Glycemic load in relation to hepatocellular carcinoma among patients with chronic hepatitis infection

P. Lagiou1,2, M. Rossi3, A. Tzonou1, C. Georgila1, D. Trichopoulos1,2 & C. La Vecchia3,4*

1Department of Hygiene, Epidemiology and Biostatistics, University of Athens Medical School, Athens, Greece; 2Department of Epidemiology, Harvard School of Public Health, Boston, USA; 3Department of Epidemiology, Mario Negri Institute for Pharmacological Research; 4Institute of Medical Statistics and Biometry and G. A. Maccacaro, University of Milan, Milan, Italy

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Background: Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are of paramount etiologic importance for hepatocellular carcinoma (HCC), but other factors are likely to be important. The association of diabetes mellitus and obesity with HCC raises the possibility that dietary glycemic load (GL) may interact with chronic hepatitis infection in the causation of HCC.

Patients and methods: We conducted a case–control study of 333 HCC patients and 360 controls in Athens, Greece. Third-generation assays were used to determine chronic HBV and HCV infection and information from a semiquantitative food frequency questionnaire to estimate dietary GL.

Results: After adjustment for possible confounding factors through multiple logistic regression, we found a nonsignificant positive association between GL and HCC, which was exclusively accounted for by a positive association between GL and HCC cases with chronic infection with hepatitis B and/or C. For the latter group of patients, the odds ratio at the highest compared with the lowest GL quintile was 1.95 (95% confidence interval 1.09–3.48). The association was strengthened after exclusion of subjects with diabetes.

Conclusion: Our results indicate that, among patients with chronic infection with HBV and/or HCV, reduction of dietary GL could reduce risk or delay development of HCC.

Key words: diabetes mellitus, diet, glycemic load, hepatitis B virus, hepatitis C virus, hepatocellular carcinoma

We have investigated the association between GL and HCC positive and negative for chronic infection with hepatitis B and/or C in a large case–control study in Greece, a country in which HCC is very common [12, 13].

patients and methods

recruitment

From January 1995 to December 1998, 374 incident cases of HCC were admitted to three teaching hospitals in Athens (Hippokration, Western Attica and Laiko General Hospital). Forty-one (11%) of the HCC cases identified in these hospitals during the study period were not enrolled, for various reasons (mainly refusals and difficulty in coordinating collection of blood samples in the context of standard medical care). For the 333 cases included in the study, HCC diagnosis was based on biopsy (n = 157), elevated α-fetoprotein level (n = 159) or echotomography and/or other methods (n = 17) [14, 15].

For each case with HCC, we attempted to select one control patient from the same hospital, matching for gender and age (±5 years). By protocol, controls had to be patients with noncancer disorders usually requiring minor surgery. We opted for controls admitted in the same hospitals for injuries or for eye, ear, nose or throat conditions, that is conditions unrelated to smoking, alcohol intake or diet. There were 25 refusals (6%), and a properly matching control could not be identified for some HCC cases. Eventually, 360 control subjects were included in the analysis.

original article

introduction

Hepatocellular carcinoma (HCC) is the major form of primary liver cancer, which ranks third among men and sixth among women in terms of cancer mortality around the world [1]. Several causal factors have been identified, including tobacco smoking and excessive alcohol drinking [2], but chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are of paramount etiologic importance [3]. Nevertheless, a minority of those infected with HBV and/or HCV develop HCC, which indicates that interacting factors may be involved. The association of diabetes mellitus with HCC, which has been demonstrated in both case–control [4, 5] and cohort [6, 7] studies, raises the possibility that dietary glycemic load (GL), a known risk factor for diabetes mellitus [8], may be involved either as an independent cause or as a factor interacting with chronic hepatitis infection. This consideration is strengthened by the fact that obesity, which appears to be positively associated with dietary GL [9], is a risk factor for liver cancer, as documented in cohort studies [10, 11].

*Correspondence to: Prof. C. La Vecchia, Department of Epidemiology, Istituto di Ricerche Farmacologiche ‘Mario Negri’, via La Masa 19, 20156 Milan, Italy. Tel: +39-02-39014-527; Fax: +39-02-33200231; E-mail: lavecchia@marionegri.it

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At the time the study was conducted, no formal institutional review board existed in the University of Athens. Nevertheless, all subjects were provided with detailed information about the study and had to consent in order to participate. The study was conducted according to the principles of the Helsinki Declaration.

All HCC patients and hospital controls were interviewed in the hospital wards. Data concerning demographic, socioeconomic and medical variables were recorded, and detailed histories of smoking habits and alcoholic drinking were taken.

dietary information

We used an interviewer-administered semiquantitative food frequency questionnaire, routinely employed in case–control studies in Greece [16, 17]. Cases and controls were asked to indicate the average frequency of consumption of ~120 food items or beverage categories per month, per week or per day, over a period of 1 year preceding the recognition of symptoms or signs of the present disease [18, 19].

For each subject, we calculated the average daily GL, by summing the products of the carbohydrate content per serving for each food, times the average number of servings of that food per day, times the food’s glycemic index [20, 21]. The glycemic index is a ranking of carbohydrate foods based on the postprandial blood glucose response which is expressed as a percent of the response to an equivalent amount of available carbohydrates from a reference food (e.g. white bread or glucose) [22]. We used white bread as a standard food. With respect to glycemic index values, we used international glycemic index tables [20]. For food items for which a glycemic index had not been determined, we assigned the glycemic index of the nearest comparable food (e.g. Greek fava beans were assigned the glycemic index of French beans). For these calculations, we used the carbohydrate content of 85 foods or recipes since 35 foods or recipes, mainly meat-, cheese- and fish-based ones, contained a negligible amount of carbohydrates [23].

serology

Sera obtained from each subject and stored at −25°C for 1–4 years were transferred on dry ice to the internationally certified Biomedicine Laboratories in Athens, Greece, for serologic testing. Coded samples were tested for hepatitis B surface antigen (HBsAg), the marker for HBV infection, using the Auszyme® monoclonal enzyme immunoassay (EIA) kit (Abbott, Chicago, IL). The presence of antibodies to HCV (anti-HCV) was determined with the Abbott HCV EIA 3.0. The anti-HCV assay detects antibodies to the HC-34 (core), HC-31 (NS3 and NS4), c100-3 (NS3 and NS4) and NS5 antigens. Results were interpreted according to the manufacturer’s instructions. Both the HBsAg and anti-HCV assays are considered third-generation tests.

statistical analyses

Odds ratios (ORs), with 95% confidence intervals (CIs), for the risk of HCC were generated using logistic regression modeling. Because individual matching was impossible for some HCC cases, it was necessary to conduct unconditional, rather than conditional, logistic regression modeling, with adjustment for the matching factors [24]. We investigated the association of GL with (i) HCC overall, (ii) HCC associated with chronic infection with hepatitis B and/or C and (iii) HCC unrelated to chronic infection with these viruses, controlling for gender, age (quinquennia, categorically), years of education (<12 and ≥12), tobacco smoking (never smokers, ever smokers <25 cigarettes/day, ever smokers ≥25 cigarettes/day, ordered), alcohol consumption (nondrinkers and tertiles of alcohol intake, ordered) and energy intake without alcohol and carbohydrates (continuously). Gender-specific control-generated quintiles were used to categorize GL, and category-specific ORs and 95% CIs were estimated, using the lowest quintile as the reference category. Moreover, trend tests over the five quintiles were carried out, and ORs and 95% CIs were also estimated for GL as a continuous variable (using as increment one control-generated standard deviation).

Among the 230 HCC cases with chronic infection with HBV and/or HCV, 107 were diagnosed with cirrhosis (42 clinically and 65 both clinically and histologically). Because patients with cirrhosis are frequently advised to reduce the intake of lipids, which would increase their intake of carbohydrates, we have evaluated the association between GL and HCC positive for chronic viral infection, separately among cases with and without cirrhosis. Moreover, as diabetes mellitus is known to be related with GL [8] and is also positively associated with HCC risk [5, 25], we repeated the analysis after exclusion of 60 diabetic cases and 39 diabetic controls.

Among the 360 controls, 12 were positive for HBsAg and one for anti-HCV [15]. Although these numbers are small and would make little difference in the analyses, we have opted to include them among controls on the basis of the study base principle (controls should include all those from which cases possibly arise) [26].

All analyses were conducted using the SAS 9.1.3 statistical package.

results

In Table 1, demographic and lifestyle characteristics of the HCC cases—overall and by evidence of chronic infection with HBV and/or HCV—and the hospital controls are presented. The data in Table 1 serve descriptive purposes, because all the indicated variables are adjusted for when evaluating the association of GL with HCC. Table 1 also presents total carbohydrate intake and GL among HCC cases (overall and by evidence of chronic infection with HBV and/or HCV). The results of these analyses are shown in Table 2. The risk of HCC was calculated by unconditional, rather than conditional, logistic regression modeling, with adjustment for the matching factors [24].

Table 1. Characteristics of 333 HCC cases (overall and by evidence of chronic infection with HBV and/or HCV) and 360 hospital controls with respect to gender, age, education, tobacco smoking, alcohol consumption, carbohydrate intake and glycemic load (Greece, 1995–1998)

<table>
<thead>
<tr>
<th>HCC cases</th>
<th>N</th>
<th>Gender, % men</th>
<th>Age (years), mean (SD)</th>
<th>Education years schooling, mean (SD)</th>
<th>Tobacco smoking, ever smokers (%)</th>
<th>Alcohol consumption, drinkers (g/day), mean (SD)</th>
<th>Carbohydrate intake (g/day), mean (SD)</th>
<th>Glycemic load, mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>333</td>
<td>85.0</td>
<td>63.9 (9.6)</td>
<td>8.2 (9.8)</td>
<td>235 (70.6)</td>
<td>196 (58.9)</td>
<td>163.6 (150.0)</td>
<td>236.9 (79.6)</td>
</tr>
<tr>
<td>HBsAg+ and anti-HCV−</td>
<td>198</td>
<td>91.9</td>
<td>62.2 (8.4)</td>
<td>7.3 (4.3)</td>
<td>143 (72.2)</td>
<td>120 (60.6)</td>
<td>183.5 (148.3)</td>
<td>247.7 (82.3)</td>
</tr>
<tr>
<td>HBsAg− and anti-HCV+</td>
<td>41</td>
<td>70.7</td>
<td>66.1 (9.2)</td>
<td>11.3 (20.6)</td>
<td>27 (65.9)</td>
<td>24 (58.5)</td>
<td>144.4 (148.7)</td>
<td>228.6 (78.7)</td>
</tr>
<tr>
<td>HBsAg+ and anti-HCV+</td>
<td>11</td>
<td>90.9</td>
<td>63.7 (9.5)</td>
<td>11.2 (7.3)</td>
<td>6 (54.6)</td>
<td>4 (36.4)</td>
<td>188.2 (177.7)</td>
<td>215.6 (63.1)</td>
</tr>
<tr>
<td>HBsAg− and anti-HCV−</td>
<td>83</td>
<td>74.7</td>
<td>67.1 (11.4)</td>
<td>8.6 (11.0)</td>
<td>59 (71.1)</td>
<td>48 (57.8)</td>
<td>121.5 (143.9)</td>
<td>217.9 (71.4)</td>
</tr>
<tr>
<td>Controls</td>
<td>360</td>
<td>82.8</td>
<td>64.0 (10.4)</td>
<td>7.9 (6.7)</td>
<td>234 (65.0)</td>
<td>222 (61.7)</td>
<td>131.7 (140.0)</td>
<td>231.1 (68.3)</td>
</tr>
</tbody>
</table>

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; SD, standard deviation; HBsAg, HBV surface antigen.
infection with HBV and/or HCV) and controls. There is evidence that the diet of HCC cases positive for HBsAg is characterized by higher GL, although interrelations among risk factors for HCC hinder conclusion about an unconfounded positive association.

Numbers are substantial only with respect to HCC cases with evidence of chronic infection with HBV and those with no evidence of chronic infection with either HBV or HCV, as well as with respect to controls in general (Table 1). Thus, in Table 2, we have opted to combine all HCC patients with chronic hepatitis (B and/or C) viral infection. This table presents multiple logistic regression-derived ORs and 95% CIs according to quintiles of GL among HCC cases (overall and by evidence of chronic infection with HBV and/or HCV) and hospital controls, adjusting for possible confounding factors. There is a nonsignificant positive association between GL and HCC in general, which is exclusively accounted for by a significant positive association between GL and HCC with chronic infection with hepatitis B and/or C. The association is statistically significant at the upper two quintiles of GL, as well as in a trend test across the five quintiles, whereas it is marginally significant \( (P = 0.056) \) in the model treating GL as a continuous variable. A test for interaction of the association between GL and chronic hepatitis infection with respect to HCC risk is marginally significant \( (P < 0.066) \).

As indicated, because patients with cirrhosis are frequently advised to reduce the intake of lipids, which would increase their intake of carbohydrates, we have evaluated the association between GL and HCC positive for chronic viral infection, separately among cases with and without cirrhosis. The OR for an increase of one standard deviation of GL was identical in the two groups, indicating that possible changes in diet following diagnosis of cirrhosis have no important confounding consequences. We also repeated the analysis after exclusion of the diabetic cases and controls. The OR for HCC cases without diabetes increased to 1.14 (95% CI 0.96–1.35) overall, 1.22 (95% CI 1.01–1.47) among hepatitis-positive and 0.88 (95% CI 0.65–1.20) among hepatitis-negative cases, possibly because diabetic patients are advised against consumption of carbohydrates, thus introducing negative confounding.

### Discussion

In a large case–control study undertaken in Athens, Greece, we found evidence that dietary GL is significantly positively associated with the risk of HCC among patients with chronic infection with HBV and/or HCV. No such evidence was found for HCC unrelated to hepatitis infection. Thus, pending confirmation, our results appear to indicate that, among patients with chronic infection with hepatitis B and/or C, reduction of dietary GL could reduce the risk or delay the development of HCC. Our results were not confounded by sociodemographic factors, tobacco smoking, alcohol drinking or energy intake and could not be explained by possible increase of carbohydrate intake by HCC patients with a diagnosis of cirrhosis, who are advised to reduce dietary intake of lipids. Moreover, exclusion of patients with diabetes mellitus, which is positively associated with both GL and HCC risk, actually strengthened the association between dietary GL and HCC among patients with chronic infection with HBV and/or HCV, possibly because diabetics are advised to reduce intake of foods with high glycemic index.

Dietary aspects of the etiology of HCC have been studied, but only the evidence concerning aflatoxin contamination of foods and cirrhosis-inducing heavy alcohol drinking have so far been convincingly implicated [27, 28]. Some earlier investigations have reported that intake of fruits and vegetables may reduce the risk of HCC, but the evidence is no more than suggestive [27, 28]. There is, however, indirect evidence implicating diet, and perhaps GL, because diabetes mellitus and obesity increase HCC risk [5, 10, 11, 25].

The findings of the present study are in broad agreement with those of a case–control study from Italy including 185

### Table 2

Multiple logistic regression-derived ORs and 95% CIs according to quintiles of glycemic load among 333 HCC cases (overall and by evidence of chronic infection with HBV and/or HCV) and 360 hospital controls (Greece, 1995–1998)

<table>
<thead>
<tr>
<th>Quintiles of glycemic load (Upper cut-off point among controls: males/females)</th>
<th>OR (95% CI)</th>
<th>( \chi^2 ) trend (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>49</td>
<td>1.34 (0.80–2.23)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1</td>
<td>1.19 (0.71–2.01)</td>
</tr>
<tr>
<td>HBsAg+ and/or anti-HCV+</td>
<td>29</td>
<td>1.62 (0.90–2.92)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1</td>
<td>1.58 (0.87–2.86)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1</td>
<td>1.50 (0.90–2.55)</td>
</tr>
<tr>
<td>Controls</td>
<td>72</td>
<td>0.64 (0.28–1.48)</td>
</tr>
</tbody>
</table>

\*Adjusted for gender, age, education, tobacco smoking, alcohol consumption and energy intake without alcohol and carbohydrates.

\*Gender-specific control-generated quintiles.

\*Estimated for an increment of intake equal to one standard deviation among controls.

\*Reference category.

OR, odds ratio; CI, confidence interval; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; HBsAg, HBV surface antigen.
HCC cases and 412 hospital controls [29]. That study found an OR of 3.25 (95% CI 1.46–7.22) for the highest compared with the lowest GL quintile among cases with chronic infection with HBV and/or HCV. The association was apparently less strong and not significant among subjects without markers of hepatitis B or C infection.

If the positive association between GL and HCC risk among patients with chronic infection with HBV and/or HCV is genuine, the differential effect on those with chronic hepatitis infection could possibly be accounted for by the likely longer natural history of HCC among those with chronic hepatitis infection, that usually progresses through chronic liver disease, cirrhosis and eventually HCC. High GL has been postulated to lead to chronically elevated insulin levels that could stimulate growth [30–32].

Our findings rely on a case–control protocol which in the past has generated several noteworthy results [13, 33, 34] that have been confirmed by other investigations. The dietary questionnaire, though not formally validated, has been used in many case–control studies in Greece [16, 17], the results of which have also been supported by other investigations. The control series included patients hospitalized for diseases unlikely to be related to diet and, in an earlier analysis, based on a subset of the study sample used in the present investigation, cases and controls were found not to differ with respect to intake of individual foods or food groups [18]. Perhaps more indicative of the lack of important selection or information bias with respect to reported dietary intakes is the fact that the association of GL with HCC was evident only among those with chronic hepatitis infection (the focus of this study) and not among those with HCC unrelated to hepatitis. Nevertheless, the study size, although large for a rare disease like HCC, is not large enough to minimize chance variation and, in an observational study, unidentified biases and residual confounding cannot be confidently excluded. In conclusion, pending confirmation, our results appear to indicate that, among patients with chronic infection with hepatitis B and/or C, reduction of dietary GL could reduce the risk for or delay the development of HCC.

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references