Dietary glycemic load and hepatocellular carcinoma with or without chronic hepatitis infection

M. Rossi1, L. Lipworth2,3, L. Dal Maso4, R. Talamini4, M. Montella5, J. Polesel4, J. K. McLaughlin2,3, M. Parpinel6, S. Franceschi7, P. Lagiou8 & C. La Vecchia1,9*

1Department of Epidemiology, ‘Mario Negri’ Institute for Pharmacological Research, Milan, Italy; 2International Epidemiology Institute, Rockville; 3Department of Medicine, Vanderbilt University Medical Center and Vanderbilt—Ingram Cancer Center, Nashville, USA; 4Epidemiology and Biostatistics Unit, Aviano Cancer Centre, Aviano; 5Department of Epidemiology, Fondazione ‘G. Pascale’, National Cancer Institute, Naples; 6Institute of Hygiene and Epidemiology, University of Udine, Udine, Italy; 7Epidemiology and Biology Cluster, International Agency for Research on Cancer, Lyon, France; 8Department of Hygiene and Epidemiology, University of Athens Medical School, Athens, Greece and 9Institute of Medical Statistics and Biometry ‘G. A. Maccacaro’, University of Milan, Milan, Italy

Received 7 January 2009; accepted 13 February 2009

Background: Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the major risk factors for hepatocellular carcinoma (HCC). The association of diabetes mellitus with HCC suggests that dietary glycemic load (GL) may influence HCC risk. We have examined the association between dietary GL and HCC.

Patients and methods: We conducted a hospital-based case–control study in Italy in 1999–2002, including 185 HCC cases and 412 controls who answered a validated food frequency questionnaire and provided blood samples. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were computed using unconditional multiple logistic regression.

Results: We observed a positive association between GL and HCC overall, with an OR of 3.02 (95% CI 1.49–6.12) for the highest quintile of GL compared with the lowest and a significant trend. The OR among HCC cases with evidence of chronic infection with HBV and/or HCV was 3.25 (95% CI 1.46–7.22), while the OR among those with no evidence of infection was 2.45 (95% CI 0.69–8.64), with no significant trend. The association was not explained by the presence of cirrhosis or diabetes.

Conclusions: High dietary GL is associated with increased risk for HCC. The positive association was most pronounced among HCC cases with HBV and/or HCV markers.

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

Introduction

Worldwide, primary liver cancer is the fifth most common cancer and the third most common cause of cancer mortality [1]. Approximately 80% of hepatocellular carcinomas (HCCs), the major form of primary liver cancer, occur in developing countries of Asia and Africa, but incidence of and mortality from this malignancy have been increasing in the United States and parts of Europe (including Italy, until the mid-1990s) [2–5]. Heavy alcohol consumption is established as an important risk indicator for HCC, but >80% of HCCs are attributable to chronic infection with either hepatitis B virus (HBV) or hepatitis C virus (HCV) [6].

With the exception of aflatoxin contamination of foods [7], dietary factors have not been consistently linked to HCC. Diabetes mellitus has been shown in several studies to increase risk for HCC [8–13]. Dietary glycemic load (GL), an indicator of the plasma glucose and insulin responses to different carbohydrates [14], plays a role in the development of diabetes mellitus [15, 16] and has been associated with several types of cancers including colorectal, breast, and endometrial cancer [15, 17]. We have examined for the first time, in a case–control study in Italy, the association between dietary GL and HCC, overall and separately according to evidence of chronic infection with HBV or HCV.

Patients and methods

A detailed description of study methods has been previously published [18]. We conducted a case–control study of HCC from January 1999 to July 2002 in the province of Pordenone in Northeast Italy and the town of Naples in the south. Cases were 258 patients under the age of 85 years with incident HCC, who had not yet received any cancer treatment at study entry. They were admitted to the National Cancer Institute, Aviano, the ‘Santa Maria degli Angeli’ General Hospital, Pordenone, and the ‘Pascale’ National Cancer Institute and four General Hospitals in Naples. Overall, 29 cases did not provide a blood sample and 44 did not provide data on dietary habits, thus leaving 185 eligible cases (median age 66 years, range 43–84
years) with available questionnaires and blood samples for the present analysis. Histologic or cytologic confirmation was available for 78.2% of HCC cases, while for the remaining cases, the diagnosis was based on ultrasound, tomography, and elevated a-fetoprotein levels.

Controls were patients <85 years of age admitted for a wide spectrum of acute conditions to the same hospitals where HCC cases had been interviewed. Patients whose hospital admission was due to diseases related to tobacco smoking or alcohol abuse were specifically excluded, as were those hospitalized for chronic diseases that might have led to substantial dietary modifications. However, comorbidity for such diseases was not an exclusion criterion. Blood samples were available for 431 of 462 controls; of these, 412 provided comprehensive questionnaire information on dietary habits and were included in the present analyses (median age 65 years, range 40–82 years). Twenty-seven percent were admitted for trauma, 24% for nontraumatic orthopedic diseases, 25% for acute surgical conditions, 13% for eye diseases, and 11% for other miscellaneous illnesses. Controls were more often female and were younger than HCC cases. All analyses were adjusted for sex, age, and study center. Overall, ~1% of cases and controls contacted refused to participate.

All study participants signed an informed consent form, according to the recommendations of the Ethical Committee of the National Cancer Institute at Aviano. Cases and controls in each study center were interviewed in the hospital by uniformly trained personnel using a standardized, structured questionnaire designed to collect information on sociodemographic characteristics, lifestyle habits, such as tobacco smoking and alcohol drinking, and personal medical history, including history of cirrhosis and diabetes. An interviewer-administered food frequency questionnaire (FFQ) shown to have high reproducibility and validity [19, 20] was used to assess participants’ habitual diet, including total energy. Average weekly frequency of consumption of 63 foods or food groups, as well as complex recipes, during the 2 years before cancer diagnosis or hospital admission for (models was assessed. To compute energy and nutrient intakes, an Italian food composition database, as well as information from additional sources, were used [21, 22].

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 (GI) [23, 24]. The GI is a ranking of specific carbohydrate-rich 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) [14]. In this analysis, white bread was used as the standard food reference. For these calculations, we used the carbohydrate content of 44 foods or recipes since the remaining 19 foods or recipes, chiefly meat-, fish-, and cheese-based foods, contained a negligible amount of carbohydrates [22]. With respect to GI values, we primarily used international tables [23]. In order to take into account Italian cooking habits (e.g. pasta ‘al dente’), Italian sources were used for a few local recipes [25]. Food items for which a GI had not been determined were assigned the GI of the nearest comparable food (e.g. tangerines were assigned the GI of oranges).

Each case and control provided a 15-ml sample of blood on the day the interview took place. Sera were screened for antibodies against HCV (anti-HCV) using a third-generation MEIA (AxSYM HCV version 3.0; Abbott Diagnostic Division, Wiesbaden, Germany) and for HBV surface antigen (HBsAg) using microparticle enzyme immunosassay (AxSYM HBsAg version 2.0, Abbott Diagnostic Division) [18, 26].

Odds ratios (OR) and corresponding 95% confidence intervals (CIs) were computed using unconditional multiple logistic regression, adjusting for sex, quinquennia of age and study center, as well as years of education (<7, 7–11, 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 (quinquies, ordered). GL was entered into the models as gender-specific quintiles based on the distribution of controls, using the lowest quintile as the reference category, or as a continuous variable using as the increment one control-generated standard deviation (SD). Trend tests for HCC risk across the quintiles of GL were based on a likelihood ratio test between models with and without a linear term for GL. We investigated the association of GL with HCC overall, HCC associated with chronic infection with hepatitis B and/or C and HCC unrelated to chronic infection with these viruses.

Because patients with cirrhosis are frequently advised to reduce intake of lipids, which would increase their intake of carbohydrates, we have evaluated the association between GL and HCC separately among cases (n = 156) and controls (n = 410) without a diagnosis of cirrhosis. Moreover, as diabetes mellitus is known to be associated with GL [15] and is also positively associated with HCC risk [10, 11, 13], we repeated the analyses after exclusion of 38 diabetic cases and 27 diabetic controls.

Among the 412 controls, four were positive for HBsAg and 45 for anti-HCV [18]. 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) [27].

**results**

Among the 185 HCC cases, 147 (79.5%) had evidence of chronic infection with HBV and/or HCV [118 and 20 had evidence of chronic infection with either HCV or HBV only, respectively, and nine were positive for both HBsAg and anti-HCV] and 38 had no evidence of chronic infection with either HBV or HCV. Table 1 presents demographic and lifestyle characteristics of the HCC cases and controls, overall and by evidence of chronic hepatitis B and/or C viral infection. HCC cases were more likely than controls to smoke cigarettes, to drink alcohol, and to have diets characterized by higher carbohydrate and total energy intake. Mean GL was 222.2 among controls and 265.5 among HCC cases overall, and appears to be highest among HCC cases positive for anti-HCV, although confounding by total energy and carbohydrate intakes is likely in these descriptive analyses. The variables presented in Table 1 were adjusted for in multivariate analyses of the association of GL with HCC.

Table 2 presents multiple logistic regression-derived ORs and 95% CIs according to quintiles of GL and continuous intake increments among HCC cases overall and by evidence of chronic infection with HBV and/or HCV, adjusting for potential confounding variables. We observed a statistically significant positive association between GL and HCC overall, with an adjusted OR of 3.02 (95% CI 1.49–6.12) for the highest quintile of GL compared with the lowest and a statistically significant trend test across the five quintiles. The number of HCC cases with evidence of chronic infection with HBV was too small for meaningful analysis; thus, we combined all HCC patients with chronic hepatitis B and/or C viral infection for subsequent analyses. The positive association between GL and HCC was statistically significant only among HCC cases with evidence of chronic infection with HBV and/or HCV, with an OR of 1.44 (95% CI 1.17–1.77) for an increase of one SD of GL in the model including GL as a continuous variable. The adjusted OR for the highest
quintile of GL compared with the lowest was 3.25 (95% CI 1.46–7.22) among those positive for HBsAg and/or anti-HCV (P value for trend across quintiles = 0.008), while the corresponding OR among those with no evidence of infection with either HBV or HCV was 2.45 (95% CI 0.69–8.64) with no significant trend across quintiles.

When we evaluated the association between GL and HCC positive for chronic HBV and/or HCV infection separately among cases and controls without cirrhosis, the risk estimates were virtually unchanged (OR for an increase of one SD of GL = 1.40; 95% CI 1.13–1.73; data not shown). After exclusion of cases and controls with a diagnosis of diabetes, the ORs for the highest quintile of GL compared with the lowest increased to 2.94 (95% CI 0.63–13.73) for HCC unrelated to hepatitis infection and to 3.99 (95% CI 1.57–10.13) for HCC positive for chronic HBV and/or HCV infection; corresponding ORs in models treating GL as a continuous variable were 1.27 (95% CI 0.80–2.01) and 1.53 (95% CI 1.22–1.91), respectively, when excluding diabetic subjects (data not shown).
Our results suggest that dietary GL is positively associated with risk of HCC among patients with evidence of chronic infection with HBV and/or HCV; among those without chronic hepatitis infection, a positive association with GL was still apparent, but it was weaker and not statistically significant. Approximately 80% of HCC cases in our study population had evidence of chronic HBV and/or HCV infection; thus, the sample size for those with HCC unrelated to hepatitis infection (N = 38) is too small to provide a reliable estimate of risk.

The findings of the present study are in broad agreement with those of a similar case–control study from Greece, including 333 HCC cases and 360 hospital controls [28], which reported an OR of 1.95 (95% CI 1.09–3.48) for the highest compared with the lowest GL quintile among cases with chronic HBV and/or HCV infection. In that study, however, no association between GL and HCC was apparent among subjects without markers of HBV or HCV infection.

The observed positive association between dietary GL and HCC was not explained by either increased carbohydrate intake among HCC patients with a diagnosis of cirrhosis or reduced intake of foods with high GI and consequently high GL among diabetics. In fact, exclusion of cases and controls with a diagnosis of diabetes mellitus, which is positively associated with both dietary GL and HCC, strengthened the association between dietary GL and HCC, particularly among HCC patients with evidence of chronic HBV and/or HCV infection.

Dietary GL combines the overall GI of a diet (the extent to which carbohydrate-rich foods increase the concentration of glucose in the blood) and the actual amount of carbohydrates consumed, to represent the total glycemic effect of the diet, which affects insulin secretion and postprandial glycemia [14, 23]. Thus, GL is an indicator of the glucose response and insulin demand induced by a serving of food. GL has been consistently linked to diabetes mellitus and has been shown to be positively associated with several types of cancer [15–17].

The proposed mechanism for a role of high GI in carcinogenesis is through increased insulin concentrations, glucose intolerance, and insulin resistance, even in the absence of diabetes mellitus [29, 30]. This increased insulin stimulates a rise in free insulin-like growth factors, which, in the case of HCC, may stimulate hepatic cell proliferation. There is no immediate explanation for why an association with dietary GL would be more pronounced among, or confined to, HCC patients with chronic hepatitis infection, if in fact this is confirmed; it could be due to accumulated liver damage over decades caused by hepatitis infection, which progresses from liver inflammation to cirrhosis, to hepatocyte death and regeneration, and then to HCC, but this remains speculative. Likewise, metabolic factors, including diabetes and obesity, have been reported to be most strongly associated with HCV-positive HCC in a cohort study from Taiwan [31].

Cases and controls in our study came from comparable catchment areas, were interviewed by uniformly trained interviewers in their respective hospital settings, and were unaware of any particular dietary hypothesis related to HCC, thereby reducing potential selection and recall bias [32]. The FFQ was satisfactorily reliable [19] and valid [20]. Participation among eligible cases and controls was virtually complete, and we excluded from the control series patients hospitalized for diseases likely to be related to tobacco smoking, alcohol consumption, or long-term dietary modifications. Our results were adjusted for the major known risk factors for HCC, including education, alcohol drinking, tobacco smoking, and energy intake.

The range of dietary GL in our study population is generally consistent with what has been observed in other epidemiologic studies [17]. If in fact GL plays a role in HCC etiology, one might expect to see an association between HCC and intake of high GI foods, such as added sugars, syrups, sweets, white bread, and soft drinks; there is little such evidence [33], and an earlier report from the present study showed an inverse association between HCC risk and added sugar (but not non-added sugar), but this is likely to reflect residual confounding or the play of chance [34]. With respect to potential confounding by other dietary factors, it is possible that the balance between glucose and insulin responses may be influenced by dietary intakes of protein, fat, and/or fiber, but none of these dietary variables have been conclusively linked to risk for HCC.

In conclusion, our results suggest that high dietary GL is associated with increased risk for HCC. The positive association was not explained by the presence of cirrhosis or diabetes and was most pronounced among those HCC patients with evidence of chronic infection with HBV and/or HCV, but the small number of patients with HCC unrelated to hepatitis infection prevents reliable risk estimates for this group.