development of cancer (32). Although few studies have examined the effect of folate on HCC risk, there has been interest in the role of 5,10-methylenetetrahydrofolate reductase (MTHFR), a critical enzyme in the folate (i.e., one-carbon) metabolism pathway. A nonsynonymous single nucleotide polymorphism in *MTHFR* at position 677 leads to an alanine-to-valine substitution in amino acid 222. Individuals who are homozygous for the variant genotype (677TT) have lower enzymatic activity than do individuals with the wild-type genotype (677CC; ref. 33). As a result, individuals with the homozygous variant are less able to convert 5,10-methylenetetrahydro-

folate to 5-methyltetrahydrofolate, which results in mild hyperhomocysteinemia, an outcome also seen with a low dietary intake of folate. Results of studies of the relationship of the *MTHFR* C677T polymorphism to HCC have been inconsistent, but a recent meta-analysis found evidence that the polymorphism was associated with increased risk of HCC (34). An Italian study reported that the association between the *MTHFR* C677T genotype and HCC was only significant among persons with alcohol-related disease, but not other types of liver disease, suggesting that the polymorphism may only increase risk in combination with alcohol exposure (35).

Even though alcohol consumption has declined over time in the United States, chronic alcohol consumption contributes to approximately one-third of HCC cases (10). Similar estimates have been reported from studies in southern Europe (36, 37). For example, the Brescia HCC Case–Control Study in Italy reported that 34% of their cases were associated with heavy alcohol intake (36, 37). While HCV (39%) is associated with a greater proportion of cases in Brescia than is alcohol, reports suggest that alcohol is a greater contributor to HCC risk in the United States than is HCV (10).

In addition to the findings concerning alcohol and folate, several other findings from the current study bear comment. Several previous studies have reported that the threshold for increased risk of HCC with alcohol consumption is 4 drinks/day. The current study, however, found that risk increased at the slightly lower threshold of any amount > 3 drinks per day. Persons drinking less than 1 drink per day had an even lower risk than nondrinkers. Though men consumed more alcohol and less folate than women in the current study, there was no difference in the effect by sex. The current study also found, that nondrinkers had higher risks of developing HCC and dying of liver disease than did persons who had less than 1 drink per day. As has been previously speculated, the nondrinking category almost certainly includes individuals who were former drinkers or whose health precluded them from drinking (39). Unfortunately, however, the study did not collect information concerning drinking patterns prior to the year before baseline. In the current study, there was also some information on self-reported health at baseline. With regard to this, we found that poor health at baseline was reported more frequently among nondrinkers than drinkers, and more frequently among person who died of liver disease than persons who did not die of liver disease.

Why the results differ for liver disease mortality and HCC incidence is not certain as the great majority of HCCs develop in persons with preexisting liver disease. A complication in comparing the results, however, is that one examined incidence of an outcome (HCC) and the other examined mortality due to an outcome (chronic liver disease). As incidence ascertained by a cancer registry is a verified outcome, while mortality based on death certificates is not, the results of the HCC analysis are somewhat better supported than the results of the liver disease

mortality analysis. However, it is possible that the results differ because the persons who died of chronic liver disease had the most advanced form of the disease, upon which folate could have little effect. As noted above, persons who died of liver disease were more likely to report being in poor health at baseline than were people who did not die of liver disease, suggesting that the liver disease in the group who died may have been rather long standing. Whether a high level of folate intake could have prevented the development of alcohol-related liver disease, could not be examined in the current study, as the date of diagnosis of liver disease was not ascertained.

The current study had several notable strengths. The NIH–AARP study is a large cohort study that collected information, prospectively, on a wide variety of factors such as demographic factors, diet, cigarette smoking, body mass index, and diabetes, among others. In addition, all outcome events in the study were confirmed either by a cancer registry (for incident HCC) or by death certificate (for liver disease mortality).

Despite these considerable strengths, the study also had several limitations. The foremost limitation was the lack of biologic samples, which precluded the determination of HBV and HCV status among the study participants. It is conceivable that chronic inflammation and the underlying carcinogenic pathways may differ between viral- and nonviral-related disease, which might affect the role of folate in persons infected with HBV or HCV. For example, a recent Chinese study reported that infected individuals had decreased levels of B vitamins and folate, particularly individuals infected with HCV (40). Another limitation was that folate intake and alcohol consumption were determined only at baseline so changes over time could not be assessed. In addition, the study had no access to the participants' medical records so had no information on the date of diagnosis of liver disease. Finally, the majority of the study members were white so the results may not be generalizable to persons in other racial/ethnic populations.

In conclusion, the current study finds evidence that higher folate intake may be able to ameliorate the effects of alcohol on the development of HCC. As there is, at present, a paucity of data examining this question, further studies of alcohol, folate, and HCC may prove highly informative.

#### Disclosure of Potential Conflicts of Interest

A.R. Hollenbeck is a volunteer member of the Love/Avon Army of Women Scientific Advisory Board and serves as a volunteer elected

Board Member on the Society of Psychologist in Management Board of Directors. No potential conflicts of interest were disclosed by the other authors.

#### Authors' Contributions

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