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Alcohol and Hepatocellular Carcinoma: The Effect of Lifetime Intake and Hepatitis Virus Infections in Men and Women

F. Donato,[1] A. Tagger,[2] U. Gelatti,[1] G. Parrinello,[3] P. Boffetta,[4] A. Albertini,[1] A. Decarli,[3] P. Trevisi,[1] M. L. Ribero,[5] C. Martelli,[1] S. Porru,[6] and G. Nardi[1]

The authors investigated the dose-effect relation between alcohol drinking and hepatocellular carcinoma (HCC) in men and women separately, also considering hepatitis B and hepatitis C virus infections. They enrolled 464 subjects (380 men) with a first diagnosis of HCC as cases and 824 subjects (686 men) unaffected by hepatic diseases as controls; all were hospitalized in Brescia, northern Italy, in 1995–2000. Spline regression models showed a steady linear increase in the odds ratio of HCC for increasing alcohol intake, for values of >60 g of ethanol per day, with no substantial differences between men and women. Duration of drinking and age at start had no effect on the odds ratio when alcohol intake was considered. Former drinkers who had stopped 1–10 years previously had a higher risk of HCC than current drinkers did. The effect of alcohol drinking was evident even in the absence of hepatitis B or hepatitis C virus infection. In addition, a synergism between alcohol drinking and either infection was found, with approximately a twofold increase in the odds ratio for each hepatitis virus infection for drinkers of >60 g per day. *Am J Epidemiol* 2002;155:323–31.

alcohol drinking; carcinoma, hepatocellular; case-control studies; hepatitis B virus; hepatitis C-like viruses; odds ratio; risk factors

Alcohol intake has been definitely recognized as a cause of chronic liver diseases, including hepatocellular carcinoma (HCC) (1, 2). Alcohol could be involved in the development of HCC through both direct (genotoxic) and indirect mechanisms. An indirect mechanism includes the development of cirrhosis, which is probably the most common pathway to liver carcinogenesis in developed countries (3). We found that 87 percent of the cases of HCC occurring in Brescia, northern Italy, developed in a cirrhotic liver, including most of those attributable to alcohol intake (4).

No agreement exists on the dose-effect relation between alcohol intake and risk of HCC. Some authors argue that the risk of developing liver disease does not increase over a threshold alcohol intake of about 75 g per day (5). The relation between alcohol and HCC also could differ for men and women; for women, a higher susceptibility to liver damage due to alcohol has been suspected on the basis of metabolic differences (6). To date, some epidemiologic studies have evaluated women's risk of cirrhosis for various levels of alcohol intake (7–9), but no known research has yet been conducted to investigate the dose-effect relation between alcohol intake and HCC in men and women separately.

Other aspects of the relation between alcohol drinking and HCC are still unresolved, namely, the effects of type of alcoholic beverage usually consumed, duration of drinking, age at start, and time since quitting. Synergisms between alcohol and hepatitis B virus infection and between alcohol and hepatitis C virus infection in increasing the risk of HCC have been suggested by epidemiologic and pathologic studies (10, 11). However, we know of no data available on the pattern of this interaction for various levels of alcohol intake.

We investigated the relation between alcohol habits and HCC in men and women separately, also taking account of hepatitis B and hepatitis C virus infections. To this end, the decision to perform the study in the Brescia area seemed particularly appropriate because of the high incidence of liver cancer in the area (12) and because alcohol intake is a major cause of HCC (13) and of cirrhosis (14, 15) as well as hepatitis B and hepatitis C virus infections in Italy.

MATERIALS AND METHODS

The study design and preliminary results for a subset of cases and controls have been reported previously (13, 16). Briefly, this hospital-based case-control study was carried

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Abbreviations: HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; OR, odds ratio.

[1] Cattedra di Igiene, Università di Brescia, Brescia, Italy.
[2] Istituto di Virologia, Università di Milano, Milano, Italy.
[3] Cattedra di Statistica Medica, Università di Brescia, Brescia, Italy.
[4] International Agency for Research on Cancer, Lyon, France.
[5] Cattedra di Igiene, Università di Milano, Milano, Italy.
[6] Cattedra di Medicina del Lavoro, Università di Brescia, Brescia, Italy.

Reprint requests to Dr. Francesco Donato, Cattedra di Igiene, Università di Brescia, Via Valsabbina 19, 25123 Brescia, Italy (e-mail: donato@med.unibs.it).

out in the province of Brescia (population about 1 million inhabitants), northern Italy. We prospectively recruited as cases 464 of 496 patients (93.5 percent of those eligible) with a first diagnosis of HCC who were admitted to the two main hospitals in the area between January 1995 and April 2000. We enrolled as controls 824 of 857 subjects (96.1 percent of those selected) who were admitted to the departments of ophthalmology, dermatology, urology, surgery, cardiology, and internal medicine of the same hospitals and were unaffected by liver disease or malignant neoplasms. Subjects hospitalized for injuries were also excluded because of the relation between such conditions and alcohol abuse. The controls were frequency matched with cases on age (±5 years), sex, and date and hospital of admission. Both cases and controls were born in Italy, lived in the province of Brescia, and were less than 76 years of age. Among HCC cases, 84.7 percent were diagnosed by histology or cytology or had alpha-fetoprotein serum levels of >500 ng/ml; the remaining cases were diagnosed on the basis of sonography or computerized tomography.

The project was approved by a local ethics committee. Written informed consent was obtained from all patients.

At the hospital, a standardized questionnaire was used to interview cases and controls about their history of alcohol drinking (17). We assessed total alcohol intake according to the average ethanol content, by volume, of wine (12 percent), beer (5 percent), and spirits (40 percent). Subjects who reported at the time of the interview that they consumed alcohol were considered current drinkers. Subjects who had stopped drinking at least 1 year previously were considered former drinkers, while those who had stopped more recently were included among current drinkers.

The questionnaire used for collecting information on history of alcohol intake has been described in detail by Corrao et al. (17). Briefly, each subject's life was divided into decades, from age 20–29 to 60 years or more, and his or her usual daily or weekly intake during the decade was collected for each type of alcoholic beverage. The dates of beginning and stopping alcohol intake were also recorded to compute duration of drinking, age at start, and time since quitting (for former drinkers).

Given the evidence of a latency period of at least 5 years for alcohol-related onset of cirrhosis (18) and of the same interval between onset of cirrhosis and HCC development (19), we evaluated two measures of alcohol intake in the past as exposure variables. First, we considered intake claimed by the subject during the decade of his or her life in which consumption was the highest, according to the structure of the questionnaire ("peak"). Second, we computed average intake of alcohol during the period of regular consumption. Both measures were computed in grams of ethanol per day. The two variables showed a very high correlation and provided similar estimates of association with HCC, but peak intake showed a better fit in statistical models. Therefore, only those results according to the latter measure are reported in this paper.

Sera were collected and were tested for hepatitis B surface antigen (HBsAg) and anti–hepatitis C virus antibodies by using commercial immunoassays. Sera positive for anti–hepatitis C virus were further investigated for hepatitis C virus RNA by using reverse transcriptase-polymerase chain reaction (RT-PCR), with nested primers of the 5′ noncoding region, as described previously (16).

The odds ratios of HCC, and their 95 percent confidence intervals, were computed as estimates of the relative risks with unconditional logistic regression analysis by the maximum likelihood method; the BMDP statistical package (BMDP Statistical Software, Los Angeles, California) was used. Sex, age, area of residence (the town and surrounding municipalities vs. the rest of the province), and HBsAg and hepatitis C virus RNA were included in regression models as possible confounders. In addition to traditional logistic regression models that included ordinal variables, unconditional logistic regression with restricted cubic regression splines was also performed (20) to investigate the shape of the dose-effect relation; the S-Plus software package (MathSoft Inc., Seattle, Washington) was used. The Akaike Information Criterion (21) was used for a data-based choice of the number of knots in the models. Four knots were defined according to this procedure: 0, 44, 88, and 176 g/day. Bootstrap techniques were also used to evaluate the robustness of the estimates (22).

The interaction between alcohol intake and hepatitis B and hepatitis C virus infections was evaluated by fitting additive models, since they enable a practical and biologic interpretation of the interactive effects of epidemiologic research, and the *S* synergy index proposed by Rothman (23) was calculated as $S = [\text{odds ratio (OR)}(AB) - 1)]/[\text{OR}(A) + \text{OR}(B) - 2]$, in which OR(*AB*) denotes the odds ratio for joint exposure, OR(*A*) the odds ratio for exposure to one single factor, and OR(*B*) the odds ratio for exposure to the other single factor; subjects unexposed to either factor were taken as the reference category for all calculations. To evaluate the interaction between alcohol intake and hepatitis B and hepatitis C virus infections on the additive scale, we dichotomized the former variable as 0–60 g/day (nondrinkers and moderate drinkers) and >60 g/day ("heavy" drinkers) according to the threshold of 3–4 units of alcohol (one unit corresponds to a glass of wine, a can of beer, or a measure of spirits and contains 10–12 g of ethanol) considered necessary for alcohol-mediated injury to develop (24).

RESULTS

A total of 464 HCC cases and 824 controls were enrolled. The distribution of cases and controls by sex, age, and residence is shown in table 1. Mean age was 64.2 years (standard deviation, 7.6) for cases and 63.8 years (standard deviation, 18.4) for controls. Fewer cases than controls resided in Brescia ($p < 0.001$).

The distribution of subjects according to alcohol drinking and to hepatitis C and hepatitis B virus infection markers, and the corresponding odds ratios and 95 percent confidence intervals, are set out in table 2. Alcohol consumption was common among men, since only 2.1 percent of cases and 6.1 percent of controls had never drunk alcohol (abstainers); for women, 28.6 percent of cases and 39.1 percent of controls

TABLE 1. Demographic characteristics of cases and controls included in a study to investigate the dose-effect relation between alcohol drinking and hepatocellular carcinoma, Brescia, Italy, 1995–2000

Characteristic	Cases		Controls	
	No.	%	No.	%
Sex				
Male	380	81.9	686	83.3
Female	84	18.1	138	16.7
Age (years)				
40–49	22	4.7	49	5.9
50–59	85	18.3	170	20.6
60–69	230	49.6	365	44.4
70–75	127	27.4	240	29.1
Residence				
City of Brescia	210	45.3	527	64.0
Rest of the province	254	54.7	297	36.0
Total	464		824	

were abstainers. Controls were affected by a variety of acute and chronic diseases, such as those involving the eye, skin, and urogenital tract, and other disorders treated in medicine and surgery departments of general hospitals. The proportion of drinkers of >60 g/day of ethanol did not vary among controls according to groups of diseases ($p = 0.15$) (data not shown). Both hepatitis C virus RNA and HBsAg positivity were found in a much higher proportion of cases than controls. Significantly increased odds ratios were found for former alcohol drinkers and for the presence of hepatitis C and hepatitis B virus infection in both men and women.

The distribution of cases and controls according to categories of alcohol intake, and the corresponding odds ratios for men and women, are shown in table 3. Of the women, few subjects claimed to have drunk >80 g/day of alcohol; therefore, the categories of consumption above that level were collapsed. For more than 21–40 g/day of alcohol intake, the odds ratio for HCC showed a monotonic increase with increasing intake for both men and women. The odds ratio was significantly higher than the null value for drinking 81–100 g/day and for higher categories of intake.

Both hepatitis B and hepatitis C virus infections negatively confounded the effect of alcohol intake. In fact, after HBsAg and hepatitis C virus RNA were excluded from the regression model, the odds ratios for 81–100, 101–120, 121–140, and >140 g/day of intake changed to 1.6, 2.7, 3.3, and 3.7, respectively, for men (for a comparison, refer to the odds ratios in table 3). Accordingly, the odds ratio for an intake of >80 g/day decreased to 7.5 for women. Among HCC cases, the proportion of subjects positive for either HBsAg or hepatitis C virus RNA decreased steadily with increasing alcohol intake, from 90.6 percent for abstainers to 40.2 percent for those drinking >140 g/day (chi-square test for linear trend: $p < 0.001$), but it did not vary among controls ($p > 0.1$) (data not shown in the tables).

The spline regression models confirmed the results of the categorical analysis, showing a monotonic trend of an increasing odds ratio with increasing alcohol intake for both sexes ($p = 0.001$, C index $= 0.85$). For men, the curve showed that the odds ratio increased steadily and was definitely over the null value for an intake of more than 60 g/day, and there was a plateau at the highest levels of intake (figure 1). Likewise, for women, a clear increase in the odds ratio was observed for an intake of 60–100 g/day, whereas no risk could be defined for higher intake because of a lack of controls in this category (figure 2). For both sexes, wider 95 percent confidence intervals were found for higher values of alcohol intake, suggesting an imprecise definition of the risk function for high levels of consumption.

Among male drinkers, all but five cases and nine controls drank wine regularly, with or without beer or spirits. Therefore, we could not compare drinkers of wine with

TABLE 2. Distribution of cases and controls according to alcohol drinking and HCV* and HBV* status of men and women, Brescia, Italy, 1995–2000

Risk factor	Men						Women					
	Cases		Controls		OR*,†	95%CI*	Cases		Controls		OR†	95% CI
	No.	%	No.	%			No.	%	No.	%		
Alcohol drinker												
Never drinker	8	2.1	42	6.1	Reference		24	28.6	54	39.1	Reference	
Former drinker	151	39.7	89	13.0	8.5	3.3, 22.3	31	36.9	19	13.8	2.8	1.0, 7.9
Current drinker	221	58.2	555	80.9	2.7	1.1, 6.8	29	34.5	65	47.1	0.9	0.3, 2.3
HCV status												
Anti-HCV–, and anti-HCV+ and HCV RNA–	248	65.3	663	96.6	Reference		31	36.9	128	92.8	Reference	
HCV RNA+	132	34.7	23	3.4	17.6	10.7, 28.8	53	63.1	10	7.2	33.3	13.6, 81.3
HBV status												
HBsAg*–	290	76.3	645	94.0	Reference		68	81.0	134	97.1	Reference	
HBsAg+	90	23.7	41	6.0	6.9	4.4, 10.7	16	19.0	4	2.9	23.2	6.2, 86.6

* HCV, hepatitis C virus; HBV, hepatitis B virus; OR, odds ratio; CI, confidence interval; HBsAg, hepatitis B surface antigen.
† Adjusted for age, residence, alcohol drinking, HBsAg, and HCV RNA by using unconditional logistic regression analysis.

TABLE 3. Percentages of cases and controls and odds ratios and their 95% confidence intervals according to lifetime alcohol intake ("peak" exposure)* for men and women, Brescia, Italy, 1995–2000

Alcohol intake (g/day)	Men			Women		
	Cases/controls (no.)	OR†,‡	95% CI†	Cases/controls (no.)	OR‡	95% CI
0	8/42	Reference		24/54	Reference	
1–20	24/56	2.3	0.7, 7.2	22/49	0.6	0.2, 1.7
21–40	27/101	0.9	0.3, 2.7	15/19	1.4	0.4, 5.4
41–60	44/130	1.6	0.5, 4.6	11/10	1.9	0.4, 8.1
61–80	33/89	2.4	0.8, 7.1	4/3	3.1	0.3, 29.7
81–100	62/112	4.2	1.5, 11.7	8/3§	16.5§	3.0, 90.1
101–120	47/50	7.7	2.7, 22.7			
121–140	48/38	9.8	3.3, 29.1			
>140	87/68	11.0	3.9, 31.0			
Total	380/686			84/138		

* "Peak" exposure, maximum intake of alcohol claimed by the subject during a decade in his or her lifetime.
† OR, odds ratio; CI, confidence Interval.
‡ Adjusted for age, residence, hepatitis B surface antigen, and hepatits C virus RNA by using unconditional logistic regression analysis.
§ ≥81 g/day.

drinkers of other beverages. Accordingly, no analysis of HCC risk according to type of alcoholic beverage could be performed for women because of the small number of subjects who claimed to have drunk beverages other than wine.

Duration of alcohol drinking, age at start, and time since stopping the habit were analyzed while also taking into account alcohol intake, which was included in the model as a categorical variable; adjacent intake categories were collapsed (from 0–20 to >140 g/day) to avoid problems of model fitting. No increase in HCC risk was found for duration of drinking and age at start when intake was considered (table 4). Among former drinkers versus current drinkers, the odds ratio for men was significantly higher for those who had stopped less than 10 years previously. For women, no clear pattern of risk for time since stopping was evident because of the small number of quitters. The effect of time since peak intake on HCC risk was analyzed to investigate the possible existence of a latency period between exposure and the disease. The dose-effect HCC risk curves for time since peak intake at various levels of alcohol intake showed a monotonic, linear increase in risk with increasing time (data not shown in the tables).

The interaction between alcohol intake and hepatitis B and hepatitis C virus infection was investigated after excluding 14 cases and one control who had both infections. A monotonic trend of increasing odds-ratio logarithm with increasing alcohol intake was observed for subjects with and without hepatitis B or hepatitis C virus infection (figure 3). For each level of intake, the dose-effect curves showed the highest odds-ratio values for subjects with hepatitis C virus infection, followed by those with hepatitis B virus infection and finally by those without hepatitis virus infection. The

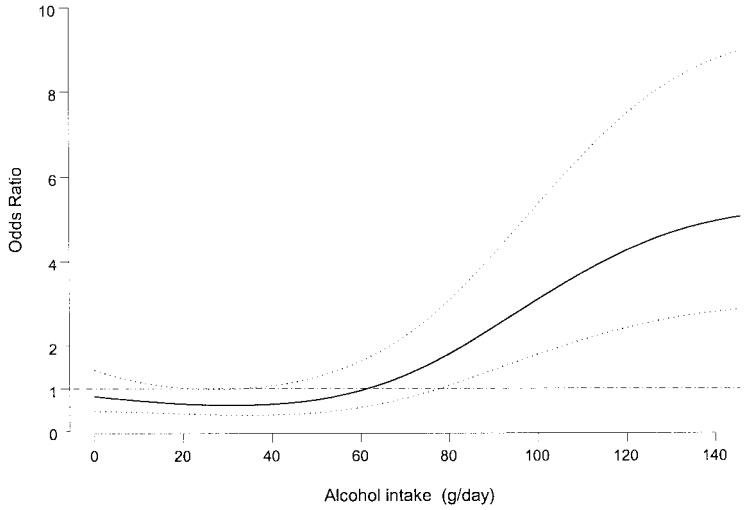

FIGURE 1. Odds ratios and their 95% confidence intervals (dotted lines) for hepatocellular carcinoma in men, according to alcohol intake, obtained by fitting spline regression models that included age, residence, hepatitis B surface antigen, and hepatitis C virus RNA as covariates, Brescia, Italy, 1995–2000.

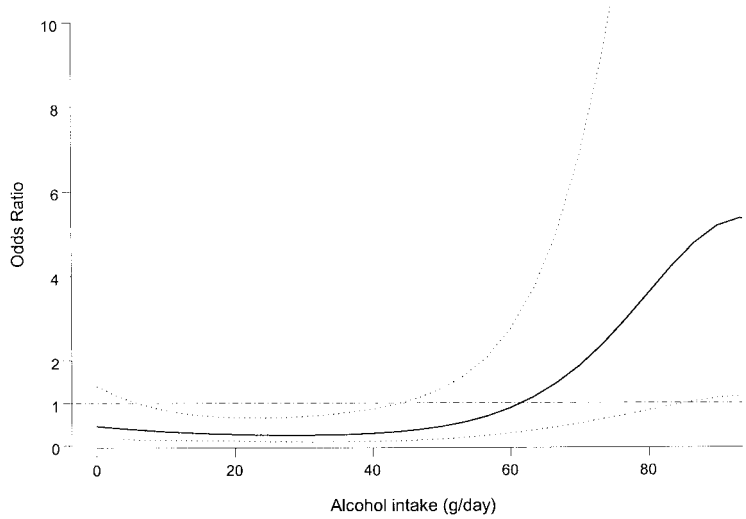

FIGURE 2. Odds ratios and their 95% confidence intervals (dotted lines) for hepatocellular carcinoma in women, according to alcohol intake, obtained by fitting spline regression models that included age, residence, hepatitis B surface antigen, and hepatitis C virus RNA as covariates, Brescia, Italy, 1995–2000.

interaction was not significant when the likelihood ratio test was used ($p > 0.1$), suggesting parallelism of the curves.

Similar results were found when alcohol intake was considered as a dichotomous variable (0–60, >60 g/day); drinkers of 0–60 g/day (abstainers and "light" drinkers) who were negative for both infections were taken as the reference, and subjects with both hepatitis B and hepatitis C virus infection were excluded. As shown in table 5, the odds ratio for drinking >60 g/day of ethanol was 7.0 for subjects negative for both infections, whereas the odds ratios for drinking 0–60 and >60 g/day were, respectively, 55 and 109 for subjects positive for hepatitis C virus RNA and 22.8 and

TABLE 4. Distribution of cases and controls and odds ratios and their 95% confidence intervals according to duration of alcohol drinking, age at start, and time since stopping the habit, Brescia, Italy, 1995–2000

Alcohol habit	Men			Women		
	Cases/controls (no.)	OR*,†	95% CI*	Cases/controls (no.)	OR†	95% CI
Duration of drinking (years)						
0	8/42	Reference		24/54	Reference	
1–20	22/33	1.7	0.5, 5.8	7/8	2.3	0.4, 14.2
21–30	55/93	1.3	0.4, 3.9	8/14	0.7	0.1, 3.2
31–40	121/199	1.4	0.5, 4.0	21/24	0.7	0.2, 2.5
>40	174/319	1.2	0.4, 3.7	24/38	0.6	0.2, 1.9
Age at start (years)						
No intake	8/42	Reference		24/54	Reference	
18–24	220/328	0.9	0.6, 1.3	31/25	0.7	0.3, 2.3
25–34	124/247	0.5	0.3, 1.1	20/37	0.7	0.1, 4.1
35–44	24/53	0.7	0.2, 2.9	5/14	1.3	0.2, 9.0
≥45	4/16	0.6	0.2, 1.9	4/8	1.2	0.4, 3.7
Time since stopping (years)						
Current drinkers	221/555	Reference		29/65	Reference	
1–5	66/30	5.0	2.9, 8.6	9/7	3.0	0.6, 15.2
6–10	51/24	4.0	2.2, 7.4	12/5	2.7	0.5, 13.6
11–15	14/12	1.6	0.6, 4.5	3/2	1.9	0.2, 19.2
>15	20/23	1.4	0.6, 3.1	7/5	8.6	1.3, 56.0
Never	8/42			24/54		

* OR, odds ratio; CI, confidence interval.

† Adjusted for age, residence, hepatitis B surface antigen, hepatitis C virus RNA, and alcohol intake by using unconditional logistic regression analysis.

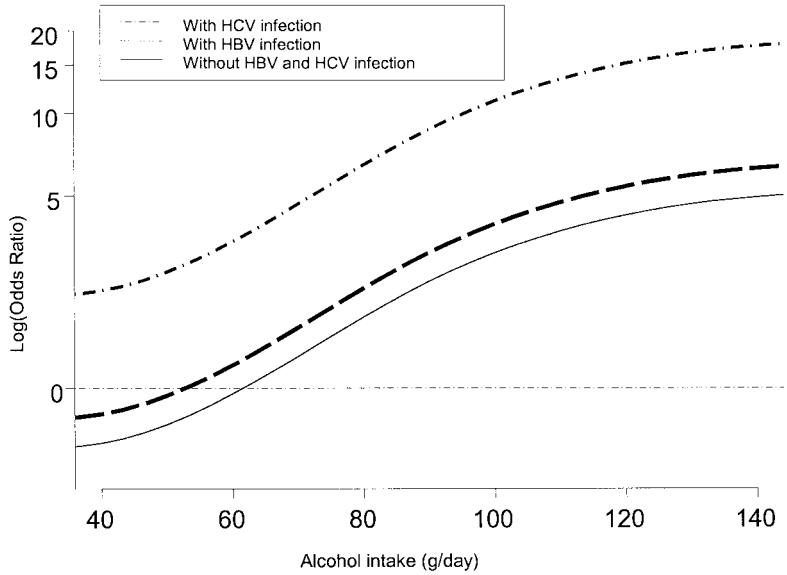

FIGURE 3. Odds ratios for hepatocellular carcinoma, according to alcohol intake and the presence of hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, obtained by fitting spline regression models that included age and residence as covariates, hepatitis B surface antigen, and hepatitis C virus RNA, Brescia, Italy, 1995–2000. Men and women were analyzed together.

48.6 for subjects positive for HBsAg. The synergy indexes for the interaction between drinking >60 g/day and the presence of hepatitis virus infection were 1.8 for hepatitis C and 1.7 for hepatitis B virus infection.

DISCUSSION

The main results of this study follow: 1) the risk of HCC increased with increasing level of alcohol intake, irrespective of duration of consumption and age at start; 2) curves for HCC risk by alcohol intake were fairly similar for men and women, showing a clear increase in the odds ratio for subjects drinking >60 g/day of ethanol; 3) for former drinkers, the risk was highest for those who had stopped drinking less than 10 years previously; and 4) a synergism between alcohol intake and hepatitis virus infections was evident, with a more than additive but less than multiplicative increase in risk.

A cause-effect relation between alcohol intake and liver disease, including HCC, has been demonstrated (1). However, no conclusive data are available on the dose-effect curve or the level of regular intake "safe" for the liver ("threshold" of no chronic damage). Most epidemiologic studies conducted on the relation between alcohol and HCC have not had enough power to investigate more than two or three categories of intake. Only one study found a linear trend of increasing risk with increasing intake (25), whereas the others found an elevated risk for arbitrarily chosen "high" levels of consumption (26–30). We addressed the issue in a geographic area with a high incidence of HCC and a relevant role of alcohol consumption as a cause of the disease. Using both a traditional, category-based modeling approach and spline regression as alternative models based on continuous variables, we found a linear increase in HCC risk with increasing alcohol intake for both sexes, from a level of about 60 g/day of ethanol. The shape of the alcohol intake curve was undefined for >140 g/day for men and >100 g/day for women because of the small number of controls claiming to have drunk so much. This finding is not surprising since controls were unaffected by liver disease, and it is reasonable to assume that very few or no subjects who have drunk >100–140

TABLE 5. Distribution of cases and controls and odds ratios and their 95% confidence intervals according to alcohol intake and the presence of HCV* and HBV* infection,† Brescia, Italy, 1995–2000

HCV or HBV infection	Alcohol intake (g/day)					
	0–60			>60		
	Cases/controls (no.)	OR*,‡	95% CI*	Cases/controls (no.)	OR‡	95% CI
Neither	30/412	Reference		157/335	7.0	4.5, 11.1
HCV infection	95/21	55.0	29.9, 101.0	76/11	109	50.9, 233.0
HBV infection	41/27	22.8	12.1, 42.8	51/17	48.6	24.1, 98.0

* HCV, hepatitis C virus; HBV, hepatitis B virus; OR, odds ratio; CI, confidence interval.
† Subjects with both infections were excluded; men and women were considered together.
‡ Adjusted for age, residence, and sex by using unconditional logistic regression analysis.

g/day are still free from liver disease at the average age of 64 years. Since most cases of HCC develop in the cirrhotic liver in this area of Italy (4), these findings are comparable with Italian studies on the etiology of cirrhosis, which also showed a linear increase in risk with increasing alcohol intake, from 31–60 (31) or 75–100 (15) g/day. A recent cohort study of patients with alcoholic fatty liver found that only those who drank >40 g/day of alcohol were at risk of developing cirrhosis (32).

We found no strong evidence for substantial differences between men and women in the HCC risk curves by alcohol intake, in contrast to the hypothesis that women are more susceptible to liver damage from alcohol. However, because of the small number of women who drank a medium-high amount of alcohol, these results should be considered cautiously, especially for controls. On the other hand, the few epidemiologic studies that have investigated the risk of cirrhosis in both men and women included only small numbers of female drinkers (7, 33). Therefore, no definite conclusion on this matter can be drawn at present, and larger studies or meta-analyses are necessary.

No influence of duration of intake or age at start was found on HCC risk when intake was taken into account, in agreement with recent studies on alcohol and cancer of the upper digestive tract (34–37) and on alcohol and liver cirrhosis (14). These findings suggest that a low intake for a long time is likely to cause less damage than a similar cumulative level of consumption distributed over a shorter period.

We found a higher risk of HCC for former than for current alcohol drinkers, probably because many people with HCC had stopped drinking some years prior to the interview because they had early symptoms of liver disease (a reverse of the cause-effect relation). These findings are in agreement with those from studies on alcohol and cancer of the upper digestive tract, which show a higher risk for former than for current drinkers (34, 37), a peak in risk immediately after stopping (37), and no clear decline in risk up to 10 years after stopping (34, 37). Taken together, these results are compatible with the role of alcohol as a cause of HCC at both the initial and final stages of the process, as occurs with other alcohol-related cancers.

This study confirmed our previous observations (13, 16) that alcohol drinking has a "pure" effect in increasing the risk of HCC and that its effect can be modified by hepatitis B or hepatitis C virus infection. Note that, among HCC cases, the proportion of those with hepatitis B or hepatitis C virus infection declined progressively with increasing alcohol intake, from 91 percent among abstainers to 40 percent among drinkers of >140 g/day, but it did not decline among controls. This finding suggests that HCC rarely, if ever, develops in the absence of either alcohol intake or hepatitis virus infection in this area of Italy. This fact may explain the negative confounding we observed between alcohol and viral hepatitis infection due to a mechanism of competition between concurrent factors. As an additional explanation, people with chronic viral hepatitis may reduce their alcohol intake to prevent liver damage, which would inflate the categories of nondrinkers and light drinkers for those who subsequently develop HCC.

As regards the interaction between alcohol consumption and viral hepatitis, we found that the odds ratios for hepatitis C and for hepatitis B virus infection approximately doubled when intake level increased from 0–60 to >60 g/day. Accordingly, the synergy index between an intake of >60 g/day and each hepatitis virus infection was 1.8 for hepatitis C and 1.7 for hepatitis B virus infection. This finding confirms our previous analyses based on lower numbers of cases and controls (13, 16), and it suggests a more than additive but less than multiplicative effect between heavy alcohol intake and each hepatitis virus infection.

Previous research on the combined effects of alcohol intake and hepatitis B virus infection was inconclusive. Alcohol intake was associated with a higher risk of HCC for HBsAg carriers in one cohort study (38) but not another (39). In a cross-sectional study, the prevalence of elevated values of transaminases in HBsAg carriers increased with alcohol consumption (40). In subjects with alcoholic disease, a higher prevalence of HBsAg was found among patients with HCC than those without (41). On the other hand, heavy drinkers in one study did not have a higher risk of cirrhosis if they were positive for hepatitis B virus serologic markers (42); in another study, hepatitis B virus infection did not increase the influence of alcohol drinking in producing cirrhosis (43). The most likely explanations for these contrasting results are the different levels of alcohol intake in different populations and the low power of these studies because of the small numbers of subjects with both hepatitis B virus infection and "heavy" alcohol intake. In fact, hepatitis B virus infection is a rare cause of liver disease in North America and northern Europe, whereas heavy alcohol intake is uncommon in east Asian countries that have a high prevalence of HBsAg carriers. In contrast, strong evidence supports the hypothesis of a synergism between hepatitis C virus infection and alcohol in causing liver disease. Among people with hepatitis C virus-related disease, those with cirrhosis or HCC had a higher alcohol intake than those with less advanced disease (44). Cohort studies conducted among subjects with hepatitis C virus liver disease have found that alcohol intake increases the rate of progression of fibrosis (45) and of carcinogenesis (46, 47). Reciprocally, studies among alcoholics showed a greater severity of liver disease and a higher risk of HCC in the presence of hepatitis C virus infection (42). Finally, a recent Italian case-control study found a more than additive effect between hepatitis C virus infection and an alcohol intake of >50 g/day on the risk of cirrhosis (15). A model of liver carcinogenesis by alcohol intake that shows both its early and late effects and its interaction with hepatitis virus infections is shown in figure 4.

Finally, some issues regarding the design of the study should be addressed in considering the validity of these findings. Regarding reliability of the information on alcohol intake, data collected through face-to-face interviews have been found to be sufficiently reliable and valid in Italy (48) also because of the pattern of drinking in the country, where most alcohol intake is accounted for by regular consumption

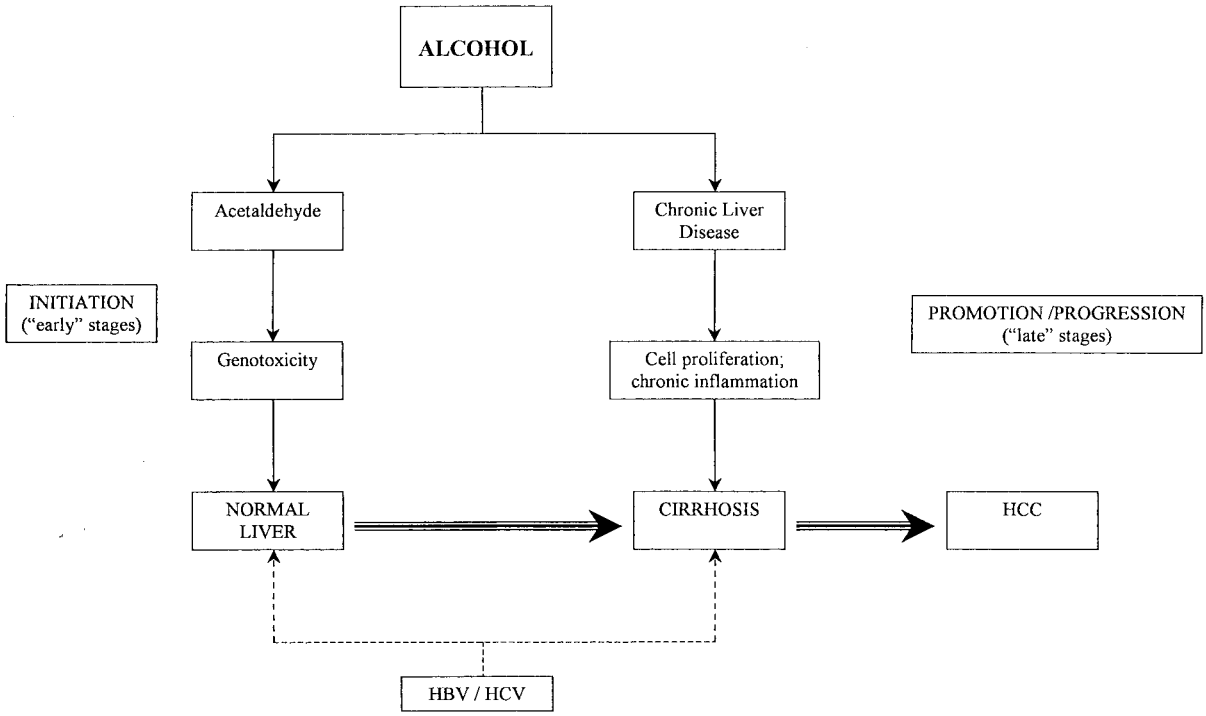

FIGURE 4. Proposed model of liver carcinogenesis by alcohol intake: the effects of early and late intake and interaction with hepatitis virus infection. HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus.

of wine with meals. Moreover, previous research has shown that when people were interviewed in the hospital setting using the same questionnaire as the one used in this study, information on lifetime alcohol intake was highly reliable: the intraclass correlation coefficient between interviews with patients and their relatives was 0.82 for lifetime alcohol intake (17).

Choosing hospital controls to estimate the prevalence of exposure in the study base is another matter of concern. However, population-based case-control studies may also be affected by selection bias if participants are asked to provide blood samples. To prevent selection bias, we excluded from the control series people hospitalized for liver disease, cancer, and other possibly alcohol-related conditions such as injuries. Controls were affected by a wide range of acute and chronic diseases, and we verified that the prevalence of drinkers of >60 g/day of ethanol did not vary according to group of diseases. However, the drinking habits of the male controls recruited for our study were similar to those found in case-control studies performed in Italy on alcohol drinking and other neoplasms (34, 35, 49): very few subjects were abstainers, about 30 percent claimed to drink at least 80 g/day of alcohol, and almost all drinkers drank wine, with or without beer or spirits. Finally, the seroprevalence of HBsAg and anti–hepatitis C virus/hepatitis C virus RNA positivity among controls is in agreement with that found in Italian case-control studies on the etiology of cirrhosis (9, 15).

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Brescia HCC Study participants: *Università di Brescia*—Drs. N. Portolani, M. Ronconi, S. Ghidoni, D. Placidi, and A. Carta; *Spedali Civili di Brescia*—Drs. L. Bettini, M. G. De Tavonatti, G. Pelizzari, E. Radaeli, M. Puoti, F. Bonetti, M. Ghirardi, and M. Favret; *Ospedale S. Orsola di Brescia*—Drs. A. Salmi, G. Lanzani, L. Biasi, A. Savio, and M. Garatti; *Ospedale Poliambulanza di Brescia*—Dr. M. Graffeo.

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