Relation between homocysteine concentrations and the consumption of different types of alcoholic beverages: the French Supplementation with Antioxidant Vitamins and Minerals Study[1–3]

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ABSTRACT

Background: Previous studies on the effects of alcohol consumption on total plasma homocysteine (tHcy) concentrations showed contradictory results. The conflicting results may derive in part from confounding by the type of alcoholic beverage consumed.

Objective: The objective of the study was to evaluate in a predominantly wine-drinking French population whether the relation between alcohol consumption and homocysteine concentrations is dependent on the type of alcoholic beverage consumed.

Design: In 1996, a cross-sectional study measuring tHcy and red blood cell folate concentrations was conducted in 1196 middle-aged women and men from the French Supplementation with Antioxidant Vitamins and Minerals Study. Intakes of alcohol, energy, coffee, and B vitamins were assessed by 6 separate 24-h dietary records from the previous year.

Results: tHcy concentrations were positively associated with wine intake ($P = 0.01$) in the women and with beer intake in the men ($P = 0.002$). No association with the consumption of spirits was observed. The association between beer consumption and tHcy concentrations in the men was modified by the consumption of wine; the association was positive in wine drinkers, whereas an inverse trend was seen in those who drank no wine.

Conclusion: Wine consumption may increase tHcy concentrations, whereas beer consumption seems to have no effect (or even an inverse effect) on tHcy.   *Am J Clin Nutr* 2003;78:334–8.

KEY WORDS   Alcohol consumption, beer, folate, homocysteine, wine

INTRODUCTION

Many epidemiologic studies have shown that alcohol consumption and the risk of cardiovascular disease are associated in a J-shaped fashion (1–3). Moderate drinkers of alcoholic beverages have a slightly reduced risk, whereas heavy drinking is associated with an increased risk (1–3). This J-shaped effect of alcohol consumption has been explained by similar effects on blood lipids and hemostatic factors (4–7). The concentration of total plasma homocysteine (tHcy) also was suggested as an explanatory factor (8, 9). The concentration of tHcy is a well-established indicator for the risk of cardiovascular disease (10–12), and it seems to be related to alcohol consumption (13–15). The nature of this relation is, however, not fully clarified. Studies performed among alcoholics showed that a chronic intake of alcohol leads to increased tHcy concentrations (16, 17). In contrast, in cross-sectional studies in general populations, inverse associations between alcohol consumption and tHcy were observed (15, 18). It has been suggested that the relation between alcohol consumption and tHcy is also J-shaped, which could explain the seemingly contrasting results from population-based studies and studies among alcoholics (9, 19). One experimental study, however, showed an increase in tHcy concentrations in healthy men after the consumption of 4 glasses of alcohol (20). More interesting, this effect depended on the type of alcohol consumed: the consumption of wine and spirits increased tHcy, but beer consumption had no such effect. It has been suggested that the vitamin B-6 content of beer, an essential factor in the breakdown of tHcy, could counteract the possible detrimental effect of alcohol on tHcy (20). On the other hand, Mar and Zeisel (21) observed that wine contains ≈10 mg betaine/L; betaine is a methyl donor in the methylation of homocysteine, and, in sufficient amounts, it can decrease tHcy. Recently, de Bree et al (22) observed an inverse relation between beer consumption and tHcy concentrations in a population-based study. No effect of wine drinking was seen, but wine consumption in the Netherlands is relatively low.

None of the above-mentioned studies was performed in a predominately wine-drinking population. We therefore studied the possible relation between tHcy concentrations and the consumption of various alcoholic beverages in an adult population in France, where wine drinking is common, to derive a more complete picture on this topic.

SUBJECTS AND METHODS

Subjects

Subjects were participants in the French Supplementation with Antioxidant Vitamins and Minerals Study, an ongoing randomized, double-blind, placebo-controlled, primary-prevention trial designed to evaluate the effects of daily antioxidant (vitamin C, vitamin E, α-carotene, selenium, and zinc) supplementation at nutritional doses on the incidence of cancer and ischemic heart disease. The cohort consists of women aged 35–60 y ($\overline{x} \pm$ SD: 46.4 ± 6.7 y) and men aged 45–60 y ($\overline{x}$: 51.1 ± 4.7 y), none of whom use vitamin supplements other than those under study. Subjects were invited to participate by a multimedia campaign conducted throughout France. Potential subjects received detailed information on the study and performed a self-test of the acceptability of the daily supplement. A total of 12 735 subjects were included at baseline in 1994, and they were followed for 8 y. The recruitment and study design was reported in detail earlier (23). tHcy concentrations were measured in a random subsample of 2000 subjects from both the intervention and placebo groups. Among these 2000 subjects, 1196 had completed ≥ 6 dietary records during the year before the tHcy measurement and were included for the present analyses. The selected subsample did not differ from the total study population in classic cardiovascular disease risk factors or in tHcy concentrations.

The study was approved by the Ethics Committee for Studies on Human Subjects (CCPPRB no. 706) of Paris-Cochin Hospital and the Comité National Informatique et Liberté (CNIL no. 334641), which requires that all medical information be kept confidential and anonymous.

Dietary assessment

Subjects completed a 24-h record every 2 mo, for a total of 6 records per year. The day of the record was randomly allocated to 2 weekend days and 4 weekdays per year in such a way that, by the end of the study, there were records for each day of the week in all seasons. Information was collected with the use of the Minitel Telematic Network (France Telecom, Paris). The Minitel is a small terminal widely used in France as an adjunct to the telephone, and the system can be considered a primitive version of the Internet. At the beginning of the study, participants received (free of charge) a tiny central processing unit specifically developed for the study and loaded with specialized software that allows them to fill out the computerized dietary record offline and to transmit data during brief telephone connections. The software allowed the subjects to communicate with the coordinating center and to ask questions, which were answered within a day by one of the investigators. This software and an instruction manual for the codification of foods guided the participants during the completion of the records. The manual contains photographs showing portions in 3 sizes; with the use of those sizes and 4 hypothetical portions—2 intermediate (volume between that in photographs A and B and volume between that in photographs B and C) and 2 extreme (less than the volume in photograph A and more than the volume in photograph C)—7 choices are available by which the participants could indicate the portion consumed. Photographs of portion sizes were previously validated by using 780 subjects in a pilot study (24). Data on variables such as cooking methods, seasoning, types of foods (eg, fresh, frozen, and canned), and place and time of consumption were also collected. Six dietary records allow the estimation of macronutrient intake with a precision of 90% (25).

Measurements

All measurements were performed at the end of the second study year (1995–1996). Weight and height were measured with subjects in underwear, and body mass index (in kg/m^2) was calculated. Information on smoking habits was obtained by means of a questionnaire. Blood samples were obtained in evacuated tubes (Vacutainer; Becton Dickinson, Le Pont de Claix, France) from participants who had been fasting for 12 h. Plasma tHcy concentrations were measured by using a BioRad kit (Hercule SA, Vitry sur Seine, France) and HPLC with fluorometric detection; the intraassay CV was 6.8% and the interassay CV was 5.7% (26). Folate concentrations were estimated by microbiological assay with the use of *Lactobacillus casei* (*Lactobacillus rhamnosus*, ATCC 7469; Institut Pasteur, Paris) and a folic acid–casei medium (Difco Labs, Detroit) (27). The CV for each assay was < 7%. Red blood cell folate values were calculated by using packed cell volume measured with the use of microcentrifugation at 7000 × *g* for 5 min at room temperature (28). Laboratory quality assurance included analysis of serum from standard pools with each run and, if available, international standards.

Statistical analysis

Intakes of energy, alcohol, vitamins B-6 and B-12, and folate were calculated from food-consumption data (including alcoholic beverages) with the use of the French computerized food-composition table CIQUAL (Agence Française de la Securité des Aliments, Maisons-Alfort, France; 29). Associations between tHcy concentration or red blood cell folate and alcohol consumption were evaluated by using linear regression analyses. These analyses were adjusted for confounding factors (ie, age, smoking, intakes of energy and B vitamins, coffee consumption, and body mass index) that were initially selected on the basis of information from the literature and that were included in the final multivariate model if they influenced the β-coefficient of the dependent variables when compared with a nonadjusted model. To test the linearity of the relations, we included second-order variables in the regression model. All statistical analyses were carried out with SAS statistical software (version 6.2; SAS Institute Inc, Cary, NC).

RESULTS

Eighty-three (13%) of the women did not drink alcohol at all, 551 (85%) of the women did not drink beer, 154 (24%) of the women did not drink wine, and 489 (76%) of the women did not drink spirits (**Table 1**). Only 26 (5%) of the men did not drink any alcohol, 350 (64%) of the men did not drink beer, 48 (9%) of the men did not drink wine, and 272 (50%) of the men did not drink spirits. The intakes of vitamin B-6 and folate due to beer intake in the beer drinkers were 0.015 mg/d and 1.28 μg/d, respectively, for the women and 0.06 mg/d and 4.67 μg/d, respectively, for the men; these intakes accounted for < 1% of the total intakes in the women and for 3.3% and 1.7% of the intakes of vitamin B-6 and folate, respectively, in the men.

In the women, the consumption of wine was positively related to tHcy; a 1-glass increase in wine consumption was associated with an increase in tHcy of 0.50 μmol/L after adjustment for possible confounders (**Table 2**). This was reflected in a positive association between total alcohol consumption and tHcy concentrations. Consumption of neither beer nor spirits was associated with

TABLE 1
General characteristics of the study population

	Women (*n* = 646)	Men (*n* = 550)
Age (y)	47.8 ± 6.4[1]	52.9 ± 4.9
Current smokers (%)	11.8	15.5
BMI (kg/m^2)	23.0 ± 3.6	24.7 ± 2.9
Energy intake (kcal)	1687 ± 447	2299 ± 542
Beer consumption (mL)	11.3 ± 40.4	40.7 ± 91.1
Non–beer drinkers excluded	78.2 ± 12.5	111.9 ± 122.1
(*n* = 95 F, 200 M)		
Wine consumption (mL)	78.9 ± 101.3	217.0 ± 193.0
Non–wine drinkers excluded	103.6 ± 104.5	237.8 ± 189.4
(*n* = 492 F, 278 M)		
Spirits consumption (mL)	3.3 ± 10.1	6.6 ± 14.5
Non–spirits drinkers excluded	13.5 ± 16.9	13.1 ± 18.2
(*n* = 157 F, 278 M)		
Alcohol consumption (g)	8.9 ± 10.6	24.1 ± 20.9
Non–alcohol drinkers excluded	10.2 ± 10.8	25.3 ± 20.7
(*n* = 563 F, 524 M)		
Coffee consumption (mL)	221.2 ± 237.9	205.5 ± 183.6
Homocysteine (μmol/L)	9.0 ± 2.9	11.0 ± 3.7
Red blood cell folate (μmol/L)	269.8 ± 88.6	273.4 ± 85.7
Vitamin B-6 intake (mg)	1.58 ± 0.47	1.99 ± 0.51
Vitamin B-12 intake (μg)	5.81 ± 4.1	7.57 ± 4.76
Folate intake (μg)	262.0 ± 85.6	308.7 ± 88.8

[1] $\bar{x}$ ± SD.

tHcy in the women. In the men, the consumption of beer was positively related to tHcy; a 1-glass increase in beer consumption was associated with an increase in tHcy of 1.48 μmol/L. This was also reflected in a positive relation between total alcohol consumption and tHcy, although it lost its significance after adjustment for confounders (Table 2).

TABLE 2
Regression coefficients and 95% CIs for the association of homocysteine (dependent variable) with different types of alcoholic beverages (independent variables in separate models) and with alcohol

	Women (*n* = 646)		Men (*n* = 550)	
	β	95% CI	β	95% CI
Beer, 1 glass[1] (μmol/L)				
Model 1[2]	0.17	−1.51, 1.85	1.55[3]	0.63, 2.47
Model 2[4]	0.53	−1.20, 2.27	1.48[3]	0.53, 2.43
Wine, 1 glass[5] (μmol/L)				
Model 1	0.62[3]	0.25, 0.99	0.15	−0.09, 0.30
Model 2	0.50[3]	0.12, 0.88	0.04	−0.11, 0.19
Spirits, 1 glass[6] (μmol/L)				
Model 1	0.54	−0.06, 1.14	0.22	−0.32, 0.76
Model 2	0.42	−0.18, 1.02	0.19	−0.36, 0.74
Alcohol, 10 g[7] (μmol/L)				
Model 1	0.44[3]	0.20, 0.68	0.17[3]	0.02, 0.32
Model 2	0.38[3]	0.13, 0.63	0.12	−0.05, 0.29

[1] 250 mL.
[2] Adjusted for age.
[3] Significantly different from 0, $P < 0.05$ (*t* test).
[4] Adjusted for age, smoking, BMI, coffee consumption, and intakes of energy, vitamin B-6, vitamin B-12, and folate.
[5] 150 mL.
[6] 25 mL.
[7] Corresponds to ≈1 glass of alcohol.

TABLE 3
Regression coefficients and 95% CIs for the association between homocysteine (dependent variable) and different types of alcoholic beverages (independent variables in one model)

	Women (*n* = 646)		Men (*n* = 550)	
	β	95% CI	β	95% CI
Model 1[1]				
Beer, 1 glass[2] (μmol/L)	−0.27	−1.42, 0.88	1.50[3]	0.55, 2.45
Wine, 1 glass[4] (μmol/L)	0.59[3]	0.21, 0.97	0.03	−0.24, 0.30
Spirits, 1 glass[5] (μmol/L)	0.39	−0.22, 0.56	0.10	−0.47, 0.67
Model 2[6]				
Beer, 1 glass (μmol/L)	0.29	−1.44, 2.02	1.50[3]	0.53, 2.47
Wine, 1 glass (μmol/L)	0.47[3]	0.08, 0.86	−0.07	−0.36, 0.22
Spirits, 1 glass (μmol/L)	0.31	−0.29, 0.91	0.17	−0.40, 0.74

[1] Adjusted for age.
[2] 250 mL.
[3] Significantly different from 0, $P < 0.05$ (*t* test).
[4] 150 mL.
[5] 25 mL.
[6] Adjusted for age, smoking, BMI, coffee consumption, and intakes of energy, vitamin B-6, vitamin B-12, and folate.

When the 3 types of alcohol were combined in one regression model, only the consumption of wine remained strongly related to tHcy in the women, whereas the consumption of beer remained strongly related to tHcy in the men (**Table 3**). The consumption of spirits was not related to tHcy in the men or in the women. The shape of the observed relation was evaluated by the inclusion of second-order variables for the different types of alcoholic beverages or for total alcohol consumption in the regression model. None of these variables was significant, and the observed associations thus seemed linear.

The positive association between beer consumption and tHcy in the men was further investigated by dividing the men into wine drinkers and those who drank no wine. The positive association between beer consumption and homocysteine was seen only in the wine drinkers, whereas the relation seemed to be inverse in those who drank no wine (**Figure 1**). When this effect modification was tested by including an interaction term for beer and wine (beer × wine) in the regression model, the *P* value of the corresponding α-coefficient was 0.096.
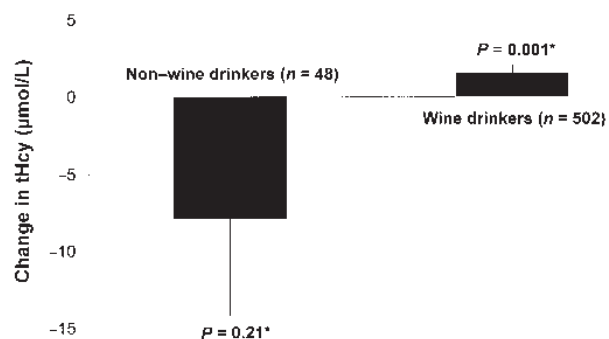
FIGURE 1. Change in mean (± SE) total homocysteine (tHcy) concentrations after a 1-glass increase in beer consumption in the men who drink wine and those who do not drink wine at all, as estimated by multiple linear regression analyses, with adjustment for age, smoking, BMI, coffee consumption, and intakes of energy, vitamins B-6 and B-12, and folate. *For β-coefficient linear regression analyses.

TABLE 4
Regression coefficients and 95% CIs for the association between red blood cell folate (dependent variable) and different types of alcoholic beverages (independent variables in separate models) with adjustment for age, smoking, BMI, coffee consumption, and intakes of energy, vitamins B-6 and B-12, and folate

	Women (*n* = 646)		Men (*n* = 550)	
	β	95% CI	β	95% CI
Beer, 1 glass[1] (μmol/L)	27.18	−33.07, 87.43	5.99	−19.82, 31.80
Wine, 1 glass[2] (μmol/L)	2.41	−14.84, 19.66	12.02[3]	3.40, 20.64
Spirits, 1 glass[4] (μmol/L)	27.34[3]	4.27, 50.41	10.92	−5.54, 27.38
Alcohol, 10 g[5] (μmol/L)	5.05	−5.71, 15.81	7.07[3]	1.79, 12.35

[1] 250 mL.
[2] 150 mL.
[3] Significantly different from 0, *P* < 0.05 (*t* test).
[4] 25 mL.
[5] Corresponds to ≈1 glass of alcohol.

In the men, a positive association was observed between red blood cell folate and wine consumption, which was reflected in a positive association with total alcohol intake (**Table 4**). The consumption of spirits was positively associated with red blood cell folate only in the women. There was no association between the beer consumption and folate status in either the men or the women.

DISCUSSION

In the present study, we have shown that the relation between alcohol consumption and tHcy concentrations is dependent on the type of alcoholic beverage consumed. Furthermore, the association between beer consumption and tHcy concentrations in men was dependent on the consumption of wine.

These results were obtained through cross-sectional analyses, and thus no conclusions about causality can be drawn. However, because the consumption of alcoholic beverages was estimated over the year before the tHcy measurement, it seems likely that alcohol consumption is causally related to tHcy concentrations. Furthermore, the relation between tHcy concentrations and the consumption of alcoholic beverages persisted after adjustment for the major lifestyle determinants of tHcy, and thus a lifestyle related to the consumption of alcoholic beverages probably does not explain the observed associations.

It is well known that tHcy concentrations are greatly elevated in persons with chronic alcoholism (16, 17). This effect is in part due to nutritional deficiencies of folic acid and vitamin B-6 in alcoholics. On the other hand, it was shown that chronic ethanol feeding inhibited methionine synthase in rats (30). There is probably a direct interference of alcohol or its metabolites with the intracellular metabolism of folic acid, vitamin B-6, and vitamin B-12 at more than one site (16).

Although studies on the effects of alcohol on tHcy in the general population showed seemingly contradicting results, it is noteworthy that the studies showing an inverse association were performed in populations that drank little wine and predominantly drank beer. Two studies in the Netherlands and one study in the United Kingdom observed an inverse association between alcohol consumption and tHcy (15, 31, 32). In addition, de Bree et al (22) found an inverse association in a mainly beer-drinking population but no association with the consumption of wine or spirits. Mayer et al (33) observed an inverse relation between beer consumption and

tHcy concentrations in adult residents of Pilsen (Czech Republic). Furthermore, Jacques et al (13) showed that the positive association of alcohol consumption with tHcy concentrations in their study was due to the consumption of wine and spirits only; beer drinking was not associated with tHcy. In a randomized crossover trial in which subjects consumed 4 glasses of wine, spirits, beer, or water, Van der Gaag et al (20) showed, more strongly, that wine and spirit consumption increased tHcy, whereas that of beer did not. Two studies in the United States, one in young women and one in elderly subjects, found a J-shaped relation between total alcohol consumption and tHcy (8, 14). Three studies in middle-aged US subjects observed a positive association between these 2 variables (13, 34, 35), whereas Gudnason et al (36), who evaluated data from various European countries, and Lussier-Cacan et al (37), who studied subjects living in Montreal, did not find any relation between alcohol intake and tHcy. In the present study, we observed a positive association between beer consumption and tHcy concentrations in men. However, when we evaluated the association in those who drank no wine, it became inverse, although not significantly so, probably because of the low numbers in that group [there were too few women who drank beer and no wine (*n* = 12) to explore this effect in women as well]. This finding confirms our hypothesis that beer drinking is inversely related to tHcy in persons who drink no wine. It is possible that the B-vitamin content of beer (vitamins B-12 and B-6 and folate) is enough to counteract the tHcy-increasing effect of the alcohol in beer, but not enough to compensate for the effects of the alcohol in wine consumed in the same period. Apparently the betaine content in wine (21) is too low to counteract the negative effects of alcohol on tHcy. This hypothesis may explain the lack of effect of total alcohol consumption in the European study in which the wine-drinking and beer-drinking populations were mixed (36). That may also have been the case in the study from Montreal (37). If we speculate even further, the observed J-shaped relation between total alcohol consumption and tHcy in 2 studies in young women and elderly subjects (8, 14) may be logical in light of this hypothesis if those subjects who were low alcohol consumers were beer drinkers and those who were high alcohol consumers were wine drinkers.

Finally, the fact that we did not find a positive effect of wine consumption on tHcy in the men can be explained by the positive association between red blood cell folate and wine consumption in these subjects. This relation may have masked the increasing effect of wine consumption. When we adjusted the model for folate (red blood cell folate) status, the association between wine consumption and tHcy indeed increased 10-fold (β-coefficient: 0.43 μmol/L per glass), but it still was not significant (*P* = 0.21). The consumption of spirits in our population was probably too low to show a positive effect on tHcy.

At the start of our study, we hypothesized that a J-shaped relation exists between alcohol consumption and tHcy concentrations. From our results and those of the above-mentioned studies, we must refute this hypothesis, because the consumption of wine and spirits seems to increase tHcy in a linear fashion as a result of the effects of alcohol. Moderate beer consumption, however, seems to have no effect (or even an inverse effect) on tHcy, probably because of the folate, vitamin B-6, and vitamin B-12 contents of beer.

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